

**ISOLATION AND CHARACTERIZATION OF FUNGI ASSOCIATED
WITH POSTHARVEST ROT OF YAM (*Diocorea rotundata* Poir) AND
POTATO (*Solanum tuberosum* L.) IN ENUGU STATE**

BY

**EZEANI, PRINCESS NNEOMA
GOU/U22/MCB/516**

**MICROBIOLOGY
DEPARTMENT OF BIOLOGICAL SCIENCES
FACULTY OF NATURAL SCIENCES AND
ENVIRONMENTAL STUDIES
GODFREY OKOYE UNIVERSITY, ENUGU NIGERIA.**

JUNE, 2026

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**A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL
SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE IN MICROBIOLOGY.**

SUPERVISOR: EMERITA PROF. C. A. OYEKA.

JUNE, 2026

APPROVAL

This research titled “isolation and characterization of fungi associated with postharvest rot of yam (*diocorea rotundata* poir) and potato (*solanum tuberosum* l.) in enugu state” has been assessed and approved by the Committee of Department of Biological Sciences and the Faculty of Natural Sciences and Environmental Studies, Godfrey Okoye University Enugu, Nigeria.

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CERTIFICATION

This is to certify that this research titled (isolation and characterization of fungi associated with postharvest rot of yam (*diocorea rotundata* poir) and potato (*solanum tuberosum* l.) in enugu state) was carried out by EZEANI, PRINCESS NNEOMA under the supervision of EMERITA PROF. C. A. OYEKA in the Department of Biological Sciences, Godfrey Okoye University Enugu, Nigeria.

EZEANI, PRINCESS NNEOMA

Signature

.....

Date

DEDICATION

This project is dedicated to the Almighty God for His infinite mercy and strength throughout the course of my studies. I dedicate this work to my family whose unconditional love, sacrifices and prayers have been the foundation of my success. Finally, this project is dedicated to Godfrey Okoye University for providing the academic environment and challenges that have shaped my intellectual growth and resilience.

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ABSTRACT

Post-harvest rot is a major cause of storage losses in yam (*Dioscorea rotundata* Poir.) and potato (*Solanum tuberosum* L.). This study aimed to identify and isolate the fungal agents that cause yam and potato rot. Five diseased yam and five diseased potato tubers were collected from Abakpa Market, Enugu State. Isolation was performed using swab and cutting techniques on Potato Dextrose Agar (PDA). Pure isolates were obtained through sub-culturing on potato slants. The fungi were identified using macroscopic observation, microscopic examination with lactophenol cotton blue stain, and slide culture technique. Data obtained were analyzed using descriptive statistics. A total of ten fungal species were isolated, with four species recovered from yam tubers: *Aspergillus clavatus*, *Aspergillus fumigatus*, *Aspergillus niger*, and *Trichophyton soudanense* and seven species from potato tubers: *Candida krusei*, *Candida tropicalis*, *Candida utilis*, *Candida famata*, *Chrysosporium sitophila*, *Trichophyton mentagrophytes*, and *Aspergillus niger*. *Aspergillus niger* was the most dominant and occurred in both crops with an isolation frequency of 20%. The isolated fungi showed varying levels of pathogenicity when tested on healthy tubers. The findings confirm that fungal infection is a major cause of post-harvest losses of yam and potato in the study area. This study highlights the need for improved storage practices, proper handling techniques, and targeted management strategies to minimize post-harvest losses and enhance food security in Nigeria.

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Dioscoreaceae family which includes the tuberous crop, yam. (*Dioscorea rotundata* Poir). or white yam continues to be the most extensively cultivated species in West Africa. For millions of farmers, processors and consumers, it is an essential source of food and income. In a similar vein, potatoes (*Solanum tuberosum* L.) are an expensive and versatile crop that contributes significantly to household income and food supply in many developed and developing countries worldwide (Koch *et al.*, 2020; Tolessa, 2018). Despite their importance, post-harvest rot pathogens constantly undermine yam tubers' capacity to make a substantial contribution to food safety and security (Adeniji, 2019). Post-harvest rot seriously hinders yams' ability to be stored, preserved and used commercially. Similarly, potato dry rot which is mostly caused by *Fusarium* species, poses a severe danger to worldwide potato production systems (Tiwari *et al.*, 2020).

