AROC In Food And Nutrition
2024;04(01):01-13

Arocjournal.com
(An affiliate of The International African Society of Sciences)

INTERNATIONAL AFRICAN SOCIETY OF SCIENCES

RESEARCH ARTICLE

Isolation and Molecular Characterization of Fungal Pathogens Associated with White Yam (*Dioscorea Rotundata*) Spoilage in Kabba/Bunu Local Government Area of Kogi State

Haruna Ademu[1], Olajumoke Josephine Matthew[1], Joy Atule Peter[1], Ahmed Saliuh[2], Adamu Mohammed Saba[3]

[1]Department of Biochemistry, Faculty of Science and Computing, Kogi State University, Kabba Nigeria
[2]Department of Biotechnology, Nigeria Defence Academy, Kaduna
[3]Department of Biological Sciences, Niger State Polytechnic Zungeru

Correspondence should be addressed to Haruna Ademu; Email: hademu@ksukabba.edu.ng

https://doi.org/10.53858/arocfn04010113

ABSTRACT

Background: Food security and scarcity are among the most viable problems challenging Agricultural development in the third world today. Yam is one of the most important staple source of carbohydrate in Africa, but its production is hindered by post-harvest deterioration by diverse microorganisms. **Methods;** This study aimed to isolate and molecularly characterize the fungal pathogens associated with white yam (*Dioscorea rotundata*) spoilage in Kabba/Bunu Local Government Area of Kogi State. A total of 12 diseased and healthy white yam tubers were purchased from three selected storage locations in Kabba/Bunu Local Government Area of Kogi State between the month of June and July, 2024. Three diseased and one healthy yam (control) each were randomly picked from the three different storage units. The collected yam from the three storage unit were labeled Benin, Okene and Kabba/Bunu yam. Fungal pathogens associated with the collected white yam were isolated and identified using standard methods. The level of pathogenicity of the isolated fungal pathogens associated with the white yam spoilage were ascertained using standard methods. The fungal pathogens isolated from the white yam spoilage were characterized and compared with the stored database analysis using online BLAST (Basic Local Alignment Search Tool). **Results;** The result of the fungal pathogens isolated and identified from the white yam spoilage across all the three selected locations in Kabba/Bunu local government area of Kogi State revealed the presence of predominant fungal pathogens associated with white yam spoilage; *Aspergilus niger, Fusarium sp, Rhizopus nigricans, Penicilium sp, Trichoderma viridae,* and *Botrodiplodia theobrome* while the level of pathogenicity result shows *Aspergilus niger, Fusarium sp,* and *Penicilium sp,* as the common virulent fungal pathogens associated with the white yam spoilage in the three different locations. The result of the molecular characterization of the fungal pathogens revealed uncommon species of fungal pathogens such as *Penicillium monomenatosum, Penicillium basilianum* and *Fusarium solani* associated with white yam spoilage across all the three locations in Kabba/Bunu Local Government Area of Kogi State. **Conclusion:** The study conclude that Benin white yam is better than Okene and Kabba white yam in respect to fungal pathogens load, resistance to disease caused by microorganisms and preferable for human consumption.

Keywords: White yam, spoilage, *Aspergilus niger, Penicillium monomenatosum*, Okene, Kabba, Benin

Received: 5 Novemeber 2024, Revised: 20 November 2024, Published: December 2024

Citation: H.,Ademu., O.J.,Matthew, J.A.,Peter, A.,Saliuh, A.M.,Saba (2024). Isolation and Molecular Characterization of Fungal Pathogens Associated with White Yam (*Dioscorea Rotundata*) Spoilage in Kabba/Bunu Local Government Area of Kogi State. *AROC In Food And Nutrition,* 4(1):01-13, https://doi.org/10.53858/arocfn04010113

1.0 Introduction

Yam (*Dioscorea spp)* is a tuber crop belonging to the family *Dioscoreaceae*, a perennial herbaceous vine cultivated mainly for the consumption of its starchy tubers [1]. They are monocotyledons and have twining vines with alternate or opposite leaves that can lobe. The leaves can be simple and usually oval to heart-shaped with petioles of the same length or slightly longer than the leaf blades [2]. It is one of the most important staple foods in the world, especially in some parts of tropics and subtropics [1]. The most cultivated species in Nigeria are *Dioscorea rotundata* (white yam), *Dioscorea cayenesis* (yellow yam) and *Dioscorea alata* (water yam). In West Africa, *Dioscorea rotundata* is also referred to as white yam or white guinea yam and the most widely cultivated followed by *Dioscorea alata* (water yam). White yam is herbaceous annual plant with climbing or trailing vines. The vines can be smooth and prickly, reaching 10 m or more in length. White yam is grown on a free draining, sandy and fertile soil. Planting of white yam is done by seed yam or cut setts from ware tubers. The plant can produce singular or several tubers that extend

from stolon of a central corm. The tubers, with rounded ends, can be cylindrical, curved or lobed, with brown, grey, black or pink skin and white flesh. They are harvested after 5 - 12 months of growth and could weigh between 2.5\25.0 kg. The harvested yam tubers have shelf lives of about 5 - 8 months, with their barks darkening during storage [3].

Globally yam is one of the most important food that serve as source of income for millions of producers, processors and consumers in some region [4]. The edible varieties of yam are important food crops and serve as sources of carbohydrate staple for millions of people in Sub-Saharan Africa, the Carribean, the Northern and Central part of South East Asia including parts of China, Malaysia, Japan and Oceania [5]. More than 90% of the yam consumed in the world is produced in West Africa. Yam production has increased steadily in the last decades from 18 million metric tonnes in 1990 to a recent estimate of over 39 million metric tonnes [6]. Nigeria alone produces three quarter of the world total output of yams (Okigbo *et al*., 2015). Ethiopia and Sudan are major yam producers in East Africa [7]. The yam plants are mostly distributed in south and south west Ethiopia around Metekel, Sidama, Wolayta, Gambella, Maji, Jimma and North Omo [8]. Ethiopia has a world share of 0.6% yam production (tons) and 0.9% area harvested (hectare) in the world [6].

