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Research Article

Identification and Characterization of Rot-Causing Fungi Affecting Yam Tubers During Storage in Calabar, Nigeria

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Abstract

Background and Objective: Post-harvest deterioration of yam by microorganisms is a major constraint militating against yam production and marketing in many yam-producing countries. This study aimed to identify the fungal pathogens causing rots and their impact during the storage of yam tubers in Calabar. **Materials and Methods:** Twenty yam tubers were collected from a warehouse at Akim Market in Calabar and kept to rot. Eighteen of the tubers developed different symptoms of dry, wet and soft rots during storage from which 15 fungal pathogens were isolated. The pathogens were isolated and characterized based on identified morphological characteristics, in addition to molecular data. **Results:** The main fungi isolated were *Aspergillus* spp., *Lasiodiplodia* spp. and *Rhizopus* spp. Nucleotide sequence identities ranging from 60.0 to 97.6% were obtained among the isolates indicating various degrees of affinity. Pathogenicity test on *Dioscorea rotundata* and *Dioscorea alata* showed that all the isolates displayed typical symptoms of different yam rots as were observed in the original rotted yams from which the pathogens were isolated among the fungi isolates. *Rhizopus* spp., was the most invasive causing up to 100% rot of the tubers. **Conclusion:** Based on the present study data, it is recommended that in addition to employing improved methods of handling and storing yam tubers, developing resistant varieties will effectively cut down on postharvest losses caused by biological agents.

Key words: *Dioscorea* spp., fungal disease, phylogeny, rots, yam

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Yam (*Dioscorea* spp.) is grown mainly in tropical and subtropical climates and it is a major staple for millions in West Africa[1-4]. The yam belt region of West Africa, which includes Nigeria, Benin, Togo, Ghana and Côte d'Ivoire, accounts for about 95% of the 72.6 million tons of global yam production; with Nigeria as the lead producer contributing over 65% of the global production[4]. As far as production is concerned, yam ranks second as the world's most important tuber crop after cassava[4]. There are well over 600 species of yams[5], but only a few, including *D. rotundata* Poir. (white yam), *D. alata* L. (water yam), *D. cayenensis* L. (yellow or Guinea yam), *D. dumetorum* (trifoliate or sweet yam), *D. bulbifera* Lam. (aerial yam), *D. japonica* (Japanese yam), *D. nummularia* (Pacific/spiny yam), *D. oppositifolia* (Chinese yam; China), *D. pentaphylla* (five-leaved yam), *D. trifida* (aja, aje, cush-cush, yampi) and *D. esculenta* (Asiatic yam) are cultivated as food sources[1,5,6].

Yam is seen as a religious, social and cultural crop in some communities in Nigeria and it is one of the favorite foods in social functions such as marriages, burials, as well as other traditional ceremonies and rituals. This perception explains why these communities celebrate annual 'New Yam Festivals'[4,7]. The supply for yam, therefore, hardly meets its demand and this is partly because its production is severely constrained by the cost and unavailability of clean planting material[8]. Pests and pathogens have been identified as key factors hindering the productivity of this staple both in farms and storage units[4]. Bacteria, fungi, viruses and nematodes are responsible for rots in yams at different stages of growth and storage[9-11]. These pathogens reduce the growth, quantity and quality of yam[10,12]. In Nigeria alone, rots initiated by these pathogens account for over 50% of losses in yam tubers in storage[13,14]. Depending on the pathogen, infected yam tubers may display dry rot, soft rot and wet or watery rot[15]. Fungi species belonging to diverse genera such as *Aspergillus*, *Botryodiplodia*, *Fusarium*, *Penicillium*, *Trichoderma*, *Rhizopus*, *Colletotrichum*, *Cladosporium*, *Cylindrocapus*, *Gliocladium*, *Geotrichum*, *Gliomatrixcon*, *Macrophomina*, *Mucor* and *Rhizoctonia* have been implicated in yam tuber rot, causing annual yield decline in storage barns[10,16-19]. Proper diagnosis of the disease and identification of the causing agent(s) are critical in designing appropriate mitigation measures to curb the menace to ensure the availability of tubers to consumers throughout the year. This research was aimed at identifying the fungal pathogens associated with post-harvest yam tuber rots and their impact on stored yam tubers in Calabar. *Aspergillus* spp., *Lasiodiplodia* spp. and *Rhizopus* spp., were identified as the fungal pathogens causing rots of yams in storage in Calabar and environs, with *Rhizopus* having the greatest impact on the stored yams.

MATERIALS AND METHODS

Study area: This study was carried out in Calabar, Cross River State from April, 2022 to February, 2023. The yam samples were collected from Akim Market in Calabar and the laboratory analyses were carried out in the Molecular Biology Laboratory of the Department of Genetics and Biotechnology, Faculty of Biological Sciences, University of Calabar, Calabar, Nigeria.

Collection of yam tubers from sampling location and categorization of rots: Twenty tubers of *D. rotundata* having no physical damage, were collected from a warehouse at Akim Market in Calabar, Cross River State, Nigeria. The *D. rotundata* is the most cultivated and consumed yam species in Cross River State. The yam tubers were transported to the laboratory in labelled, sterile polythene bags. The tubers were removed from the bags and placed on open shelves in the laboratory at room temperature where they were observed daily for two weeks.

Decayed yams were distinguished by visual examination, as being withered in appearance and/or delicate to touch. Where there was uncertainty, the peeling off of the skin at the infected location usually uncovered brown decayed tissue for confirmation. The scheme of Amusa *et al.*[15] was used to categorize the rots as follows: Dry rot: Infected tissues got hard and dry with different discolorations depending on the causative agent; Soft rot: Infected tissues got delicate and ramified by the fungal mycelium; Wet rot: This was exemplified by the exudation of whitish liquid from the infected yam tissue when squeezed between the fingers. All decayed tubers were removed from the shelves and kept independently for identification of fungal pathogens.

Isolation of fungal pathogens: Yam tubers with decaying indications were washed with running tap water to remove residual soil particles. Each tuber was surface decontaminated in 5% sodium hypochlorite for 5 min and rinsed a couple of times with sterile distilled water. After that, 3-4 mm yam pieces were picked from the decaying edge with sterilized forceps and inoculated in potato dextrose agar (PDA) medium supplemented with chloramphenicol (1000 mg/L). The plates were incubated at 28±2°C and checked every day for seven days for fungal growth. To get pure cultures, any observed fungal growth was sub-cultured on a new PDA plate. The pure cultures were stored on PDA slant in the refrigerator until needed.

Identification of fungal isolates using morphological features: Morphological characteristics of mycelia mounted in lactophenol were observed with a compound Olympus microscope. Identification of fungal isolates was based on the following culture characteristics on PDA: Mycelial growth rate (measured daily with a meter rule in cm from the center of the plate to the edge (radius)), mycelial colour and branching pattern; spore colour, type, size and shape; aggregation and arrangement of sporangiophore, conidiophore, conidia and sporulating structures as described by researchers[20-26]. The measurements and observations were made on three culture plates for each isolate.

The structures of the isolates were compared with the standard reference atlas of imperfect fungi by Barnet and Hunter[22] and with literature on the identification of yam storage fungi by Visagie *et al.*[27]. Isolates were subsequently subjected to a pathogenicity test.

Molecular characterization of fungal isolates

Extraction of fungal genomic DNA: As 100 mg of seven day old fungal cultures were freeze-dried in liquid nitrogen, pulverized with mortar and pestle and then transferred into 2 mL Eppendorf tubes. Approximately 500 µL of Cetyltrimethylammonium Bromide (CTAB) extraction buffer and 10 µL of β-mercaptoethanol were added to the tube before incubation for 1 hr at 60°C. As 1000 µL of Phenol:Chloroform:Iso-amyl alcohol in the ratio 25:24:1 was added and vortexed for 5 min, then allowed to stand for 5 min. The samples were centrifuged at 12,000 rpm for 10 min after which the supernatants were transferred to new tubes and 1000 µL of Chloroform:Iso-amyl alcohol (24:1) was added and centrifuged at 12,000 rpm for 5 min. The supernatants were transferred to a new tube and 500 µL of ice-cooled isopropanol was added and incubated at -20°C for 15 min to precipitate DNA. The DNA was recovered by centrifuging at 12,000 rpm for 5 min. The pellets were washed with 70% ethanol, air-dried and re-suspended in 20 µL Tris-EDTA (TE) buffer.

Amplification of fungal genomic DNA by PCR: Polymerase chain reaction was performed using New England BioLabs Taq DNA polymerase kit in a 50 µL reaction volume containing 1 µL DNA, 5µL of Taq polymerase enzyme with standard buffer, 5 µL of Magnesium Chloride ($MgCl_2$), 1 µL of dNTP, 1 µL of 10 µM each of forward and reverse primer and 36 µL nuclease-free water. The PCR amplification conditions were performed as follows: Initial denaturation step at 94°C for 3 sec, followed by 35 cycles of denaturation at 94°C for 20 sec, annealing at 58°C for 60 sec, extension at 68°C for 1 min; final extension at 68°C for 5 min and holding at 4°C. The primers used are shown in Table S1. The amplified products were separated in 1% agarose gel stained with ethidium bromide for 15 min and visualized under a UV transilluminator.

Sequencing of the PCR products: The PCR products obtained above were purified by ultrafiltration using Centricon®-100 columns (PN N930-2119) following instructions in the user manual. The purified products were used to constitute a PCR sequencing reaction as follows: 13.5 µL nuclease-free water, 2.5 µL PCR product, 2 µL sequencing buffer (5X), 1 µL of Big Dye Terminator ready reaction premix and 1 µL of 10 mM of either forward or reverse primer for each of the primer pair (LT347-F&R, ASP_GEN_MTSSU-F&R and RRF1-F&R).