Potatoes have been an essential component of the human diet for many ages. They were an essential part of the Inca diet and helped keep people alive during Europe's industrial revolutions in the nineteenth century (Jansky *et al.*, 2019). Potatoes include dietary antioxidants that may help prevent disease in addition to a strong supply of essential protein, vitamins and minerals (Tolessa, 2018). Similar to this, at least 60 million people especially in West Africa depend on yam for their livelihood and food security. In essence, they are food crops that are high in energy and contain bioactive compounds that have positive health effects such as antibacterial and anti-tumor properties (Samuel *et al.*, 2018; Tareen *et al.*, 2025). Despite their significance, rot disease negatively impacts these crops' production resulting in a decrease in yam yield and significant storage damage (Ndifon and Lum, 2021; Tiwari *et al.*, 2020). Molds

are the primary pathogens of stored sweet potatoes, resulting in significant post-harvest and financial losses for Nigerian farmers. Approximately 45% of the world's produce is lost, resulting in global economic losses between \$10 billion and \$100 billion. Over 25% of yams are lost annually due to numerous pests and illnesses in Nigeria, which supplies 70% of the world's yams (Ano, 2019). Fungal agents are the main cause of rots (Tiwari *et al.*, 2020). *Fusarium* species are responsible for dry rot in potatoes and in Algeria, thirteen species of *Fusarium* and *Neocosmospora* have been linked to tuber dry rot and potato wilt (Azil *et al.*, 2021). *Aspergillus flavus*, *A. niger*, *Fusarium oxysporum*, *Penicillium notatum* and *Rhizopus stolonifer* are the common fungal isolates that cause yam rot (Ekpenyong *et al.*, 2024). *Botryodiplodia theobromae*, *Colletotrichum sp.*, *Curvularia eragrostidis* and *Penicillium purpurogenum* are other fungal pathogens that have been discovered from decaying yam tubers.

1.2 Statement of the Problem

The widespread post-harvest rot of yam and potato tubers caused by fungal pathogens results in significant losses and threatens food security. However, the specific fungal agents responsible and the efficacy of current control methods are not fully consolidated. This knowledge gap hinders the development of suitable management strategies, leading to chemical resistance and health risks. Therefore, this study aims to identify the fungal species associated with these rots, providing insights to improve tuber preservation and reduce waste.

1.3 Aim and Objectives

This study **aims** to isolate and identify the fungal agents causing yam and potato rots.

The **objectives** are to:

1. To isolate and identify the various fungal species linked to yam and potato tuber rots.

2. To examine the documented pathogenicity of the isolated fungal species of yam and potato tubers.

CHAPTER TWO

LITERATURE REVIEW

2.1 Yam and Potato: Importance and Uses

Roots and tuber crops such as potatoes (*Solanum tuberosum* L.) and yams (*Dioscorea rotundata* Poir.) are key actors in regional and global food systems, especially in developing countries, which utilize them as sources of revenue and food and as symbols of culture. Yams, which are one of the non-cereal food crops, are mostly cultivated in West Africa, Asia and some parts of Latin America and the Caribbean. The tubers are a good source of carbohydrates and therefore millions of people depend on them as their primary source of energy. These nutritional qualities make yams an important part of balanced diets in places where cereal is either expensive or scarce. Nigeria, the world's top producer of yams, regularly produces more than 35 million metric tons annually, accounting for more than 65% - 70% of the world's total. Yams' economic importance is shown by their enormous production volume, which sustains the livelihood of smallholder farmers, traders and processors. In many communities, especially in southeastern and middle-belt Nigeria, where it is widely used in festivals, marriage rituals and customary rites, yam is more than just a subsistence crop. In order to add value and lessen seasonal shortages, yams are industrially produced into flour (also called elubo) chips and other derivatives. By prolonging shelf life, the processing sector also improves food security and generates job opportunities. In contrast, potatoes are the most significant non-cereal food crop in the world in terms of consumption and adaptability. It is grown in temperate, subtropical and highland tropical regions. In recent decades, Asia and Africa have seen notable increases in output (Jansky *et al.*, 2019). High-quality carbohydrates, significant levels of vitamin C (typically higher than many fruits), vitamin B6, potassium, magnesium, iron and dietary fiber are all found in potato tubers. Furthermore, potatoes provide bioactive substances such as