Yam is an important source of carbohydrate for many people of the Sub-Saharan Region, especially in the yam zone of West Africa [9]. [10] reported that yam contributes more than 200 dietary calories per capita daily for more than 150 million people in West Africa and serves as an important source of income to the people. Yam has some inherent characteristics, which make it attractive, first, it is rich in carbohydrate especially starch consequently has a multiplicity of end use. Secondly, it is available all year round making it preferable to other seasonal crops [6]

Yam shows nutritional superiority over other tropical root and tuber crops. Yams are the most nourishing plants in the diet of many inhabitants of inter-tropical region. The nutritional value of yam confirms its importance to mankind. It is a major source of carbohydrate and is one of the cheaper sources of carbohydrate to man [11]. Yam contains protein and substantial amount of vitamins (Thiamine, Riboflavin and Ascorbic acid) and some other minerals like calcium, phosphorus and iron than any other common tuber crop. By dry weight yam is composed of 80% starch, 7% protein, 7% minerals, 3% fibre and 1.7% lipids; 100 g of the yam give 385kcal energy [12]. Yam has other uses other than food. The yam tuber is a good source of energy mainly from their carbohydrate contents since it is low in fat and protein. Yam tuber is said to contain some pharmacologically active substances such as dioscorine, saponin and sapogenin. Dioscorine which is the major alkaloid in yam is medicinally a heart stimulant [13]. Also it has been reported that yam is a good source of industrial starch whose quality varies with the species [14].

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. During the past four decades, food production has failed to keep pace with population growth in many African countries. One group of commodities that holds much potential for reversing this trend is the roots and tubers. But in Africa, data compiled by researchers showed that more than 40% of these root and tuber crops are lost to rot annually [15]. Hence the most challenging part of this problem is the hunger that result from inadequate agricultural storage and lack of produce preservative as well as microbial spoilages.

Fungi which are mainly terrestrial organisms are found virtually every habitat on the earth where organic materials exist. Some species including *Aspergillus niger*, occur only on a particular strain of one genus of plants whereas others are extremely versatile in what they can attack and use as a source of carbon and energy. Yam plants are susceptible to infection by fungi, bacteria and viruses at all stages of growth and also during storage of tubers. Tuber rot is a major factor limiting the post-harvest life of yams and losses can be very high which is estimated at about 26% in the world [4].

There are several constraints to yam industry in Nigeria. Of these constraints, diseases contribute to high yield loss before and after harvest. Yam plant are prone to infection by fungi, bacteria, and viruses at all stages of growth and also during storage of tubers. Yam (*Dioscorea spp)* is seriously threatened in the South Eastern and other parts of Nigeria as a result of its high susceptibility to fungal soft rots [16]. Rot is a major factor limiting the post-harvest life of yams and losses can be very high. Losses due to post-harvest rot significantly affect farmers' and traders' income, food security and seed yams stored for planting. The quality of yam tubers are affected by rots, which makes them unappealing to consumers. In Nigeria, over 60% of white yam varieties gets rotten when stored for more than six months [17]. Because of their poor storage, farmers sell produce just after harvest to avoid losses, and this result in low income or reduced profits as well as scarcity. This practice also affects farmers' food security particularly in the lean season.

The use of chemicals such as fungicides in controlling yam diseases have proved effective against some species of the fungi but not without challenges [1]. Chemicals are not just expensive, not readily available but they are also not eco-friendly and some of these chemicals have an adverse effects due to problems such as chemical residues, biodegradation, pollution, high cost, non-availability and hazard to the environment as well as causing health related problems and it has made some of the farmers not adopting this control measures [18]. There is need in search for safer, more efficacious, economical, and non-polluting methods to control plant diseases instead of chemical products [16]. Hence alternative control methods are employed.

The present study isolated and identified some fungal pathogens of yam and investigate the effect of their infection on the nutrient composition and shelf life. Hence, the study will assist in providing the framework for developing preventive measures that would reduce yam spoilage, and reduce health problems associated with these fungi, increasing yam production as well as contributing to scientific knowledge research.

2.0 Methodology

2.1 Materials

2.1.1 Sample Collection

A total of 12 diseased and healthy white yam tubers were purchased from three different storage locations in Kabba/Bunu Local Government Area of Kogi State between the month of June and July, 2024. In each storage unit in Kabba/Bunu Local Government Area of Kogi State, the labeled Benin, Okene and Kabba white yam were collected randomly. Three diseased and one heathy white yam were collected from each of the storage unit. The three locations where the sample were taken within Kabba/Bunu Local Government Area were (shop No 17, Kabba/Bunu central market, Oluwatosin stores, Kabba/Bunu central market and Bukola stores, Kabba/Bunu, Kogi State). The samples were collected with Whirl – Pak sample bags using sterile forceps and then taken to Microbiology laboratory, Ahmadu Bello University, Zaria for analysis.

2.1.2 Equipment
List of equipment used in the course of this study are as follows:
PCR Machine, Whirl pak, Sterile loop, Sterile forceps, Petri dishes, Culture media, Autoclave, Sterile, knife, Sterile scalpel, Electrophoresis machine, Whatman paper and Tissue paper.

2.1.3 Chemicals
The list of chemicals used in the course of this study are;
Distilled water, Ethanol, Potato dextrose agar and Sodium hypochlorite solution.

2.2 Methods
2.2.1 Sample Identification
Both the healthy and diseased yam tubers were identified and authenticated by Dr. Lukman Oyedeji in the Department of Plant Biology, Ahmadu Bello University, Zaria and the fungi were isolated and authenticated by Dr. Temi Olukoya in the Department of Microbiology, Ahmadu Bello University, Zaria.

2.2.3 Sample Preparation and Isolation
Isolation of fungi was based on the method described by [19]. (5g) portion of the both healthy and diseased white yam tubers were cut-off by using a sterile forceps and then washed with distilled water to remove dust, debris adhering to them and the surface was sterilized with 70% ethanol for a minute. They were then sliced into small pieces of 1x1 cm under aseptic conditions using a sterile scalpel and then immersed in 4% sodium hypochlorite solution for 3 minutes. This was followed by rinsing with 70% alcohol for 1 minute. The samples were finally rinsed in a series of changes with sterile distilled water and blotted dry on sterile tissue paper. Sample were then inoculated into different agar plates, labelled appropriately. Potato dextrose agar (PDA) was used for the isolation of fungi, and was prepared according to the manufacturer's instructions, in which 42 g of potato dextrose agar (PDA) was weighed and distilled water was added to make 1000 ml. The medium was autoclaved at 121 °C for 15 minutes, and allowed to cool to about 50 °C before the addition of 0.1 g/L of streptomycin. The mixture was stirred by gently swirling the flask, before pouring into 9 mm diameter sterile Petri dishes. The medium was allowed to solidify before inoculation [20]. After the inoculation, agar plates was incubated at room temperature and the growth was monitored and recorded daily. The developing cultures were purified by repeated subculture technique.

2.2.4 Sub-Culturing of the Fungal Isolates

A small portion of growth from the developing cultures was picked with a sterile whirl loop and placed in 10 ml of sterile water, which was then swirled gently to mix. Sample obtained was diluted serially to obtain a dilution of 10-1 dilution factor. One milliliter of the samples were pipetted from dilution tube (10-3) into a sterile petri dish, thereafter, 20 ml of Nutrient agar and Potato dextrose agar were added and heated which were allowed to cool to 45^{o}C.