A thermal cycler was used for the PCR amplification with initial denaturation step at 96°C for 1 min followed by 40 cycles of denaturation at 96°C for 10 sec, annealing at 50°C for 5 sec, extension at 60°C for 4 min, final extension at 72°C for 4 min. The sequencing product was purified by adding the following: 50 µL ethanol (100%), 2 µL sodium acetic acid (3 M) and 2 µL of EDTA (125 mM), mixed by inverting 4 times and incubated for 15 min at room temperature. Following the incubation period, the mixture was centrifuged at 1400×g for 45 min at 20°C.

The mixture was centrifuged at 1400×g for 45 min at 20°C. As 70% ethanol was used to clean the pellets, then left to dry in the hood for 20 min. The samples were re-suspended in an injection buffer and then sequenced using ABI Prism 3700 DNA analyzer (Applied Biosystems, California, USA). The SnapGene® software version 7. 0.2 (Dotmatics, Boston, USA) was used to assemble, edit and analyze the sequences. For each isolate, 2 replicates each for forward and reverse primers were sequenced.

Phylogenetic analyses: To identify the most similar sequences available in the database, the nucleotide sequences for each isolate were used in BLASTn searches against the GenBank database.

Sequences in the GenBank with >60% similarity to the isolates were selected for alignment. Sequence alignment was done using the CLUSTALW alignment[28]. The CLUSTALW alignments were used for inferring phylogenetic trees using the UPGMA method[29]. To determine the confidence values for the grouping within a tree, a bootstrap analysis was performed using 1000 resampling's of the data. Phylogenetic and molecular evolutionary analyses were done using MEGA version 7[30].

Pathogenicity test: To ascertain the virulence of the isolated fungi, a pathogenicity test was performed by axenic inoculation of fresh and healthy yam tubers (*D. rotundata* and *D. alata*) according to the method described by Okafor[31]. Healthy yam tubers (20 *D. rotundata* and 20 *D. alata*) free from bruises, cuts, rots or any visible defects were collected from Akim Market in Calabar, washed in running tap water for 10 min, surface sterilized by immersion in 5% sodium hypochlorite solution for four minutes, rinsed in four successive changes of sterile distilled water (SDW) and blotted-dry. A disc of five days old culture of the fungal isolates obtained from rotted yam tubers grown on PDA was the source of inoculum for the pathogenicity test. A 5 mm diameter cork borer was used to remove 5 mm tissue from the healthy yam tuber surfaces aseptically. A sterile 5 mm diameter cork borer was used to cut mycelia plugs from the edge of five days old cultures of each isolate. These fungal plugs were put in the holes created in the yam tubers. The mouths of the wells were wiped with 95% ethanol and the wells occluded with yam disk before sealing inoculation points with petroleum jelly[22]. The same procedure was used for the control except that disks of un-inoculated PDA were placed in the holes created in the tubers. There were three replicate tests of each isolate and the control. The tubers were partitioned into equal sizes (7 cm per partition) such that the 15 fungal isolates were randomized on three tubers, while a separate tuber was used for control[31]. The inoculated yam tubers were placed at room temperature in a completely randomized design for five weeks at 28±2°C under sterile condition.

At the end of the study, the tubers were cut longitudinally through the inoculation points and the symptoms of rot recorded and compared with those observed in the original infected tubers from where the isolates were gotten. To establish the patho-system and fulfill the Koch's postulate[32], the rot-causing organisms were re-isolated and identified from inoculated yam tubers and compared to the original isolates. An isolate was confirmed to be pathogenic in the case were it caused rot comparable to that observed on the infected tubers from where it was isolated.

Estimation of percentage rot: To estimate the extent of rot on tubers caused by each pathogen, *D. rotundata* and *D. alata* tubers were cut open longitudinally at five weeks and the surface area of both rotted and entire yam was calculated as length multiplied by breadth using vernier caliper. Percentage rot was determined by dividing the surface area of rotted yam by the surface area of the entire yam tuber and multiplied by 100.

Statistical analysis: A completely randomized experimental design was used. Data collected were subjected to an Analysis of Variance (ANOVA) test using GenStat Discovery Edition 12. Significant means at 5% ($p<0.05$) were separated using the least significant difference (LSD) test.

RESULTS

Identification and characterization of rots and the associated fungal isolates: Twenty days after storage, 90% of the yam tubers showed various symptoms of rots and were accordingly classified as dry (Fig. 1a), soft rots (Fig. 1b) and wet (Fig. 1c), with 15 different fungal isolates (Table 1). The fifteen fungal isolates obtained (YSF1-15) (Fig. 2) were described based on the cultural and morphological characteristics (Table 2). Mycelia growth ranged from slow (isolates YSF1-YSF4 and YSF15), covering up to 4 cm from the center of the Petri dish in 48 hrs, to fast (isolates YSF5-YSF14), covering the entire Petri dish in 48 hrs (Table 2). Mycelia colour

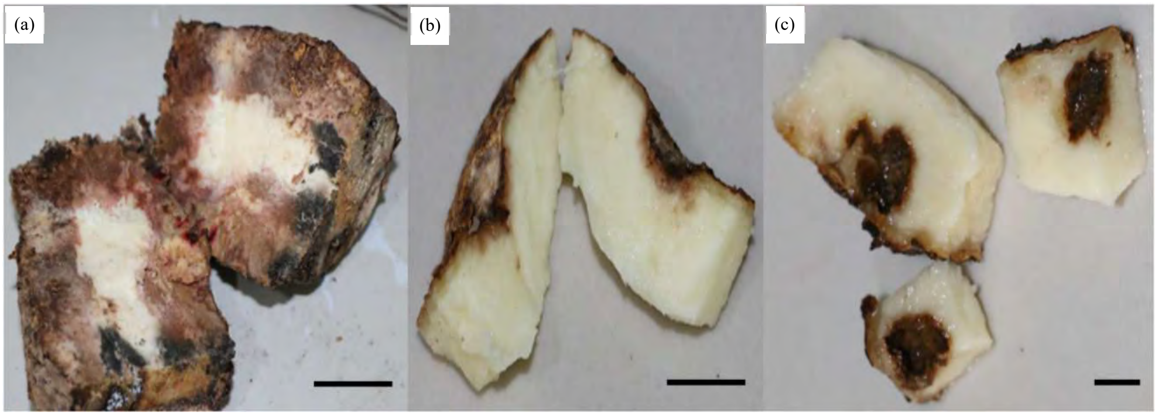


Fig. 1(a-c): Rotted yam tubers, (a) Dry rot, (b) Soft rot and (c) Wet rot
Scale bar: 20 mm

Table 1: Identification and characterization of rots and the associated fungal isolates from rotted yams in storage

Type of rot in stored tubers	Occurrence of rot in stored tubers (%)	Number of fungal isolates	Physical appearance
Dry	60	4	Dry and woody tubers with dark-brown to black colour
Soft	10	9	Tissues appear foamy and soft with colour ranging from light brown to dark-brown
Wet	20	2	Tissues appear marshy and moist, yellowish and sometimes creamy in colour
No rot	10	-	-

Table 2: Cultural characterization of the different fungal isolates

Isolate	Mycelia growth rate at 48 hrs (cm)	Colour of mycelia	Morphology of mycelia	Spore conc. (×10^4)	Sporulating structures
YSF 1	2	White	Cottony mycelia with yellowish centric pigment, elevation raised, margin undulate and wrinkled on the reverse. Alternate, branched, non-septate hyphae with smooth and round conidiophore	112	Brownish sand-like spores
YSF 2	2	White	Cottony mycelia with entire margin and partitions elevation flat. Non-septate hyphae, highly branched towards the tip, sporangiophore rough with numerous sticky spore	163	Brownish sand-like spores
YSF3	1.2	White	Cottony mycelia with lobed margin. Thread-like, branched, hyaline, septate and colourless hyphae, dark rough conidia	253	Brownish-black spores
YSF4	4.2	Yellow	Short hair-like mycelia with flat surface and entire margin. Thread-like, non-branched and non-septate hyphae with sporangia	254	Black spores
YSF5	Covered the entire Petri dish	Black	Woolly/filamentous mycelia with crateriform elevation, margin entire. Short branched and hyaline mycelia, sporangia globose to somewhat cylindrical	8	Brown spores
YSF6	Covered the entire Petri dish	Transparent	Rhizoid form with raised elevation, filiform margin translucent and glistering surface. Branched mycelia, non-septate stolon, sporangia globose	16	Brown spores
YSF7	Covered the entire Petri dish	Transparent	Filamentous in form, convex elevation, filiform margin, smooth surface. Branched mycelia with non-septate hyphae, smooth round sporangiophore	19	Black spores
YSF8	Covered the entire Petri dish	Transparent	Rhizoid form with umbonate elevation, entire margin, wrinkled surface. Short multi-branched non-septate hyaline mycelia, sporangia present	23	Brown spores
YSF9	Covered the entire Petri dish	Transparent	Irregular form, convex elevation, undulate margin and rough surface. Thread-like non-septate mycelia, long and smooth hyaline conidia with dark apex	9	Brown spores
YSF10	Covered the entire Petri dish	Transparent	Rhizoid form, raised elevation, filiform margin with glistering surface. Short multi-branched mycelia with non-septate hyphae, sporangia globose	22	Greyish black spores
YSF11	Covered the entire Petri dish	Transparent	Circular convex elevation, entire margin, smooth surface. Branched mycelia, non-septate hyphae with oval and globose sporangia	5	Brown spores
YSF12	Covered the entire Petri dish	Translucent	Circular umbonate elevation, entire margin, with wrinkled surface. Simple branched non-septate mycelia, sporangia attached to stolon connecting the rhizoid	40	Brown spores
YSF13	Covered the entire Petri dish	Opaque	Irregular form, raised elevation, undulate margin and wrinkled surface. Simple short branched mycelia, coarsely uncoloured conidiophore with vesicles	12	Black spores
YSF14	Covered the entire Petri dish	Transparent	Irregular form, flat elevation, undulate margin with rough surface. Simple short branched mycelia, coarsely uncoloured conidiophore with vesicles	29	Brown spores
YSF15	0.5	Opaque	Filamentous convex elevation with filiform margin, smooth surface. Highly branched mycelia, conidiophores often branched and green	601	Green spores