flavonoids, carotenoids and phenolic acids that support antioxidant activity and general health advantages (Beals, 2019). Potatoes have become a key crop for reducing food insecurity and poverty in emerging nations, especially in China and India which are currently the world's top two producers (Wang and Wang, 2019). The transformation of potatoes into fries, chips, starch and dried goods allows making a substantial contribution to the development of the agro-industrial sphere and creates employment opportunities (Koch *et al.*, 2019). In Africa, there has been a rapid increase in potato production in such countries as South Africa, Egypt and Algeria where it has increased the food diversity and supplemented grains. Both potatoes and yams are highly flexible to the various agro-ecological settings. Whereas potatoes can be cultivated in colder areas of the highlands and with irrigation in semi-arid areas, yams can tolerate poor soil and drought (Tolessa, 2018). These properties render them particularly useful in marginal farming areas where cereal production is hard to achieve. Moreover, the fact that they are integrated into mixed farming enhances the diversification of farm revenue and soil fertility (Otalora *et al.*, 2024). Despite these advantages, the highly perishable nature of the two crops poses a great challenge to marketing, transportation and storage. Post-harvest losses usually amount to 20-50 percent of economic returns, drastically reducing food security in the household, especially in resource-limited regions (Abewoy, 2018). The only way to achieve this is to ensure that these losses are eliminated through enhanced handling, storage and disease control to make the most out of the potential of yams and potatoes and help in sustainable development and nutritional well-being.

2.2 Post-Harvest Diseases of Root and Tuber Crops

One of the biggest obstacles to the use of root and tuber crops globally is post-harvest infections, especially in humid tropical regions where high temperatures and relative humidity hasten microbial deterioration. Post-harvest losses of sweet potato (a related tuber) have been estimated at 30-50% by fungal rots, and yams in tropical areas; this is why improved pathogen

identification and control measures are needed (Paul *et al.*, 2021; Jahan, 2020). The importance of the topic of fungal rot is indicated by the annual losses in the economies of West Africa (including Nigeria) of more than 500 million dollars annually which explains the urgency to study the agents of rot and their management (Gwa and Nwankiti, 2018). Food availability, market value and seed quality for upcoming planting seasons are all jeopardized by these diseases, which mostly attack stored tubers and cause quantitative and qualitative losses. Yam production methods are greatly limited by post harvest rot. In studies conducted over West Africa losses of 20% to as high as 50% of the stored tubers have been recorded in the first 6 months of storage, depending on storage conditions and pathogen pressure. In contrast, the soft rot is produced by fast tissue maceration, has a mushy consistency and a bad odor. When exposed to high humidity, the injured section becomes dark brown/black, and the tuber completely collapses within days to weeks (Dania *et al.*, 2019). Wet rot is a more advanced form which may have secondary bacterial invasion following the initial attack by the fungi leading to complete liquefaction and fluid leakage. The rot in yams usually begins at the natural holes or where the damage occurred. Spores germinate and hyphae penetrate the periderm; cell walls are digested by enzymes and necrosis progresses inwards and radially. Complete disintegration occurs with soft and moist rots and with dry rots, some structural integrity remains. Rot patterns are similar in potato crops, although there are some obvious differences. Pods of potatoes have been associated with *Fusarium* spp. causing potato rots which are characterized by concentric, depressed areas of withered tissue covered by white to pinkish mycelial growth. As the rot progresses towards the inside, the fungal spore-filled chambers are formed (Tiwari *et al.*, 2020). Soft rot is a slimy, foul-smelling decay that rapidly occurs in warm, moist conditions, and is caused by bacteria and some fungi. Late blight rot is caused by *Phytophthora infestans* leading to solid, discolored lesions that soften. This is often followed by a characteristic white spore formation on the top in moist weather. The main difference

between potato rots and yam rots is the bacteria that is predominant and the rate at which the attack occurs. Potato rots commonly involve field pathogens (*Phytophthora*, *Alternaria*) that persist in storage, whereas yam rots are often dominated by storage molds (*Aspergillus*, *Penicillium*, *Rhizopus*) (Azil *et al.*, 2021). Strategies for managing disease are significantly impacted by these differences.