The media were poured separately into each of the plates in triplicates and the plates were gently swirled and allowed to solidify. The nutrient agar was incubated at 27^{0}C for 96 hours while potato dextrose agar plates were incubated at 25^{0}C for 3-5 days. After incubation, the fungal colonies were observed and counted using a colony counter. Representative fungi colonies were selected and sub cultured on fresh fungal media until pure cultures were obtained. A pure culture was obtained and maintained by sub – culturing each of the different colonies that emerged onto the SDA plates and incubated at 28^{0}C for 5 days. Isolates were later subjected to pathogenicity test.

2.2.5 Pathogenicity Test and Identification of Isolates

The needle injection inoculation method of [21] was used to test the pathogenicity of the isolated organisms. The fungal isolates obtained from the rotten yam tubers were tested for their ability to cause the same rot condition in healthy white yam tubers. The healthy yam tubers were washed with sterile water and the surface sterilized with 70% ethanol solution. With the aid of a sterile syringe, the pure culture of isolates was inoculated into a healthy yam tuber at different points. The inoculated tuber was kept in a micro humidity chamber at room temperature and examined daily for rot development for 7 days. On establishment of rot condition, re-isolation was carried out to obtain pure cultures of the inoculated isolate which was compared with original isolates before being characterized and identified as the casual organism. The pathogenic organisms were subjected to microscopic examination during which their structural features were observed under the hand lens before being mounted on a slide mount and stained with cotton blue lacto-phenol and viewed under the microscope. The characteristic features observed were compared with those contained in and identified accordingly. A pathogenicity test of each isolate was replicated three times. On appearance of symptoms, the area of infection were measured in millimeters using a meter rule and the mean percentage infection (disease severity) was calculated using the formula cited in [22].

Thus;

$$\text{Disease severity (Area)} = \frac{\text{Area of plant tissue affected } \times 100}{\text{Total area}}$$

2.2.6 Cultural Characterization

From each fungus isolated in pure culture, 5 mm mycelia discs were transferred to the centre of sterile Petri dishes containing fresh PDA. Inoculated plates were incubated for 7 days. Colony diameter was measured using a graduated 30 cm ruler and the mean diameter was obtained through the following formula [20].

$$\text{Mean colony diameter} = \frac{\text{Sum of the colony diameter in the plates}}{\text{Total number of the replicates}}$$

2.2.7 Morphological Characterization

For macroscopic observation, the cultural appearances (colony color, texture, margin, form, elevation and aerial hyphae) were observed on PDA. Colonies were determined using a book of description of medical fungi by [23] Laboratory manual and a pictori33al atlas for identification of fungi by [24] was equally used in the description of colony morphology.

2.2.8 Colonial morphology

Isolated colonies of purified fungal strains grown on solidified agar plates were observed for data using cultural and morphological features such as colony growth pattern, pigmentation, texture and conidial morphology.

2.2.9 Molecular Identification of the Fungi

After cultural and microscopic characterization of fungal pathogens, the sequencing of the nucleotide sequence of the identified fungal pathogens were used to confirm their respective identities from database system [25]. The fungal pathogens DNA were extracted from 200 mg of mycelia scraped from a 3-day-old culture using a commercial isolation kit (Power Lyser R Power Soil rDNA). The region of amplification was amplified in a PCR mix made up of PCR buffer, deionized water, $MgCl_2$, dNTP, Taq polymerase, genomic DNA extract, and primers ITS1 (Forward primer) and ITS4 (Reverse primer). The thermal cycler (T3000, BiometraR) was set at 95°C for 5 minutes (Initial denaturation), followed by a 35 cycles of 95°C for 5 minutes, 35 cycles of 95°C for 30 seconds, 49°C for 30 seconds, and 72°C for 2 minutes after which the reaction was kept at 72°C for 5 minutes. PCR amplicons were purified using purification protocol with Nucleo SpinR Gel and PCR Clean-Up kit and sequenced. The amplified rDNA gene was sequenced by BMC (Boulder Medical Center) Laboratory using the gene specific primer.

The BLAST algorithm was then used to find similar sequences of those obtained from fungal isolates. The identification of fungal isolates were based on the similarity between fungal isolates sequences found and to those of reliable reference included in the NCBI Genbank public nucleotide databases.

2.2.10 Electrophoresis of the Extracted Genomic DNA.

2.2.10.1 Agarose gel electrophoresis

One gram (1 g) of agarose (QD LE Agarose, Green Bio-Research, USA) was weighed into a conical flask and mixed with appropriate volume of 0.5 % SB/TBE buffer. The mixture was allowed to dissolve properly by stirring with the

stirrer and the agarose was melted inside microwave for 3 minutes, then allowed to cool down for 5 minutes. Then 5 µL of safe view DNA stain was added to the agarose solution and stirred. The agarose was slowly poured into the balanced gel tray with the well combs in place which was allowed to solidify for 20-30 minutes. The solidified gel was placed into the gel tank and the buffer poured until the gel is completely covered. The comb was gently removed, the ladder and sample was gently loaded into the well. The electrodes were connected to power pack and the gel ran at 100 Volts for 1 hour 30 minutes. The power was turned off and the gel carefully removed from the tray. The buffer was drained from the gel and the gel placed on the gel documentation system to capture the gel image.

2.2.10.2 Polymerase chain reaction (PCR)
The primers that were used for the PCR is shown in Table 2.1. All the components in Table 2.2 were mixed together in PCR tube making a final volume of 25 µl. The PCR tubes were centrifuged at 11,000rpm for 30 seconds and placed in the thermo cycler for amplification as described by [26].

Table 2.1: Oligonucleotide primers used in the PCR assay for the detection of fungi species.

PCR identification	Forward primer (5`-3`)	Reverse primer (5`-3`)
OMP-31	GGCTCGGTGCCAATATCA	GACTGCGTAAAGCCGGACTC

[27, 28]

Table 2.2: Concentration for the isolated fungal genome amplification (PCR)

Concentration	Volume (µL)
10 ng DNA	3
10 µM Forward Primer	1
10 µM Reverse Primer	1
10x buffer	2.5
2.5 mM Deoxynucleotide triphosphates (dNTPs)	2
25 mM magnesium dichloride ($MgCl_{2)}$	2
Dimethylsulfoxide (DMSO),	1
5 U/µl thermos aquaticus (Taq),	0.48
Distilled water	12.02

[29]

GeneAmp PCR system 9700 was used which was initialized and programme set as described in Table 2.2 The first amplicons were done with touchdown of 55 ^{0}C [29] and later optimized with conditions as shown in Table 2.3.

2.2.10.3 Agarose gel electrophoresis
The amplicon after optimization was run on Agarose gel electrophoresis with the same condition above to check the size. To check the amplicon size, 1000 base pair ladder was run with amplicons.