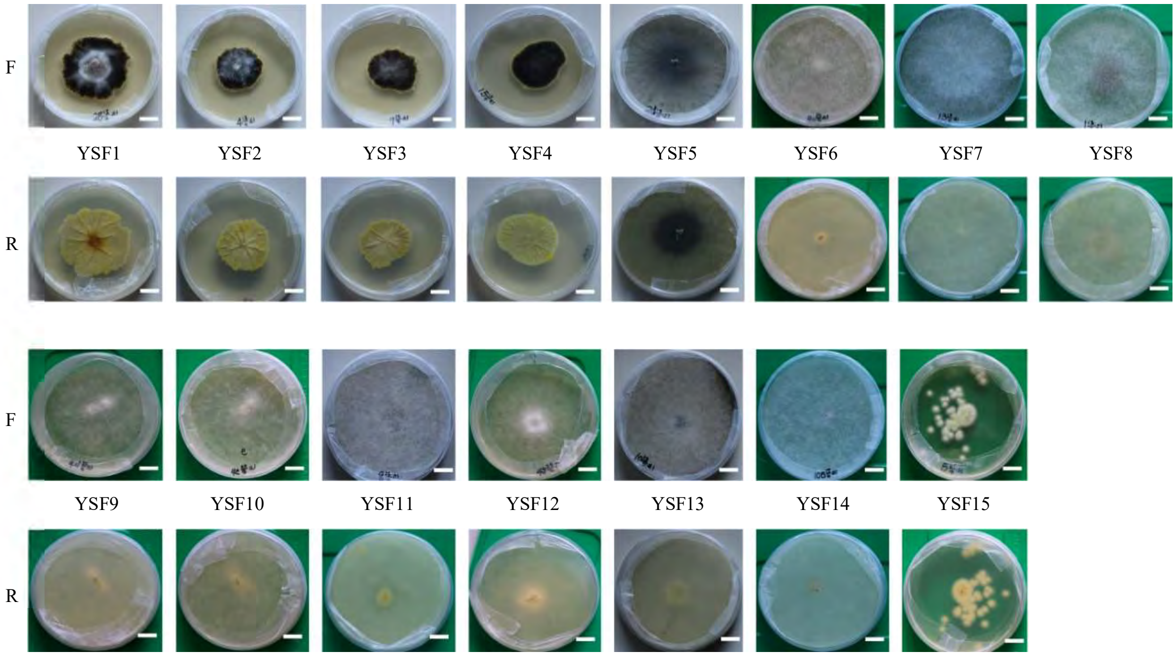


Fig. 2: Morphological variability of 15 fungal isolates obtained from the rotten yam tubers

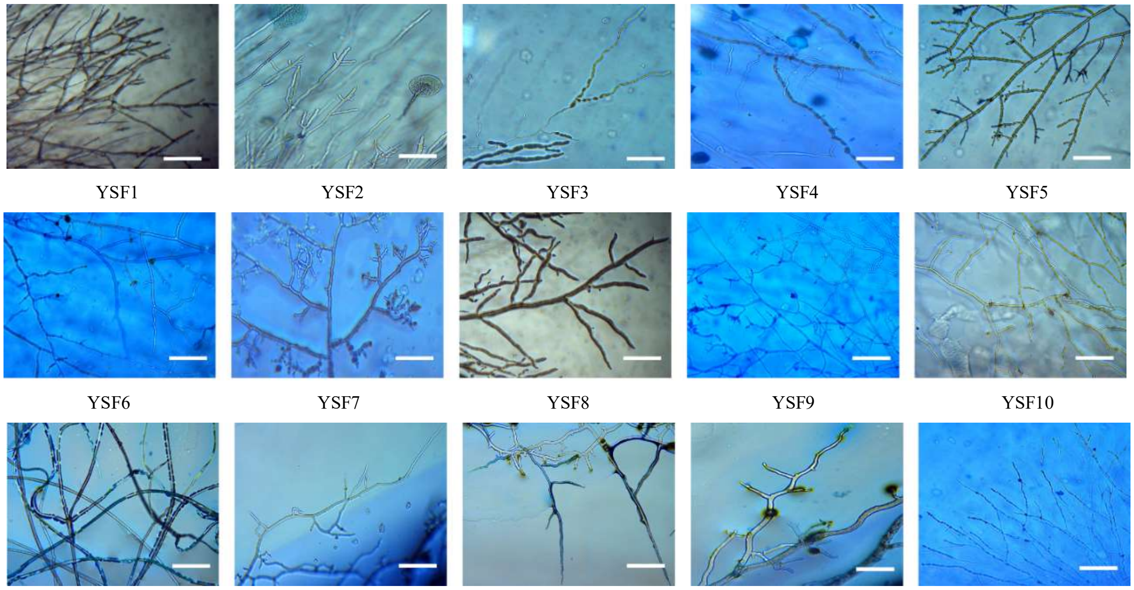
Top row (F): Upper side of the colony, Bottom row (R): Reverse side of the colony and Scale bar: 20 mm

was white in isolate YSF1-3, yellow in isolate YSF4, black in YSF5, transparent in isolates YSF6-YSF11, translucent in YSF12 and opaque in YSF13-YSF15. Spores were observed to be brownish, black and green across the different isolates. The morphology of the mycelia varied greatly across the isolates with different characteristic features. The mycelia were cottony as observed in isolates YSF1, YSF2 and YSF3; rhizoid in isolates YSF6, YSF8 and YSF10; filamentous in isolates YSF7, YSF8 and YSF15 and irregular in isolates YSF9, YSF13 and YSF14. The hyphae were observed to be mostly branched and non-septate as seen in isolates YSF1, YSF2, YSF4, YSF6, YSF7, YSF10 and YSF11; while the fruiting bodies were globose in isolates YSF5, YSF6, YSF10 and YSF11; smooth as in isolates YSF1, YSF7 and YSF9 and rough in isolates YSF2 and YSF3 (Table 2, Fig. 2-4).

PCR amplification: Initially, 7 pairs of primers targeting specific genes were screened in the genera *Lasiodiplodia*, *Aspergillus*, *Penicillium*, *Rhizopus* and *Alternaria*. Of these, only 3 pairs showed amplification and these were selected for further analysis. The 3 pairs are: For ITS1 gene, Primer LT347-F&R for *Lasiodiplodia* spp.[33], for 18S rRNA gene, primer RRF1-F&R for *Rhizopus* spp.[34] and mtDNA_SSU rRNA gene, primer ASP_GEN_MTSSU-F&R for *Aspergillus* spp.[35]. When the 15 isolates were subjected to PCR analysis using these three primers, only nine isolates were amplified. Primer LT347-F&R amplified one isolate (isolate YSF5) (Fig. 5a), primer RRF1-F&R amplified seven isolates (YSF6, YSF7, YSF11, YSF12, YSF13, YSF14 and YSF15) (Fig. 5b), while primer ASP_GEN_MTSSU-F&R amplified isolate YSF3 (Fig. 5c), with PCR fragments of 347, 631 and 480 base pairs, respectively. This result indicates that the isolated fungi are *Lasiodiplodia* spp., *Aspergillus* spp. and *Rhizopus* spp. and are the organisms responsible for the manifestation of rots in the yam tubers in storage.

Sequence analysis: The PCR products obtained using primers LT347-F&R, RRF1-F&R and ASP_GEN_MTSSU-F&R were purified and sequenced. The nucleotide sequences of each isolate are presented in Table S2. The EMBOSS matcher-Pairwise Sequence Alignment was used to determine whether the isolates were different. Alignment of the nucleotide sequences of the isolates revealed nucleotide sequence identities ranging from 60.0 to 97.6% among the isolates (Table 3). The five *Rhizopus* spp., isolates share >89% nucleotide identity (Table 3) indicating that the isolates are closely related.

The nucleotide sequences were then compared with those of published ITS, 18S rRNA and mtDNA_SSU RNA sequences of several *Lasiodiplodia*, *Rhizopus* and *Aspergillus*, respectively.