2.2.11 Optimization Reactione
The second round of PCR with the conditions shown in Table 2.3 below was repeated followed by gel electrophoresis and optimization.

Table 2.3: Conditions for the isolated fungal pathogens genome amplification (PCR)

Step	Temperature (^{o}C)	Time	Cycles
Pre-denaturation	94	5 minutes	
Denaturation	94	30 seconds	40
Annealing	55	45 seconds	40
Extension	72	50 seconds	40
Final extension	72	5 minutes	
Hold	10	Infinity	

[29]

2.2.11.1 Agarose gel electrophoresis
The amplicon after optimization was run on Agarose gel electrophoresis with the same condition above to check the size. To check the amplicon size, 50 base pair ladder was run with amplicons.

2.2.11.2 Optimization Reaction
The second round of PCR with the conditions shown in Table 2.2 and 2.3 above was repeated followed by gel electrophoresis for optimization.

2.2.12 Isolated Fungal DNA Sequencing
2.2.12.1 Ethanol precipitation

For each sample, a 0.5 mL tube was labeled. Following manufacturer's instructions, a fresh stop solution/glycogen mixture was prepared briefly as follows, 5 µL of the stop solution/glycogen mixture was added to each of the labeled tubes. The sequencing reaction was transferred into each of the labeled tubes and mixed thoroughly. Sixty microlitre of ethanol (95 % (v/v)) was added into the tubes and mixed thoroughly. After that, it was immediately centrifuged at 14,000 rpm at 4^0C for 15 minutes. The supernatant was carefully discarded with a micropipette. The pellet was then rinsed with 200 µL of cold 70 % (v/v) ethanol and centrifuge for 2 minutes at 14,000 rpm at 4^0C. The supernatant was carefully removed with a micropipette. It was vacuum dried for 10 minutes. The samples were re-suspended in 40 µL of the sample loading solution (provided in the kit) [30].

The re-suspended samples were transferred to the appropriate wells of the sample plate. Each of the re-suspended samples were overlaid with one drop of mineral oil (provided in the kit). The sample plates were then loaded into the instrument [30].

2.2.12.2 DNA sequencing procedure (Dye terminator cycle sequencing with quick start kit)
Positive, purified PCR products were used as templates for sequencing in the Big-Dye Terminator version 3.1 cycle sequencing kit (Applied Biosystems, Foster City, California, USA) reaction. While preparing the sequencing reactions all reagents were kept on ice. The mixture was prepared in a 2 ml tube. The reaction volume contains 9.5 ul of deionized water, 2 ul of primers, 10 ul of ng DNA and 8 ul of DTCS Quick start mix. The sequencing reaction in the PCR machine was set at 96 ^{0}C for 20 seconds, 50 ^{0}C for 20 seconds for 30 cycles and 60 ^{0}C for 4 minutes. Samples were analyzed on an automated sequencer (ABI PRISM 310 genetic analyzer, Applied Biosystems, Foster City, California, USA; Model 310). The products were sequenced from both strands to get consensus sequence. The sequence from nucleotide (NT) 9 to 252 (244 NT) was taken for analysis [30].

2.2.13 Phylogenetic Analysis
DNA sequence analysis was carried out to confirm the genotypes identified by PCR analysis. The chromatographs were edited using Finch TV (version 1.4.0) software. All the nucleotide sequences obtained in this study were screened using the online BLAST (Basic local alignment search tools) to search for sequence similarities to previously reported sequences in the database (https://blast.ncbi.nlm.nih.gov/Blast.cgi?megaBlast&BLAST_SPEC=MicrobialGenomes).
All isolates sequenced in the present study were aligned with the representative number of sequences for each major genotype and subtype selected from fungi database and Gen Bank using Multiple Sequence Alignment programme, ClustalO. Pair-wise comparison for percent nucleotide homology and evolutionary distance were made (https://www.ebi.ac.uk/Tools/msa/clustalo/).

The phylogenetic analysis of the isolated fungal was done with MEGA 6.0 software [31]. Maximum composite likelihood algorithms were utilized, and phylogenetic trees were constructed using the Neighbor-Joining method [32]. Test of phylogeny that is, the percentage replicated tree in which the associated taxa clustered together was performed with Bootstrap replication of 1000 and branch support values of > 60 %.

3.0 Results
3.1. Isolation and Identification of Fungal Pathogens Associated with Diseased White Yam
The table 3.1a below shows the common fungi species isolated from the diseased white yam obtained from Shop No 17, Kabba/Bunu central market storage unit that contained the labeled Benin, Okene and Kabba white yam. The fungi species isolated from the diseased white yam were subjected to identification and morphological characterization. The result revealed the presence of *Aspergilus niger, Fusarium sp, Rhizopus nigricans, Penicilium sp,* and *Trichoderma viridae* in the diseased white yam while no fungi species were isolated from the healthy white yam.

Table 3.1a: Shows the Fungi Species Isolated from the Diseased White Yam Obtained from Shop No 17, Kabba/Bunu Central Market Storage Unit

Species of fungi isolated from diseased white yam	Benin white yam	Okene white yam	Kabba white yam	Healthy white yam (control)
Aspergilus niger	*Aspergilus niger*	*Aspergilus niger*	*Aspergilus niger*	-
Fusarium sp	-	*Fusarium sp*	*Fusarium sp*	-
Rhizopus nigricans	-	*Rhizopus nigricans*	*Rhizopus nigricans*	-
Penicilium sp	-	*Penicilium sp*	*Penicilium sp*	-
Trichoderma viridae	*Trichoderma viridae*	*Trichoderma viridae*	*Trichoderma viridae*	-

The table 3.1 b below showed the common fungi species isolated from the diseased white yam obtained from Oluwatosin Stores, Kabba/Bunu central market storage unit that contained the labeled Benin, Okene and Kabba white

yam. The isolated fungi species were subjected to identification and morphological characterization. The result obtained revealed the presence of *Aspergilus niger, Fusarium sp, Rhizopus nigricans, Penicilium sp, Botrodiplodia thoebromae,* and *Trichoderma viridae* in the diseased white yam.

Table 3.1 b: Shows the Fungi Species Isolated from the Diseased White Yam Obtained from Oluwatosin Stores, Kabba/Bunu central market Storage Unit

Species of fungi isolated from diseased white yam	Benin white yam	Okene white yam	Kabba white yam	Healthy white yam (control)
Aspergilus niger	-	*Aspergilus niger*	*Aspergilus niger*	-
Fusarium sp	*Fusarium sp*	*Fusarium sp*	*Fusarium sp*	-
Rhizopus nigricans	-	*Rhizopus nigricans*	*Rhizopus nigricans*	-
Penicilium sp	*Penicilium sp*	*Penicilium sp*	*Penicilium sp*	-
Botrodiplodia theobrome	*Botrodiplodia theobromae*	*Botrodiplodia thoebromae*	*Botrodiplodia thoebromae*	-
Trichoderma viridae	-	*Trichoderma viridae*	*Trichoderma viridae*	-

The table 3.1 c below presents the common fungi species isolated from the diseased white yam obtained from Bukola Stores, Kabba/Bunu, Kogi State storage unit that contained the labeled Benin, Okene and Kabba white yam. The fungi species isolated were subjected to identification and morphological characterization and the results of the analysis indicates the presence of *Aspergilus niger, Fusarium sp, Rhizopus nigricans, Penicilium sp, Botrodiplodia thoebromae,* and *Trichoderma viridae* in the diseased white yam while no fungal pathogens were isolated from the healthy white yam (control)

Table 3.1 c: Shows the Fungi Species Isolated from the Diseased White Yam Obtained from Bukola stores, Kabba/Bunu central market Storage Unit

Species of fungi isolated from diseased white yam	Benin white yam	Okene white yam	Kabba white yam	Healthy white yam (control)
Aspergilus niger	-	*Aspergilus niger*	*Aspergilus niger*	-
Fusarium sp	*Fusarium sp*	*Fusarium sp*	*Fusarium sp*	-
Rhizopus nigricans	-	*Rhizopus nigricans*	*Rhizopus nigricans*	-
Penicilium sp	*Penicillium sp*	*Penicillium sp*	*Penicillium sp*	-
Botrodiplodia thoebromae	_	*Botrodiplodia thoebromae*	*Botrodiplodia thoebromae*	-
Trichoderma viridae	*Trichoderma viridae*	*Trichoderma viridae*	*Trichoderma viridae*	-

3.2 Pathogenicity Test

The table 3.2a below presents the level of pathogenicity test of the fungi species isolated from the diseased white yam obtained from Shop No 17, Kabba/Bunu central market storage unit that contained the labeled Benin, Okene and Kabba white yam.

Table 3.2a: Shows the Level of Pathogenicity Test of the Fungi Species Isolated from the Diseased White Yam Obtained from Shop No 17, Kabba Central Market Storage Unit

Species of fungi isolated from diseased white yam	Benin white yam	Okene white yam	Kabba white yam	Healthy white yam (control)
Aspergilus niger	+	++	++	
Fusarium sp	-	++	++	
Rhizopus nigricans	-	+	+	
Penicilium sp	-	++	++	

Trichoderma viridae	+	++	+

Key: + = mildly pathogenic (> 10< 50 mm in diameter); ++ = highly pathogenic (≥50 mm in diameter).

The table 3.2b below shows the level of pathogenicity test of the fungi species isolated from the diseased white yam obtained from Oluwtosin Stores, Kabba/Bunu central market storage unit that contained the labeled Benin, Okene and Kabba white yam.

Table 3.2b: Shows the Level of Pathogenicity Test of the Fungi Species Isolated from the Diseased White Yam Obtained from Oluwatosin Stores, Kabba/BUNU Central Market Storage Unit

Species of fungi isolated from diseased white yam	Benin white yam	Okene white yam	Kabba white yam	Healthy white yam (control)
Aspergilus niger	-	++	++	-
Fusarium sp		++	++	_
Rhizopus nigricans	-	+	+	_
Penicilium sp	++	++	++	_
Botrodiplodia thoebromae	+	+	+	_
Trichoderma viridae	-	+	+	_

Key: + = mildly pathogenic (> 10< 50 mm in diameter); ++ = highly pathogenic (≥50 mm in diameter).

The table 3.2c below presents the level of pathogenicity test of the fungi species isolated from the diseased white yam obtained from Bukola Stores Kabba/Bunu central market storage unit containing the labeled Benin, Okene and Kabba white yam.

Table 3.2c: Shows the Level of Pathogenicity Test of the Fungi Species Isolated from the Diseased White Yam Obtained from Bukola Stores, Kabba/Bunu Central Market Storage Unit

Species of fungi isolated from diseased white yam	Benin white yam	Okene white yam	Kabba white yam	Healthy white yam (control)
Aspergilus niger	_	++	++	-
Fusarium sp	+	++	++	-
Rhizopus nigricans	-	+	+	-
Penicilium sp	++	++	++	-
Botrodiplodia theobrome	-	+	+	-
Trichoderma viridae	+	+	+	-

Key: + = mildly pathogenic (> 10< 50 mm in diameter); ++ = highly pathogenic (≥50 mm in diameter).

3.3 Molecular Characterization

The table 3.3a below presents the molecular characterization of fungi species isolated from the diseased white yam obtained from Shop No 17, Kabba/Bunu central market storage unit that contained the labeled Benin, Okene and kabba white yam. This analysis gives the details specification of the fungi species causing yam spoilage which morphological analysis were not able to reveal as a result of similarities in morphological characteristics. Therefore, morphological characterization is not enough to ascertain the specific fungi causing the yam rots in the selected study areas. There is diversity in gene sequences within isolated fungal pathogens with respect to the data base aligned in NCBI Gene Bank. The result of the molecular characterization presented in the table 4.3a below also revealed the *Fusarium sp* and *Penicillium sp* isolated from both labeled Okene and Kabba yam

Table 3.3a; Isolated and Identified Fungal Strains in Yam Obtained from Shop No 17, Kabba/Bunu central market storage unit that contained the labeled Benin, Okene and Kabba diseased white yam as compared to the NCBI Gene Bank

Species of fungi isolated from diseased white yam	Benin white yam	Okene white yam	Kabba white yam	Healthy white yam (control)
Aspergilus niger	*Aspergilus niger*	*Aspergilus niger*	*Aspergilus niger*	-
Fusarium oxysporum	-	*Fusarium oxysporum*	*Fusarium oxysporum*	-
Rhizopus nigricans	-	*Rhizopus nigricans*	*Rhizopus nigricans*	-
Penicilium monomenatosum	-	*Penicilium monomenatosum*	*Penicilium monomenatosum*	-
Botrodiplodia theobrome	-	*Botrodiplodia theobrome*	*Botrodiplodia theobrome*	-
Trichoderma viridae	*Trichoderma viridae*	*Trichoderma viridae*	*Trichoderma viridae*	-

Table 3.3b below presents the result of molecular characterization of the fungal pathogens isolated in the white yam obtained from Oluwatosin Stores, Kabba/Bunu central market storage unit that contained the labeled Benin, Okene and Kabba white yam. Similar strain of fungal pathogens were molecularly characterized in the labeled Okene and Kabba yam. A difference species of *Fusarium* and *Penicillium* were molecularly characterized in the labeled Benin yam

Table 3.3b; Isolated and Identified Fungal Strains in Yam Obtained from Oluwatosin Stores, Kabba/Bunu Central Market Storage Unit containing the labeled Benin, Okene and Kabba diseased white yam as compared to the NCBI Gene Bank