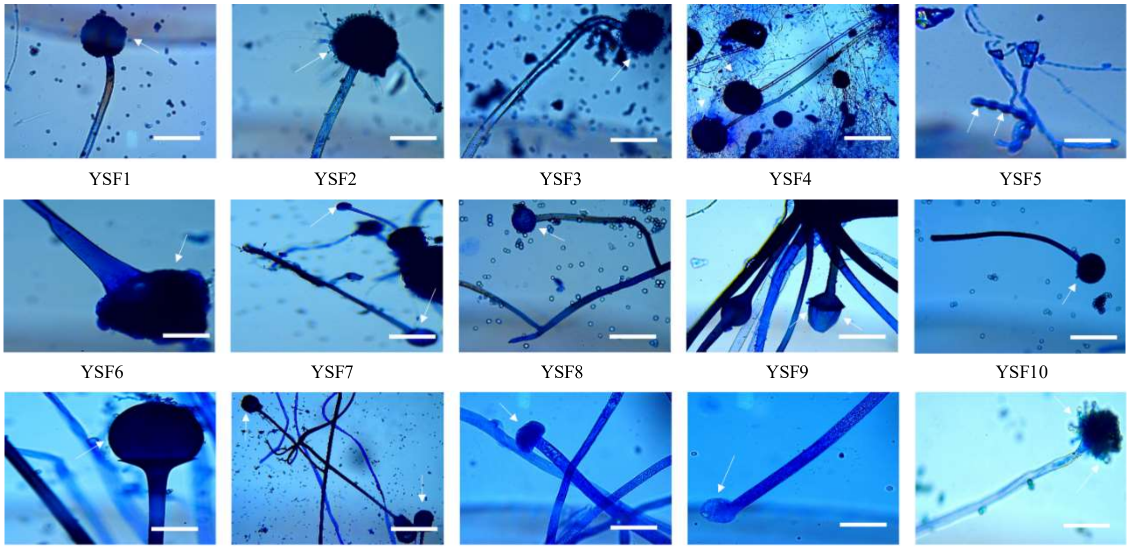


YSF1 YSF2 YSF3 YSF4 YSF5

YSF6 YSF7 YSF8 YSF9 YSF10

YSF11 YSF12 YSF13 YSF14 YSF15

Fig. 3: Photomicrographs of day-old cultures showing growth and branching pattern of the hyphae of the 15 fungal isolates
Scale bar: 100 nm



YSF1 YSF2 YSF3 YSF4 YSF5

YSF6 YSF7 YSF8 YSF9 YSF10

YSF11 YSF12 YSF13 YSF14 YSF15

Fig. 4: Photomicrograph showing fruiting body (sporangiophore) of the 15 fungal isolates
Scale bar: 100 nm

Table 3: Percentage nucleotide similarity among fungal isolates from yam

Isolate/strain	YSF3	YSF5	YSF6	YSF7	YSF11	YSF12	YSF13
YSF3	100	78.6	62.57	62.5	62.5	62.1	62.5
YSF5		100	60.0	71.4	71.4	65.2	63.6
YSF6			100	93.2	97.6	90.2	90.1
YSF7				100	94.6	89.9	91.7
YSF11					100	91.7	92.5
YSF12						100	89.1
YSF13							100

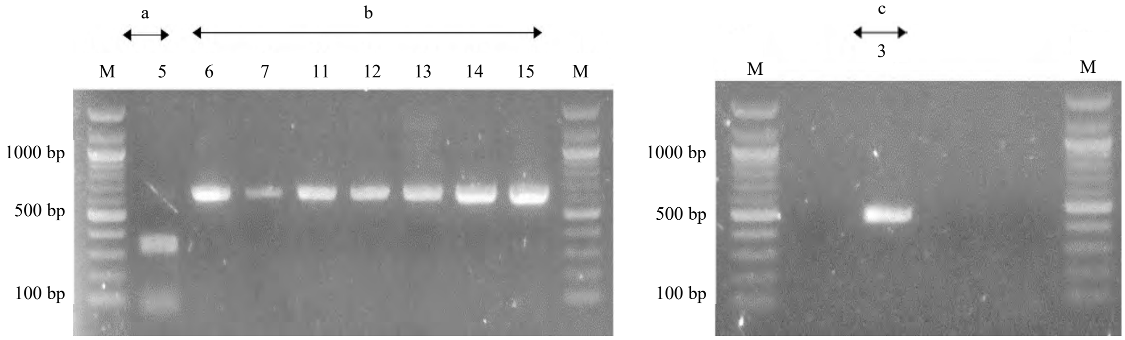


Fig. 5: PCR amplification of ITS1, 18S rRNA and mtDNA SSU rRNA gene from the fungal isolates, (a) PCR analysis of ITS1 gene using Primer LT347-F&R (*Lasiodiplodia* spp.) as previously reported[33], 5 is fungal isolate, (b) PCR analysis of 18s rRNA gene using primer RRF1-F&R (*Rhizopus* spp.) as previously reported[33], 6, 7, 11, 12, 13, 14, 15, are fungal isolates and (c) PCR analysis of mtDNA SSU rRNA gene using ASP_GEN_MTSSU-F&R (*Aspergillus* spp.)[35]

3 is fungal isolate and M: 100 bp DNA marker

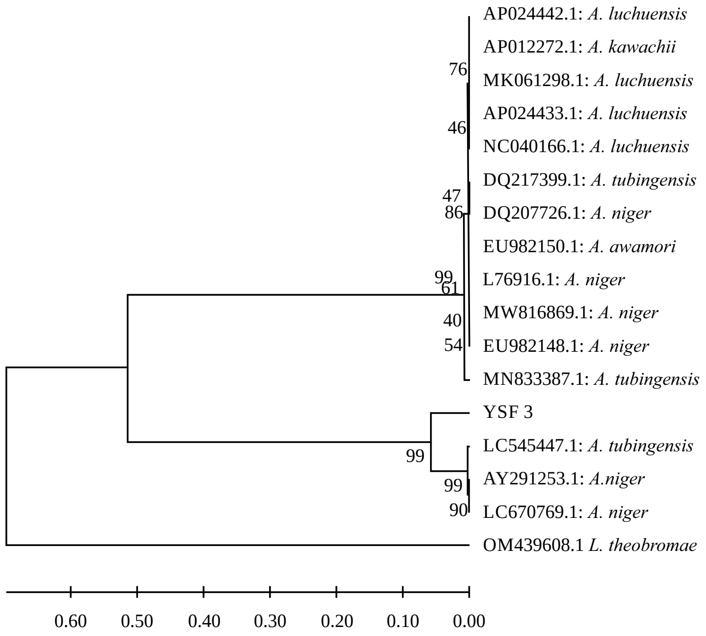


Fig. 6: Phylogenetic tree showing relationships of closely related accessions with our isolates using Maximum Likelihood Method and based on the mtDNA SSU rRNA gene sequences

YSF3 isolate was obtained from this study

To infer the evolutionary history, the UPGMA method was used, while the Maximum Composite Likelihood method was used to compute the evolutionary distances which were in the units of the number of base substitutions per site. This analysis involved 30 nucleotide sequences for trees built with sequence data of 18S region and 17 nucleotide sequence data each for trees of ITS and mtDNA sequences.

Nucleotide sequences of YSF3 clustered together with *A. niger* (AY291253.1 and LC670769.1) and *A. tubingensis* (LC545447.1), while others clustered together (Fig. 6). Isolate YSF5 was distinguishable from the database sequences (Fig. 7). Isolates YSF6, YSF7, YSF11, YSF12 and YSF13, shared >90% nucleotide identity based on sequence alignment (Fig. S1). All the *Rhizopus* spp., except *Rhizopus lyococcus* (KJ408542.1), were placed in one cluster with a percentage nucleotide identity >90%, while isolates YSF6, YSF7, YSF11, YSF12, YSF13 and *R. lyococcus* (KJ408542.1) formed separate clusters (Fig. 8).

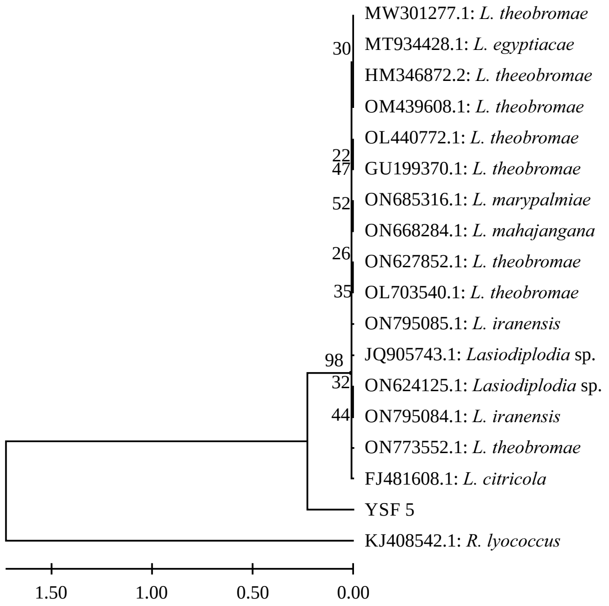


Fig. 7: Phylogenetic tree showing relationships of closely related accessions with our isolates using Maximum Likelihood method and based on the ITS1 gene sequences

YSF5 isolate was obtained from this study

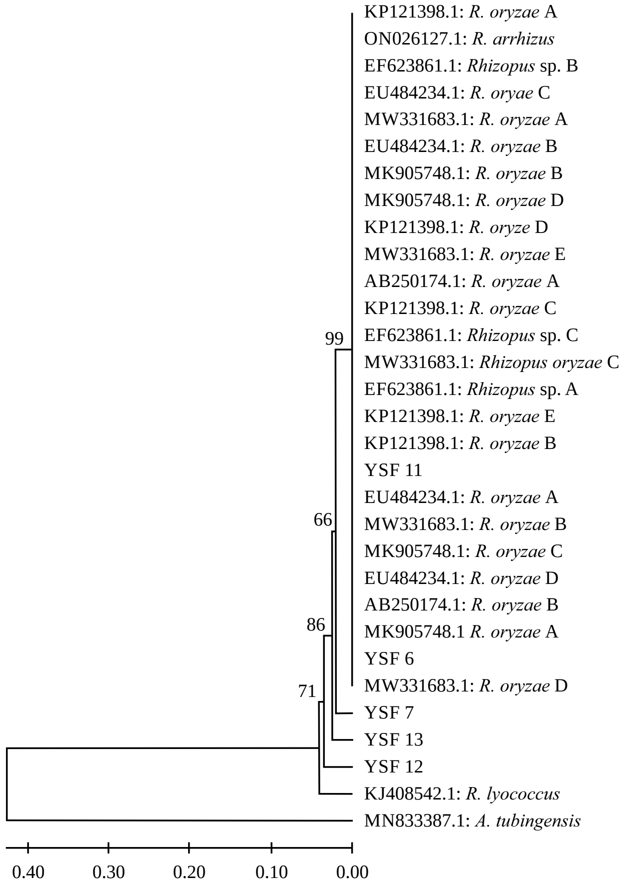


Fig. 8: Phylogenetic tree showing relationships of closely related accessions with our isolates using Maximum Likelihood Method and based on the 18S rRNA gene sequences