Species of fungi isolated from diseased white yam	Benin white yam	Okene white yam	Kabba white yam	Healthy white yam (control)
Aspergilus niger	-	*Aspergilus niger*	*Aspergilus niger*	-
Fusarium sp	*Fusarium solani*	*Fusarium oxysporum*	*Fusarium oxysporum*	-
Rhizopus nigricans	-	*Rhizopus nigricans*	*Rhizopus nigricans*	-
Penicilium monomenatosum	*Penicillium brasilianum*	*Penicilium monomenatosum*	*Penicilium monomenatosum*	-
Botrodiplodia theobrome	-	*Botrodiplodia theobrome*	*Botrodiplodia theobrome*	-
Trichoderma viridae	-	*Trichoderma viridae*	*Trichoderma viridae*	-

Table 3.3c below presents the result of molecular characterization of the fungal pathogens isolated in the yam obtained from Bukola, Kabba/Bunu central market storage unit containing the labeled Benin, Okene and Kabba. Similar strain of fungal pathogens were molecularly characterized in the labeled Okene and Kabba yam. A difference species of *Fusarium* and *Penicillium* were molecularly characterized in the labeled Benin yam.

Table 3.3c: Isolated and Identified Fungal Strains in Yam Obtained from Bukola stores, Kabba/Bunu Central Market Storage Unit containing the labeled Benin, Okene and Kabba diseased white yam as Compared to the NCBI Gene Bank

Species of fungi isolated from diseased white yam	Benin white yam	Okene white yam	Kabba white yam	Healthy white yam (control)
Aspergilus niger	-	*Aspergilus niger*	*Aspergilus niger*	-
Fusarium sp	*Fusarium solani*	*Fusarium oxysporum*	*Fusarium oxysporum*	-
Rhizopus nigricans	-	*Rhizopus nigricans*	*Rhizopus nigricans*	-
Penicilium monomenatosum	*Penicillium brasilianum*	*Penicilium monomenatosum*	*Penicilium monomenatosum*	-
Botrodiplodia theobrome	-	*Botrodiplodia theobrome*	*Botrodiplodia theobrome*	-

Trichoderma viridae	*Trichoderma viridae*	*Trichoderma viridae*	*Trichoderma viridae*	-

4.0 Discussion

Isolation and Identification of Fungal Pathogens Associated with Diseased White Yam

Myriad microorganisms are responsible for the spoilage of white yam and significant of this microbial agents that causes white yam (*Dioscorea rotundata*) rot are mostly fungal pathogens which reduces the yield, quality, and shelf life of yam which brings a wide gap between demand and supply of yam [33]. Morphological characteristics of microorganisms is one of the methods used to identify microorganisms based on their colony growth pattern, pigmentation, texture and conidial morphology. This method was adopted in this study as it was described by [34]. In this study the fungal pathogens isolated from the diseased white yam in respective of their locations are *Aspergilus niger, Fusarium sp, Rhizopus nigricans, Penicilium sp, Trichoderma viridae,* and *Botrodiplodia theobrome.*
A total of five fungal pathogens were isolated from the diseased white yam obtained from Shop No 17, Kabba/Bunu central market storage unit that contained the labeled Benin, Okene and Kabba white yam. They are *Aspergilus niger, Fusarium sp, Rhizopus nigricans, Penicilium sp,* and *Trichoderma viridae.* This findings is in agreement with the report of [35] who also isolated similar fungal pathogens in diseased white yam bought from Okedayo, Nigeria. Out of the fungal pathogens isolated only two fungal pathogens (*Aspergilus niger* and *Trichoderma viridae*) were associated from Benin diseased white yam while all the five fungal pathogens were isolated from Okene and Kabba diseased white yam. The differences in the labeled yam may be attributed to the differences in geographical locations and the method used in storing the yam. This indicates that Benin white yam are better than Okene and Kabba white yam in terms of microbial resistance.

Six fungal pathogens were isolated in the labeled Benin, Okene and Kabba diseased white yam selected from Oluwatosin stores, Kabba/Bunu central market storage unit. They are *Aspergilus niger, Fusarium sp, Rhizopus nigricans, Penicilium sp, Botrodiplodia thoebromae,* and *Trichoderma viridae.* Three of the fungal pathogens that is *Fusarium sp, Penicilium and Botrodiplodia thoebromae* were predominant in the labeled Benin, Okene and Kabba white yam while the rest fungal pathogens were isolated in the labeled Okene and Kabba yam. The non-significance difference between the fungal pathogens isolated from the labeled Okene and Kabba yam may be due to similarity in geographical locations as both yam are transported from the North Central part of Nigeria. This result is in line with the findings of [36]. Also, similar result were obtained from the report of [37].

The most common fungal pathogens isolated from the labeled Benin, Okene and Kabba white yam that were picked at random from Bukola Stores, Kabba/Bunu central market Storage Unit are *Fusarim sp, Penicilium sp* and *Trichoderma viridae*. This similarity in fungal pathogens may be attributed to the method of storage adopted in the storage unit. The fungal pathogens *Aspergilus niger, Rhizopus nigricans,* and *Botrodiplodia thoebromae* were only isolated from the labeled Okene and Kabba yam. The significance difference in the fungal pathogens isolated from Benin and labeled Okene and Kabba yam suggests the differences in geographical location where they are been transported from. The differences may also be attributed to the level of water content of the edible yam. Microbial agents thrive well in moisture environment than dried places [38]. [1] reported that fungal pathogens associated with yam varies in regards to the differences in their location.

4.1 Pathogenicity Test for the Isolated Fungal pathogens from Diseased White Yam

Pathogenicity test is a test used to determine the degree at which a pathogenic agent can cause disease. The isolated fungal pathogens tested are *Aspergilus niger, Fusarium sp, Rhizopus nigricans, Penicilium sp, Botrodiplodia thoebromae,* and *Trichoderma viridae.* Of all the results obtained for the pathogenicity test in the diseased white yam obtained from No 17, Kabba/Bunu central market storage unit that contained the labeled Benin, Okene and Kabba yam. It revealed a non-significance difference in the level of pathogenicity of the isolated fungal in Okene and Kabba yam which may be attributed to the similarity of their geographical location as well as species of fungi isolated. All the fungal pathogens isolated from Okene and Kabba yam are pathogenic while only two of the fungal pathogens isolated from Benin yam are mildly pathogenic. This result is in agreement with the result reported by [19].

The result of the pathogenicity test for the fungal pathogens isolated from labeled Okene and Kabba yam collected from the Oluwatosin Stores, Kabba/Bunu central market storage unit revealed a non-significant difference in the level of pathogenicity. All the fungal pathogens isolated from the labeled Okene and Kabba yam are highly pathogenic with only exception of *Rhizopus nigricans, Botrodiplodia thoebromae* and *Tricoderma viridae* that are mildly pathogenic while *Fusarium* and *Botrodiplodia thoebromae* isolated from the labeled Benin yam are mildly pathogenic too. But *Penicilium sp* isolated from the labeled Benin yam are highly pathogenic. This differences in fungal pathogens isolated from the labeled yam may be as result of the differences in diversity of where the yam are been transported from to the storage unit where they are obtained for the experimental study. The result of this work is not in agreement with the report of [39].

The predominant fungal pathogen associated with the labeled Benin, Okene and Kabba yam bought from Bukola Stores, Kabba/Bunu Central Market Storage Unit is *penicilium sp* and the pathogenicity test result revealed a highly pathogenic level. All the fungal pathogens isolated with the labeled Okene and Kabba are highly pathogenic with the

exception of *Rhizopus nigrians, Botrodiplodia thoebromae* and *Trichoderma viridae* that are mildly pathogenic. There is no significant difference between the pathogenicity test of the isolated fungal pathogens from the labeled Okene and Kabba yam. The non-significant differences may be attributed to the soil microorganisms of the area where the yam are harvested from before transporting to the study area. Also, the reason may be due to the storage method used in the storing unit. This is in agreement with the findings of [40].

4.2 Molecular Characterisation and Genome diversity of the Isolated Fungal Pathogens Associated with Diseased White Yam

The recent advance in molecular methods provides an additional means of detection and identification in epidemiology and diagnosis of diseases. In this study, the molecular characterization of all the isolated fungal pathogens associated with the spoilage of white yam obtained from three different locations in Kabba/Bunu Local Governnment Area, Kogi State were carry out and the result were used to compare with the sequenced genes in the Gen bank. The result was able to give all the specific name fungal pathogens associated with white yam and there is diversity of genes across the three labeled white yam with respect to their locations. The diversity in genes and isolated fungal pathogens may be attributed to the differences in the fungi species. Also, the diversity in genes may be a subject of environmental factors of where they are been transported from to the storage unit [39].

The result of sequencing of the ITS1-5.8S rDNA-ITS2 of the isolated fungal pathogens as compared to the morphological characteristic result in respect to the genes in the Gen Bank confirmed the presence of *Fusarium solani, Penicillium monomenasotum* and *Fusarim basilianum* in addition to the morphological characterization result. The identification and confirmation of the fungal pathogens were possible due to the gene sequencing method adopted which the morphological characterization alone would not have been possible. This findings agreed with the earlier work done by [41].

5.0 Conclusion

The predominant fungal specie causing diseases in white yam across all the three locations in Kabba/Bunu Local Government of Kogi state are ; *Aspergilus niger, Fusarium sp, Rhizopus nigricans, Penicilium sp, Trichoderma viridae,* and *Botrodiplodia theobrome.* Of all the fungal pathogens causing diseases in white yam across all the three locations in Kabba/Bunu Local Government, the most commom and virulent among them are; *Aspergilus niger, Fusarium sp,* and *Penicilium sp,* while the uncommon specie of fungal that also cause diseases in white yam across all the three locations in Kabba/Bunu Local Governmet are; *Penicillium monomenatosum, Penicillium basilianum* and *Fusarium solani.* The results of this study conclude that Benin white yam is better than Okene and Kabba white yam in terms of fungal load and resistance.

Conflict of interest

The authors declare that there is no conflict interest

Abbreviation

PDA= Potato Dextrose Agar

BLAST = Basic Local Alignment Search Tools

PCR = Polymerase Chain Reaction

NCBI = National Centre for Biotechnology Information

References

[1] Okigbo, R.N., & Ogbonnaya, U.O. (2014). Antifungal effects of two tropical plant leaf extracts of Ocimum gratissimum and Aframomum melegueta on post-harvest yam (*Dioscorea spp*) rot. *African Journal of Biotechnology*, 5(9), 727-731.

[2] Okereke, G. O., Igbabul, B. D., & Ikya, J. K. (2023). Potentials Of Yams' Utilizations In Food, Medicine And Allied Fields. *International Journal of Current Science*, 13, 2.

[3] Bassey E. E. & Akpan U. S. (2015). Evaluation of guinea white yam (*Dioscorea rotundata Poir*) for yield and yield components in Nigeria. *American Journal of Experimental Agriculture* 8, 216–233

[4] Amusa, N. A. (2023). Concentric leaf spot of yam (*Discorea* spp) in South Western Nigeria. *Mycopathologia,* 148, 33-36

[5] Coursey, D. G. (2019). Yam storage I. A review of storage practices and information on storage losses. *Journal of Stored Product Research*, 2, 227-244.

[6] FAO (2021). Food and Agriculture organization of the United Nations: Production 1995, vol.50.Rome, Italy

[7] Musa, Y. H., Onu, J., Vosanka, I. P. & Anonguku, I. (2011). Production efficiency of yam in Zing Local Government area of Taraba State, Nigeria. *Journal of Horticultur and Forestry* 3, 372-378

[8] Umeta, M., West, C. E. & Fufa, H. (2015). Content of zinc, iron, calcium and their absorption inhibitors in foods commonly consumed in Ethiopia. *Journal of Food Composition and Analysis,* 18, 803-817.

[9] Akissoe, N., Mestres, C., Hounhouigan, J., & Nago, M. (2023). Biochemical origin of browningduring the processing of fresh yam (*Dioscorea* spp.) into dried product. *Journal of Agriculture and Food Chemistry* 53, 2552-2557.

[10] Babaleye, T. (2013). "West Africa; Improving Yam Production Technology". ANB-BIA supplement Issue/Edition Nr 463.

[11] Izekor, O. B., & Olumese, M. I. (2020). Determinants of yam production and profitability in Edo State, Nigeria. *African Journal of General Agriculture,* 6, 205-210.

[12] Olaoye, J. O., & Oyewole, S. N. (2012). Optimization of some "poundo" yam production parameters. *Agricultural Engineering International Journal,* 14, Manuscript No. 2063.

[13] Eka, O. U. (1985). The Chemical Composition of Yam Tubers, in *Advances in Yam Research*, 61-83, (Osuji, G., ed.). Frontline Publishers, Enugu-Nigeria

[14] Shehu, J. F., Iyortyer, J. T., Mshelia, S. I., & Jongur, A. A. U. (2010). Determinants of yam production and technical efficiency among yam farmers in Benue State, Nigeria. *Journal of Social Science,* 24, 143-148

[15] Anon, A. (2019). West African Agricultural Productivity Programme. Baseline survey report Ghana.