YSF6, 7, 11, 12 and 13 isolates were obtained from this study

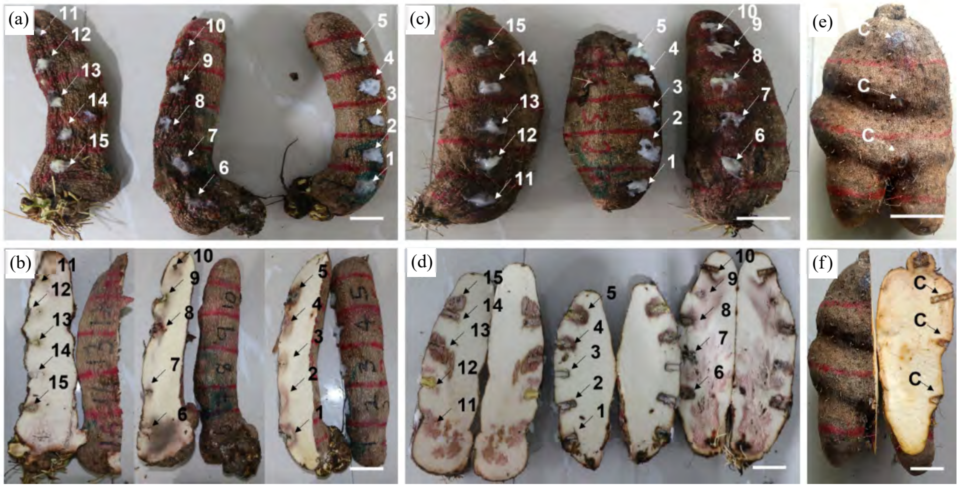


Fig. 9(a-f): Pathogenicity test, (a) Inoculated yam tubers of *D. rotundata* showing points of inoculation of the 15 fungal isolates covered with petroleum jelly, 1-15 represent the 15 fungal isolates YSF1-YSF15, (b) Longitudinal section of tubers of *D. rotundata* 14 days after inoculation with the 15 fungal isolates (YSF1-YSF15), (c) Inoculated yam tubers of *D. alata* showing points of inoculation of the 15 fungal isolates covered with petroleum jelly, 1-15 represent the 15 fungal isolates YSF1-YSF15, (d) Longitudinal section of tubers of *D. alata* 14 days after inoculation with the 15 fungal isolates (YSF1-YSF15), (e) Negative control showing points of inoculation of sterile growth media (PDA) covered with petroleum jelly, the Cs represent control group and (f) Longitudinal section of tuber used as negative control 14 days after inoculation with sterile PDA

Scale bar: 20 mm

Pathogenicity test (Koch's Postulate): The 15 isolates were subjected to a pathogenicity test using white yam (*D. rotundata*) and water yam (*D. alata*). After one week of inoculation, tubers were cut open to examine the different types of rot in the laboratory. All 15 isolates displayed typical symptoms of different yam rots as were observed in the original rotted yams from which the pathogens were isolated (Fig. 9a-d). No symptoms were observed in the negative control which was inoculated with sterile PDA (Fig. 9e-f). Isolate YSF1-3 caused dry rot, isolate YSF5 caused wet rot and isolate YSF6-15 caused soft rot (Table 1). Morphological characterization of re-isolated micro-organism were similar to those used for the inoculation. Symptoms of rots were not observed on the negative control yam tubers.

Estimation of percentage rot: The percentage rot on tubers of *D. rotundata* and *D. alata* infected with the fifteen different fungi isolates were evaluated at five weeks post-inoculation and the results are shown in Fig. 9. The analysis of variance showed a highly significant difference ($p<0.001$) in the rotting effect of the 15 fungal isolates and the control on both *D. rotundata* and *D. alata* (Table S3). Results obtained for *D. rotundata* showed that YSF 6 and 11 had the highest rot at 100%, while the least was recorded for both YSF7 (0.56%) and YSF8 (0.84%). For *D. alata*, the highest percentage of rot was found in isolates YSF7, YSF12 and YSF13 with 100% rot, while the least was recorded in YSF2 and YSF3 with 0.60 and 0.88%, respectively. On the other hand, the control for both *D. rotundata* and *D. alata* showed no symptoms of rot (Fig. S2, Table S3).

DISCUSSION

Fungal pathogens are the main causative agents of rot in stored yam tubers in yam-producing areas, reducing the yield and productivity of yam per annum.

The pathogens enter through wounds within the tubers and infect the internal tissues. Such wounds are more often than not caused by insects, nematodes and Improper handling of the tubers before, during and after harvest[15].

In the present study, the fungal pathogens identified as the cause of yam tuber rot in storage are members of the genera *Aspergillus*, *Rhizopus* and *Lasiodiplodia*. *Aspergillus* spp., were isolated from yam tissues showing symptoms of dry rot while *Rhizopus* and *Lasiodiplodia* spp., were isolated, respectively from tissues showing symptoms of soft rot and wet rot. These pathogens had previously been implicated with post-harvest yam tuber rot in different locations in Nigeria[10,36-39].

Members of the genus *Aspergillus* are reported as the most common and frequent fungi causing rot in yams[40]. These reports however are not supported by the present findings which show *Aspergillus* spp., with a percentage rot of less than 20% in both *D. rotundata* and *D. alata* with a single isolate compared to *Rhizopus* spp., which had seven isolates from the original infected tissues. Tissues infected with *Aspergillus* spp., showed characteristic symptoms of dry rot as they became hard and dry and subsequently turned brownish-black in colour. The progress of the infection on yam tissues as well as the growth rate of the isolate on culture media was relatively slow. The characteristic white cottony mycelia with septate hyphae of the isolate in culture suggested that it is a member of the genus *Aspergillus*[41]. Based on nucleotide similarity, the *Aspergillus* spp., isolated in this study is highly similar to *A. niger* which is known to produce aflatoxins B1 and B2[42]. This is of great concern given the associated serious health risks due to aflatoxins produced by this fungus, as aflatoxins are known carcinogenic and hepatotoxic agents[43]. Some effects of mycotoxins in humans when consumed, even at low dosages, include delayed growth and development, immune system malfunction and altered DNA processes[44,45].

Generally, mycotoxins can withstand high temperatures experienced during food processing, for example frying, roasting and baking. Consequently, they can survive in the finished food products that in the long run reach the customers. Hence, individuals can be exposed to mycotoxins by eating plant and animal products produced with contaminated raw materials.

Other fungi including *Rosellinia bunodes*, *Lasiodiplodia theobromae*, *Penicillium oxalicum*, *Penicillium cyclopium*, *Fusarium oxysporum*, *Fusarium moniliforme* and *Fusarium solani*, as well as the nematode *Scutellonema bradys* have been reported to cause dry rot of yam tubers in storage showing various symptoms of dry rot[15].

Five isolates (YSF6, YSF7, YSF11, YSF12 and YSF13) were confirmed to be members of the genus *Rhizopus* based on macromorphological features and molecular characterization. *Rhizopus spp.* reported in this study was the major cause of rot with severe impact on the yam tubers. They had the highest frequency of occurrence (10 isolates) and were highly invasive, causing a high level of deterioration in infected tissues (up to 100%). Tissues infected with these isolates became ramified by the fungal mycelia, turned brown and became soft and at times wet due to a rapid collapse of the cell walls. The mycelial growth pattern of the isolates on culture was rapid, covering the culture plate within 48 hrs. Other cultural features included multi-branched and transparent mycelia with non-septate hypha, globose sporangia and the production of brownish-black spores. These macromorphological features have previously been used to identify members of the genus *Rhizopus* implicated with the rotting of yam tissues[38]. *Rhizopus oryzae* has been reported as an agent of spoilage, causing soft rot disease in yam and other root tubers in storage[46]. They do so through pectinolytic enzyme activities which often lead to cell separation, maceration and tissue disintegration[47]. Wounds arising from mechanical injuries predispose the roots of these tubers to opportunistic *Rhizopus* spp., that cause rots in storage[48]. Other fungi found to be associated with soft rot in yams include *Mucor circinelloides*, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Armillariella mellea*[15,49]. Also, the identification of *Rhizopus* spp., a ubiquitous member of the Mucorales, poses a serious health concern as Mucorales are linked to mucormycosis with a high death rate[45]. Mucormycosis is an invasive mycosis mostly contracted when an individual ingests foods contaminated with the pathogens[45,50].

Lasiodiplodia sp., was isolated from tissues with wet rot, typified by exudates of whitish to creamy fluid from infected yam tissues when pressed. Only one isolate was identified as a member of the genus *Lasiodiplodia*, showing a low occurrence of wet rot on yam-infected tissues examined. The cultural and morphological features of the isolate, particularly the uniseptate nature of the mature spores, brown walled with longitudinal striations on the conidial walls, helped in the identification of the isolate as belonging to the genus *Lasiodiplodia*[51-53]. In the present study, *Lasiodiplodia* sp., is seen to cause moderate rotting as also reported by Ramirez and Martinez[24] and Gwa and Ekefan[38]. In the same manner, the mycelia growth pattern of the fungus on the culture plate was relatively slow. These physiological characteristics have been reported in yam tubers infected with the same pathogens[29]. In contrast, *Lasiodiplodia* spp., has been reported to be a major cause of the decay of yams in West Africa[16,31,54]. Also, Ogundana *et al.*[55] generally considered the fungus as the most common biological-deteriorating agent and most severe rotter of stored yams. Okigbo *et al.*[10], reported *Lasiodiplodia theobromae* as the most virulent microorganism when compared to *Sclerotia rolfsii*, *Fusarium oxysporum* and *Aspergillus niger* isolated from rotten white yam tubers. Their report also revealed that *Aspergillus niger* was a secondary pathogen to the rotten white yam with the possibility of multiple infections resulting in severe rotting. Another organism often associated with wet rot is the bacterium *Erwinia carotovora* pv carotovora[15]. This bacterium was not isolated in this study as our focus was only on fungal pathogens. Wet rot has also been associated with the progression of soft rot as it causes the infected tissues to become soft, ramified by the fungal mycelium and turn brown[56].