[16] Stangarlin, J. R., Kuhn, O. J., Assi, L, & Schwan-estrada, K. R. F. (2021). Control of plant diseases using extracts from medicinal plants and fungi. In: Méndez-vilas A. (ed). *Science against Microbial Pathogens: Communicating Current Research and Technological Advances*. Badajoz: *Formatex*. 2: 1033-1042

[17] Yusuf, C., & Okusanya, B. A. O. (2019). Fungi associated with the storage rot of yam (Dioscorea rotundata Poir) in Yola State. *Journal of Sustainable Development in Agriculture and Environment,* 2, 9

[18] Okigbo, R. N., & Odurukwe, C. N. (2019). Occurrence and control of fungal rot pathogens of yam (*Discorea rotundata* Poir) with leaf extracts of *Chromolena odorata, Carica papaya* and *Aspilia Africana. Nigerian Journal of Mycology,* 2(1), 154-165

[19] Okigbo, N. R., Ekweremadu, C. E., Agu, C. E., Irondu, R. C., Okeke, B. C., Awah, S. N., Anaukwu, C. G., Okafor, I. O., Ezenwa, C. U., Iloanusi, A. C. (2015). Control of white yam (*Dioscorea rotundata*) rot pathogen using peel extract of white yam (*Dioscorea alata*). *Advances in Applied Science Research*, 6(10), 7- 13

[20] Bechem, E. T., & Afanga, Y. A. (2017). Morphological and molecular identification of fungi associated with corm rot and blight symptoms on plantain (*Musa paradisiaca*) in macro-propagators. *International Journal of Biological and Chemical Sciences*, 11(6), 2793-2808.

[21] Okigbo, R.N., & Ikediugwu, F. E. (2020). Studies on biological control of post-harvest rot yams (Dioscorea spp) with *Trichoderma viride*. *Journal of Phytopathology*, 148, 351-355.

[22] Umeta, M., West, C. E. & Fufa, H. (2015). Content of zinc, iron, calcium and their absorption inhibitors in foods commonly consumed in Ethiopia. *Journal of Food Composition and Analysis,* 18, 803-817.

[23] Kidd, S., Halliday, C. L., Alexiou, H., & Ellis, D. (2016). Descriptions of medical fungi (Vol. 3). David Ellis

[24] Watanabe, T. (2010). Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species. CRC press

[25] White, T.J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M.A. Innis, D. H. Gelfand, J.J. Sninsky, and T.J. White (eds.) PCR protocols: a Guide to Methods and Applications. Academic Press Inc., San Diego, Californie, pp 315-322.

[26] Sabrina, R., Taha, M. H., Bakir, M., Asma, M., & Khaoula, B. (2018). Detection of fungi spp. in milk from seronegative cows by real-time polymerase chain reaction in the region of Batna, Algeria. Veterinary World, 11, 363-367

[27] Probert, W. S., Schrader, K. N., Khuong, N. Y., Bystrom, S. L., & Graves, M. H. (2004). Real-Time Multiplex PCR Assay for Detection of fungi spp., B. *Aspergilus niger*. *Journal of Clinical Microbiology*, 42(3), 1290–1293. doi:10.1128/JCM.42.3.1290–1293.2004.

[28] Zhang, B., Wear, D. J., Stojadinovic, A., & Izadjoo, M. (2013). Sequential Real-Time PCR Assays Applied to Identification of Genomic Signatures in Formalin-Fixed Paraffin-Embedded Tissues: A Case Report About Brucella-Induced Osteomyelitis. Military Medicine, 178(1), 88-94.

[29] Ali, S., Akhter, S., Neubauer, H., Melzer, F., Khan, I., Abatih, E. N., El-Adawy, H., Irfan, M., Muuhammad, A., Akbar, M. W., Umar, S., Ali, Q., Iqbal, M. N., Mahmood, A., & Ahmed, H. (2017). Seroprevalence and risk factors associated with yam rots in the Potohar Plateau, Pakistan. BMC Research notes, 10(73), 20-31. DOI 10.1186/s13104-017-2394-2

[30] Halling, S. M., Peterson-Burch, B. D., & Bricker, B. J. (2005). Completion of the genome sequence of species of fungal and comparison to the highly similar genomes of isolated fungal pathogens. *Journal of Bacteriology*, 187, 2715–2726.

[31] Tamura, K.., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA6: Molecular evolution genetic analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. *Molecular Biology and Evolution*, 28(10), 2731-2739. doi.org/10.1093/molbev/msr121.

[32] Saitou, N., & Nei, M. (1987). The Neighbor-Joining Method- a new method for reconstructing phylogenetic trees. Molecular Biology Evolution, 4, 406-425

[33] Sangoyomi, T, E., Ekpo, E. J. A. & Asiedu, R. (2019). Fungitoxic effects and attributes of *Allium sativum* and *Ocimum gratissimum* extracts on *Rhizoctonia solani,* the causal agent of yam (*Dioascorea rotundata* Poir) rot disease. *Nigerian Journal of Mycology,* 2(1), 166-177.

[34] Alves, A., Crous, P.W., Correia, A. & Phillips, A. J. I. (2018). Morphological and molecular data reveal cryptic speciation in lasiodiplodia theobromae. *Fungal diversity*, 28, 1-13

[35] Nweke, F. U., & Enujeke E. C. (2008). Fungi associated with spoilage of stored and market white yam tubers in Umunde, Nigeria. *Global Journal of Agricultural Science*, 5, 1, 11-12

[36] Akanbi, W. B., Olaniran, O. A., & Olaniyan, A. B. (2017). Effects of Integrated use of organic and inorganic nutrient sources on growth and tuber characteristics of white yam (*Dioscorea rotundata*). *African Crop Science Conference Proceedings*, 8, 359 – 363.

[37] Ogaraku, A. O., & Usman H. O. (2018). Storage Rot of Some Yams (Dioscorea spp) in Keffi and Environs, Nasarawa State, Nigeria. *Production Agriculture and Technology Journal*, 4 (2), 22-27

[38] Zagory, D. (2021). Effects of post-processing handling and packaging on microbial populations. *Postharvest Biology and Technology* 15, 313-321

[39] Adeniji, M. O. (2020). Fungi associated with storage decay of yam in Nigeria. *Phytopathology*, 60, 590-592

[40] Lile, C. N. M., Modeste, L. S., Severin, N. T., Pierre, E., Rufin, M. K. T., Alessandra A., Pierre, G. T. G., & Fabrice, F. B. (2020). Molecular Identification of Fungal Pathogens Associated with Post-harvest Yam Tubers Rot in Mbam et Kim Division (Cameroon) with Emphasis on *Penicillium monomenatosum* (Frisvad, Filt. & Wicklow) as a First Report. *American Journal of Microbiological Research*, 2020, 8, 2, 73-78

[41] Kim, W.K., Hwang, Y.S., & Yu, S. H. (2008). Two species of *Penicillium* associated with blue mold of yam in Korea. *Mycobiology*, 36, 217-221.