Although morphological features such as conidial measurements are invaluable in discriminating among fungal species, the strategy ought to be complemented with molecular methods[25,51,57]. In addition to the morphological identification of the fungal isolates obtained from infected yam tubers in this study, analysis of the nucleotide sequences of a few phylogenetically informative genes was carried out.

The nucleotide sequences of the 18S, ITS1 and mtDNA regions, were obtained from the isolates in this study and compared to those of species reported worldwide, using a BLAST search. It was observed that isolate YSF3 obtained from this study could be a strain of *A. niger* as its nucleotide identity matches that of *A. niger,* GenBank accession number AY291253.1; LC670769.1 with percentage nucleotide identity >88%. Isolate YSF5 was in a separate cluster from species/strains of *Lasiodiplodia* obtained from the GenBank, even though its nucleotide sequence matched those from the GenBank with a percentage nucleotide identity of 99%, suggesting the isolate is an independent strain. On the other hand, YSF6, YSF7, YSF11, YSF12 and YSF13, shared >90% nucleotide identity indicating that they are closely related. However, the phylogenetic analysis placed all the *Rhizopus* spp., except *R. lyococcus* (KJ408542.1), from the GenBank in one cluster with percentage nucleotide identity >90%, while isolates YSF6, YSF7, YSF11, YSF12, YSF13 and KJ408542.1 formed separate clusters, indicating that they are independent and distinguished from other Rhizopus species.

This was comparable to the report by Abe *et al.*[58], who in an endeavor to set up the molecular phylogeny of the genus *Rhizopus*, analyzed three molecules of the ribosomal RNA-coding DNA (rDNA), 18S, ITS and 28S D1/D2 from all the species of the genus. The phylogenetic tree revealed three major clusters corresponding to the three groups within the current morphological scientific classification, microspores-group, stolonifer-group and *Rhizopus oryzae*. *Rhizopus stolonifer* var. lycoccus clustered separately from the major clusters.

When the different fungal isolates were inoculated into healthy *D. rotundata* and *D. alata* tubers, they were able to colonize and induce rot symptoms on the inoculated yam tubers. This was expected because yam tissues are good growth media for various microorganisms owing to their high nutrient and relative water content[59]. The pathogenicity test revealed that *Rhizopus* spp., were the most virulent fungi causing rot in the healthy white and water yam tubers compared to the less virulent *Lasiodiplodia* sp. and *Aspergillus* sp., obtained in this study. Similarly, *Rhizopus* spp., caused high levels of deterioration, up to 100%, in both inoculated tubers of *D. rotundata* and *D. alata*, compared to *Lasiodiplodia* spp. and *Aspergillus* spp., which showed lower levels of deterioration. This is in line with the report of Amusa *et al.*[15] on the deterioration effect of yam and other tubers by members of the genus *Rhizopus*. The findings of this study suggest that *Rhizopus* spp., could be the leading cause of postharvest decay of yam tubers in various parts of Calabar where the rotted yam tubers were collected. Yam varieties cultivated in different climes vary among themselves arising from their inherent properties. The combined effect of these properties and other environmental factors determine to a greater extent, the type of microorganisms that cause spoilage in these yam varieties as well as the level of deterioration[60]. However, in this study, *Rhizopus* spp., *Lasiodiplodia* sp. and *Aspergillus* sp., were observed to have similar effects in tissues of both *D. rotundata* and *D. alata*.

CONCLUSION

In this study, members of the genera *Aspergillus*, *Rhizopus* and *Lasiodiplodia* were identified as the major fungal pathogens causing yam tuber rot in stored yam tubers in Calabar, Cross River state. *Rhizopus* spp., had the highest frequency of occurrence and was highly invasive, causing high levels of deterioration in infected tissues. The findings of this study give an insight into the level of post-harvest losses incurred by yam farmers and traders on a daily basis. Adopting improved methods of handling, transporting and storing of yam tubers will, to an extent, ensure the elongation of the shelf life and quality of the tubers. However, developing resistant varieties will effectively cut down on postharvest losses caused by biological agents and consequently ensure food security and safety.

SIGNIFICANCE STATEMENT

Several fungi species have been implicated in yam tuber rot, leading to annual yield decline. Proper diagnosis of the disease and identification of the causing agent(s) are important steps in designing appropriate mitigation measures. This study has identified members of the genera-*Aspergillus*, *Lasiodiplodia* and *Rhizopus* as the major fungal pathogens causing tuber rots in yams in storage within Calabar metropolis. *Rhizopus* spp., were the most invasive, causing high levels of deterioration in infected tissues. The *Rhizopus* spp., *Lasiodiplodia* sp. and *Aspergillus* sp., obtained in this study had similar effects on tissues of both *Dioscorea rotundata* and *D. alata* contrary to the expected difference stemming from their inherent properties and other environmental factors.

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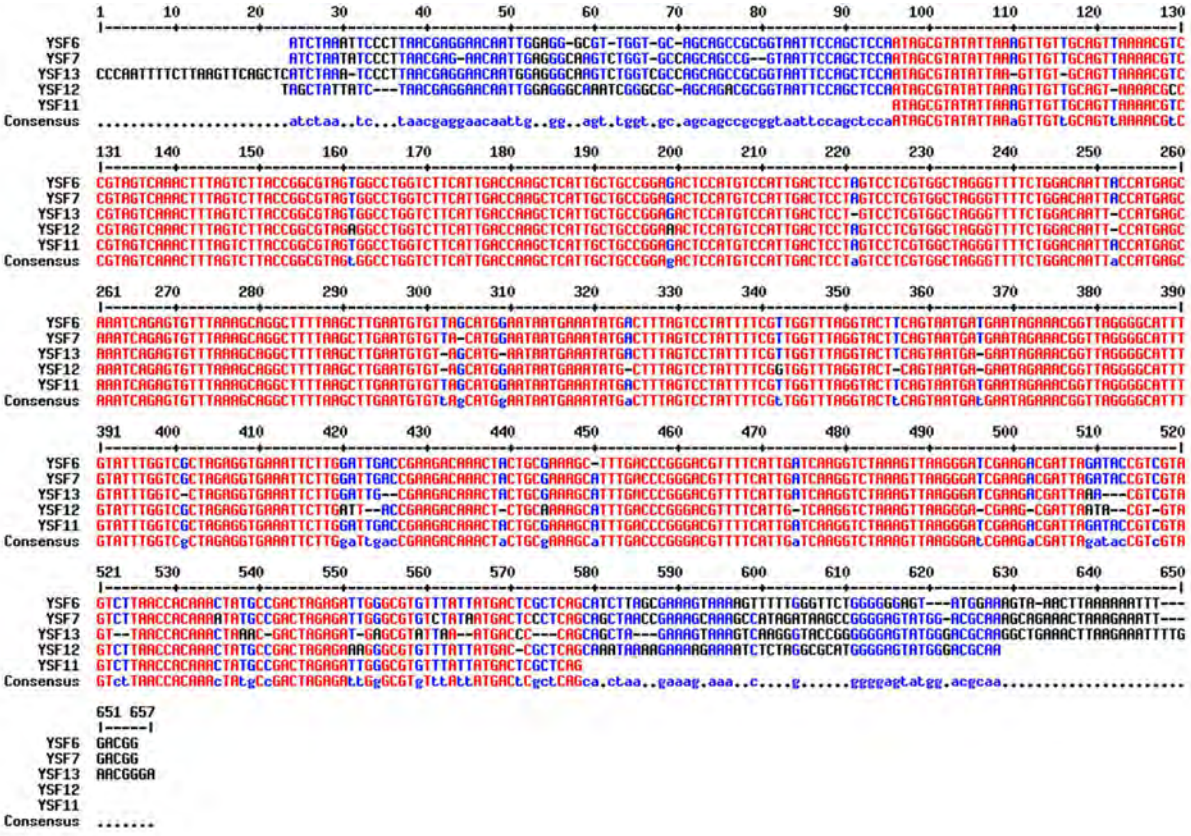
SUPPLEMENTARY INFORMATION

Table S1: List of primers synthesized and used for identification of yam storage rot causing fungi

Gene	Primer	Sequence 5'-3'	Amplicon size (base pairs)
ITS1	LT347-F	AACGTACCTCTGTTGCTTTGGC	347
	LT347-R	TACTACGCTTGAGGGCTGAACA	
18S rRNA	RRF1-F	ATCTAAATCCCTTAACGAGGAACA	631
	RRH1-R	CCGTCAATTTCTTTAAGTTTCAGCCTT	
mtDNA SSU rRNA	ASP_GEN_MTSSU_F	GCCATATTACTCTTGAGGTGGAA	480
	ASP_GEN_MTSSU_R	CCGAAAGGCTGAACCAGTAA	



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          1       10        20        30        40        50        60        70        80        90       100       110       120       130
          |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
     YSF6                        ATCTAAATTCCCTTAACGAGGAACAATTGGAGG-GCGT-TGGT-GC-AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAACGTC
     YSF7                        ATCTAATATCCCTTAACGAG-AACAATTGAGGGCAAGTCTGGT-GCCAGCAGCCG--GTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAACGTC
    YSF13 CCCAATTTTCTTAAGTTCAGCTCATCTAAA-TCCCTTAACGAGGAACAATGGAGGGCAAGTCTGGTCGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAA-GTTGT-GCAGTTAAAACGTC
    YSF12                       TAGCTATTATC---TAACGAGGAACAATTGGAGGGCAAATCGGGCGC-AGCAGACGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGT-AAAACGCC
    YSF11                                                                                                ATAGCGTATATTAAAGTTGTTGCAGTTAAAACGTC
Consensus .......................atctaa..tc...taacgaggaacaattg..gg..agt.tggt.gc.agcagccgcggtaattccagctccaATAGCGTATATTAAaGTTGTtGCAGTtAAAACGtC

          131    140       150       160       170       180       190       200       210       220       230       240       250       260
          |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
     YSF6 CGTAGTCAAACTTTAGTCTTACCGGCGTAGTGGCCTGGTCTTCATTGACCAAGCTCATTGCTGCCGGAGACTCCATGTCCATTGACTCCTAGTCCTCGTGGCTAGGGTTTTCTGGACAATTACCATGAGC
     YSF7 CGTAGTCAAACTTTAGTCTTACCGGCGTAGTGGCCTGGTCTTCATTGACCAAGCTCATTGCTGCCGGAGACTCCATGTCCATTGACTCCTAGTCCTCGTGGCTAGGGTTTTCTGGACAATTACCATGAGC
    YSF13 CGTAGTCAAACTTTAGTCTTACCGGCGTAGTGGCCTGGTCTTCATTGACCAAGCTCATTGCTGCCGGAGACTCCATGTCCATTGACTCCT-GTCCTCGTGGCTAGGGTTTTCTGGACAATT-CCATGAGC
    YSF12 CGTAGTCAAACTTTAGTCTTACCGGCGTAGAGGCCTGGTCTTCATTGACCAAGCTCATTGCTGCCGGAAACTCCATGTCCATTGACTCCTAGTCCTCGTGGCTAGGGTTTTCTGGACAATT-CCATGAGC
    YSF11 CGTAGTCAAACTTTAGTCTTACCGGCGTAGTGGCCTGGTCTTCATTGACCAAGCTCATTGCTGCCGGAGACTCCATGTCCATTGACTCCTAGTCCTCGTGGCTAGGGTTTTCTGGACAATTACCATGAGC
Consensus CGTAGTCAAACTTTAGTCTTACCGGCGTAGtGGCCTGGTCTTCATTGACCAAGCTCATTGCTGCCGGAgACTCCATGTCCATTGACTCCTaGTCCTCGTGGCTAGGGTTTTCTGGACAATTaCCATGAGC

          261    270       280       290       300       310       320       330       340       350       360       370       380       390
          |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
     YSF6 AAATCAGAGTGTTTAAAGCAGGCTTTTAAGCTTGAATGTGTTAGCATGGAATAATGAAATATGACTTTAGTCCTATTTTCGTTGGTTTAGGTACTTCAGTAATGATGAATAGAAACGGTTAGGGGCATTT
     YSF7 AAATCAGAGTGTTTAAAGCAGGCTTTTAAGCTTGAATGTGTTA-CATGGAATAATGAAATATGACTTTAGTCCTATTTTCGTTGGTTTAGGTACTTCAGTAATGATGAATAGAAACGGTTAGGGGCATTT
    YSF13 AAATCAGAGTGTTTAAAGCAGGCTTTTAAGCTTGAATGTGT-AGCATG-AATAATGAAATATGACTTTAGTCCTATTTTCGTTGGTTTAGGTACTTCAGTAATGA-GAATAGAAACGGTTAGGGGCATTT
    YSF12 AAATCAGAGTGTTTAAAGCAGGCTTTTAAGCTTGAATGTGT-AGCATGGAATAATGAAATATG-CTTTAGTCCTATTTTCGGTGGTTTAGGTACT-CAGTAATGA-GAATAGAAACGGTTAGGGGCATTT
    YSF11 AAATCAGAGTGTTTAAAGCAGGCTTTTAAGCTTGAATGTGTTAGCATGGAATAATGAAATATGACTTTAGTCCTATTTTCGTTGGTTTAGGTACTTCAGTAATGATGAATAGAAACGGTTAGGGGCATTT
Consensus AAATCAGAGTGTTTAAAGCAGGCTTTTAAGCTTGAATGTGTtAgCATGgAATAATGAAATATGaCTTTAGTCCTATTTTCGtTGGTTTAGGTACTtCAGTAATGAtGAATAGAAACGGTTAGGGGCATTT

          391    400       410       420       430       440       450       460       470       480       490       500       510       520
          |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
     YSF6 GTATTTGGTCGCTAGAGGTGAAATTCTTGGATTGACCGAAGACAAACTACTGCGAAAGC-TTTGACCCGGGACGTTTTCATTGATCAAGGTCTAAAGTTAAGGGATCGAAGACGATTAGATACCGTCGTA
     YSF7 GTATTTGGTCGCTAGAGGTGAAATTCTTGGATTGACCGAAGACAAACTACTGCGAAAGCATTTGACCCGGGACGTTTTCATTGATCAAGGTCTAAAGTTAAGGGATCGAAGACGATTAGATACCGTCGTA
    YSF13 GTATTTGGTC-CTAGAGGTGAAATTCTTGGATTG--CGAAGACAAACTACTGCGAAAGCATTTGACCCGGGACGTTTTCATTGATCAAGGTCTAAAGTTAAGGGATCGAAGACGATTAAA---CGTCGTA
    YSF12 GTATTTGGTCGCTAGAGGTGAAATTCTTGATT--ACCGAAGACAAACT-CTGCAAAAGCATTTGACCCGGGACGTTTTCATTG-TCAAGGTCTAAAGTTAAGGGA-CGAAG-CGATTAATA--CGT-GTA
    YSF11 GTATTTGGTCGCTAGAGGTGAAATTCTTGGATTGACCGAAGACAAACTACTGCGAAAGCATTTGACCCGGGACGTTTTCATTGATCAAGGTCTAAAGTTAAGGGATCGAAGACGATTAGATACCGTCGTA
Consensus GTATTTGGTCgCTAGAGGTGAAATTCTTGgaTtgacCGAAGACAAACTaCTGCgAAAGCaTTTGACCCGGGACGTTTTCATTGaTCAAGGTCTAAAGTTAAGGGAtCGAAGaCGATTAgatacCGTcGTA

          521    530       540       550       560       570       580       590       600       610       620       630       640       650
          |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
     YSF6 GTCTTAACCACAAACTATGCCGACTAGAGATTGGGCGTGTTTATTATGACTCGCTCAGCATCTTAGCGAAAGTAAAAGTTTTTGGGTTCTGGGGGGAGT---ATGGAAAGTA-AACTTAAAAAATTT---
     YSF7 GTCTTAACCACAAAATATGCCGACTAGAGATTGGGCGTGTCTATAATGACTCCCTCAGCAGCTAACCGAAAGCAAAGCCATAGATAAGCCGGGGAGTATGG-ACGCAAAGCAGAAACTAAAGAAATT---
    YSF13 GT--TAACCACAAACTAAAC-GACTAGAGAT-GAGCGTATTAA--ATGACCC---CAGCAGCTA---GAAAGTAAAGTCAAGGGTACCGGGGGGAGTATGGGACGCAAGGCTGAAACTTAAGAAATTTTG
    YSF12 GTCTTAACCACAAACTATGCCGACTAGAGAAAGGGCGTGTTTATTATGAC-CGCTCAGCAAATAAAAGAAAAGAAAATCTCTAGGCGCATGGGGAGTATGGGACGCAA
    YSF11 GTCTTAACCACAAACTATGCCGACTAGAGATTGGGCGTGTTTATTATGACTCGCTCAG
Consensus GTctTAACCACAAAcTAtgCcGACTAGAGAttGgGCGTgTttAttATGACtCgctCAGca.ctaa..gaaag.aaa..c....g......ggggagtatgg.acgcaa......................

          651 657
          |-----|
     YSF6 GACGG
     YSF7 GACGG
    YSF13 AACGGGA
    YSF12
    YSF11
Consensus .......
```

Fig. S1: Sequence alignment for isolates YSF6-YSF13 which were amplified using the same primer (primer RRF1-F&R)

Table S2: Partial nucleotide sequences of the different isolates

Sample ID	Sequences
YSF3	TGCCATATTACTCTTGAGGTGGAATGCTTACAATGGCAGGGATGAATAAATATCAAATCTAGGCTATGACAATCATCAATGTCTGCTTGGACTACTAGGGTATCTAATCCTTATCGCTACCCGAGCCTTCGTCCCTCAACGTCAGTTTTACATAGAAGGACGCCTTCGCCGTTATCAGTCCTTCTGGTATTTGCGTATTTTATCCCTACTCCAGAAGTTCTTCCTTCTCACATAAAACTCTAGAAAAAAAGTACTCATTTAGAGTTTAATTTACCGTCTAGGTACCCTTTAAACCTAATAAAGATGACTAACACTAGTCTTCTACGTATCCGAAAAGGCAAGCAAGTAATTTGAACAAGACAAATAAATAAAAAAATACAATAACCATTAACCCATATAAAATTCTAAACAAGATAATCATAATGACAATCCCAGAATACATTTACATTCTTCCAAGTTACTGGTTAGGCCCTTTCGGA
YSF5	GCTTCCGGGCTTAAAACCACCGGCCGAGGGCATTTGGGCCGCTTCGCCGTGGAGGCCGTGCCCCATTTCCAGCCAAACCTGAGGGTTTTTATTACCCCTGCATGACCATGCCCCCGAAATGCCATAAGGAATGTGAATTGTTGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTACAACCCTCAAGCTCTGCTTGGAATTGGGCACCGTCCTCACTGCGGACGCGCCTCAAAGACCTCGGCGGTGGCTGTTCAGCCCTCAAGCGTAGTAACCAAAACAACCAAGTTTCTTTAAA
YSF6	ATCTAAATTCCCTTAACGAGGAACAATTGGAGGGCGTTGGTGCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAACGTCCGTAGTCAAACTTTAGTCTTACCGGCGTAGTGGCCTGGTCTTCATTGACCAAGCTCATTGCTGCCGGAGACTCCATGTCCATTGACTCCTAGTCCTCGTGGCTAGGGTTTTCTGGACAATTACCATGAGCAAATCAGAGTGTTTAAAGCAGGCTTTTAAGCTTGAATGTGTTAGCATGGAATAATGAAATATGACTTTAGTCCTATTTTCGTTGGTTTAGGTACTTCAGTAATGATGAATAGAAACGGTTAGGGGCATTTGTATTTGGTCGCTAGAGGTGAAATTCTTGGATTGACCGAAGACAAACTACTGCGAAAGCTTTGACCCGGGACGTTTTCATTGATCAAGGTCTAAAGTTAAGGGATCGAAGACGATTAGATACCGTCGTAGTCTTAACCACAAACTATGCCGACTAGAGATTGGGCGTGTTTATTATGACTCGCTCAGCATCTTAGCGAAAGTAAAAGTTTTTGGGTTCTGGGGGGAGTATGGAAAGTAAACTTAAAAAAATTTGACGG
YSF7	ATCTAATATCCCTTAACGAGAACAATTGAGGGCAAGTCTGGTGCCAGCAGCCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAACGTCCGTAGTCAAACTTTAGTCTTACCGGCGTAGTGGCCTGGTCTTCATTGACCAAGCTCATTGCTGCCGGAGACTCCATGTCCATTGACTCCTAGTCCTCGTGGCTAGGGTTTTCTGGACAATTACCATGAGCAAATCAGAGTGTTTAAAGCAGGCTTTTAAGCTTGAATGTGTTACATGGAATAATGAAATATGACTTTAGTCCTATTTTCGTTGGTTTAGGTACTTCAGTAATGATGAATAGAAACGGTTAGGGGCATTTGTATTTGGTCGCTAGAGGTGAAATTCTTGGATTGACCGAAGACAAACTACTGCGAAAGCATTTGACCCGGGACGTTTTCATTGATCAAGGTCTAAAGTTAAGGGATCGAAGACGATTAGATACCGTCGTAGTCTTAACCACAAAATATGCCGACTAGAGATTGGGCGTGTCTATAATGACTCCCTCAGCAGCTAACCGAAAGCAAAGCCATAGATAAGCCGGGGAGTATGGACGCAAAGCAGAAACTAAAGAAATTGACGG
YSF11	ATCTAATATCCCTTAACGAGGAACAATTGGAGGGCAAGTCTGGGGCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAACGTCCGTAGTCAAACTTTAGTCTTACCGGCGTAGTGGCCTGGTCTTCATTGACCAAGCTCATTGCTGCCGGAGACTCCATGTCCATTGACTCCTAGTCCTCGTGGCTAGGGTTTTCTGGACAATTACCATGAGCAAATCAGAGTGTTTAAAGCAGGCTTTTAAGCTTGAATGTGTTAGCATGGAATAATGAAATATGACTTTAGTCCTATTTTCGTTGGTTTAGGTACTTCAGTAATGATGAATAGAAACGGTTAGGGGCATTTGTATTTGGTCGCTAGAGGTGAAATTCTTGGATTGACCGAAGACAAACTACTGCGAAAGCATTTGACCCGGGACGTTTTCATTGATCAAGGTCTAAAGTTAAGGGATCGAAGACGATTAGATACCGTCGTAGTCTTAACCACAAACTATGCCGACTAGAGATTGGGCGTGTTTATTATGACTCGCTCAGCATCTTAGCGAAAGTAAAGTTTTTGGGTTCTGGGGGGAGTATGGACCAAGGTGAAACTTAAGAAATGGA
YSF12	TAGCTATTATCTAACGAGGAACAATTGGAGGGCAAATCGGGCGCAGCAGACGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTAAAACGCCCGTAGTCAAACTTTAGTCTTACCGGCGTAGAGGCCTGGTCTTCATTGACCAAGCTCATTGCTGCCGGAAACTCCATGTCCATTGACTCCTAGTCCTCGTGGCTAGGGTTTTCTGGACAATTCCATGAGCAAATCAGAGTGTTTAAAGCAGGCTTTTAAGCTTGAATGTGTAGCATGGAATAATGAAATATGCTTTAGTCCTATTTTCGGTGGTTTAGGTACTCAGTAATGAGAATAGAAACGGTTAGGGGCATTTGTATTTGGTCGCTAGAGGTGAAATTCTTGATTACCGAAGACAAACTCTGCAAAAGCATTTGACCCGGGACGTTTTCATTGTCAAGGTCTAAAGTTAAGGGACGAAGCGATTAATACGTGTAGTCTTAACCACAAACTATGCCGACTAGAGAAAGGGCGTGTTTATTATGACCGCTCAGCAAATAAAAGAAAAGAAAATCTCTAGGCGCATGGGGAGTATGGGACGCAA
YSF13	CCCAATTTTCTTAAGTTCAGCTCATCTAAATCCCTTAACGAGGAACAATGGAGGGCAAGTCTGGTCGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAGTTGTGCAGTTAAAACGTCCGTAGTCAAACTTTAGTCTTACCGGCGTAGTGGCCTGGTCTTCATTGACCAAGCTCATTGCTGCCGGAGACTCCATGTCCATTGACTCCTGTCCTCGTGGCTAGGGTTTTCTGGACAATTCCATGAGCAAATCAGAGTGTTTAAAGCAGGCTTTTAAGCTTGAATGTGTAGCATGAATAATGAAATATGACTTTAGTCCTATTTTCGTTGGTTTAGGTACTTCAGTAATGAGAATAGAAACGGTTAGGGGCATTTGTATTTGGTCCTAGAGGTGAAATTCTTGGATTGCGAAGACAAACTACTGCGAAAGCATTTGACCCGGGACGTTTTCATTGATCAAGGTCTAAAGTTAAGGGATCGAAGACGATTAAACGTCGTAGTTAACCACAAACTAAACGACTAGAGATGAGCGTATTAAATGACCCCAGCAGCTAGAAAGTAAAGTCAAGGGTACCGGGGGGAGTATGGGACGCAAGGCTGAAACTTAAGAAATTTTGAACGGGA

Table S3: Mean percentage rot and the associated pathogen at five weeks of storage

Isolates	Percentage rot in *D. rotundata* (X±SE)	Percentage rot in *D. alata* (X±SE)
YSF1	14.040±0.99	2.40±0.23
YSF2	18.716±0.81	0.60±0.11
YSF3	9.60±0.94	0.88±0.05
YSF4	5.75±0.87	6.26±0.83
YSF5	9.12±1.09	4.00±0.11
YSF6	100.00±0.00	44.23±4.00
YSF7	0.56±0.09	100.00±0.00
YSF8	0.84±0.24	10.80±1.33
YSF9	5.92±0.42	1.12±0.47
YSF10	1.44±0.34	8.60±1.01
YSF11	100.00±0.00	14.88±1.17
YSF12	53.40±3.15	100.00±0.00
YSF13	44.00±2.30	100.00±0.00
YSF14	70.55±1.36	8.64±1.08
YSF15	22.07±1.15	1.20±0.23
Control	0.00±0.00	0.00±0.00
LSD (0.05)	3.605	3.532

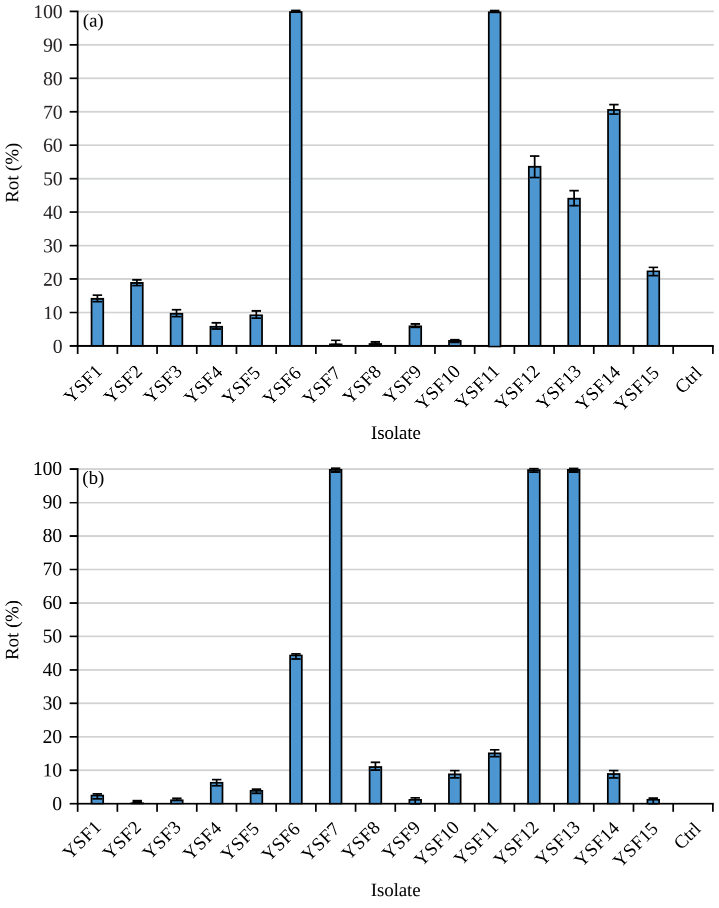

Fig. S2(a-b): Estimated percentage rot and the associated pathogen at five weeks of storage, (a) *D. rotundata* (b) *D. alata*, YSF1-YSF15 are fungal isolates

Ctrl: Control, All the 15 isolates and control were replicated three times. The error bars are standard error of means