2.3 Fungal Agents Associated with Yam Rots

The degeneration of yam tubers during storage is caused by a wide variety of fungal species, each of which has distinct morphological characteristics, infection tactics and economic importance.

2.3.1 Procedures for fungal recovery of diseased tubers

Recovery of diseased fungi on rotted yams (*Dioscorea rotundata* Poir.) and potato (*Solanum tuberosum* L.) tubers is an essential step in the identification of the causal agents of post-harvest rot. Literature has reported various means through which efficient isolation of pathogenic fungi can be done, in order to reduce contamination by surface microbes or secondary organisms. Surface sterilization can be a typical preliminary step to remove epiphytic or surface dirt. Tubers are usually sprayed with running tap water to take off the soil and debris, after which they are dipped sequentially into 70 percent ethanol for 30 to 60 seconds and then in 5 percent sodium hypochlorite (NaOCl) for 2 minutes. Four volumes of sterile distilled water are then used to rinse the tissues at a time to eliminate the sterilant (Gwa and Nwankiti, 2018; Paul *et al.*, 2021). This will be done to avoid excessive proliferation of non-pathogenic fungi and bacteria on isolation plates. The advancing margin of the rot (the point of transition between healthy and diseased tissue) is sterilized and small tissue pieces (3- 5 mm) are aseptically removed with a sterile scalpel or cork borer. These fragments are directly cultured on solidified Potato Dextrose Agar (PDA) in Petri plates, typically 3 to 4 pieces per plate, to permit separate

growth of colonies. The most common medium is PDA since it contains the nutrients that support the growth of fungi; the medium is prepared by dissolving 39 g of dehydrated PDA powder in 1 liter of distilled water and adjusting pH to 5.6 ± 0.2 , by autoclaving at 121 C within 15 minutes and pouring it into sterile Petri dishes. Plates are incubated between 25 and 28 C, and within the span of 5-7 days, fungal colonies grow out of the pieces of tissue. Other possible methods of recovery are tissue plating on selective media with or without antibiotics (e.g., streptomycin or chloramphenicol) to curb the growth of bacterial contaminants, or serial dilution plating to quantitatively determine the fungal load (Paul *et al.*, 2021). Pure isolates are obtained through sub-culturing by transfer of the hyphal tips or individual spores of the emerging colonies onto fresh plates of PDA. This is followed by morphological identification based on the macroscopic (colony colour, texture, growth rate) and microscopic (conidiophores, spores, hyphae) features that can be seen through a compound microscope after lactophenol cotton blue is used as a stain.

These recovery techniques allow a steady isolation of the main pathogenic agents including *Aspergillus flavus*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Colletotrichum* spp. to give dependable material to confirm pathogenicity and additional investigations on the etiology and management of diseases. The method used is also determined by the type of rotation (dry, soft, or wet) and the necessity of differentiating between primary pathogens and secondary invaders.

2.3.2 *Aspergillus flavus*

Aspergillus flavus is commonly known as one of the most common and devastating economically damaging fungal pathogens concerning post-harvest rot in yam tubers (*Dioscorea rotundata* Poir. and other species) in storage systems in the tropics. This fungus is a member of the genus *Aspergillus*, a group of ubiquitous filamentous fungi that have saprophytic and opportunistic pathogenic lifestyles. *Aspergillus flavus* is specifically infamous

in yam-producing areas of West Africa, such as Nigeria, where it prospers in warm and humid climates of traditional storage barns and marketplaces. Macroscopically, *Aspergillus flavus* forms typical velvety-yellowish to olive-green colonies on potato dextrose agar (PDA) and most commonly with a white to cream-colored reverse. The colonies grow rapidly in terms of radial growth, attaining a diameter of 40-70mm in 7 days at 25-30 °C. Mycelium is thickly cottony or floccose and powdery in appearance owing to a high presence of conidial heads. Microscopically, the fungus forms long, smooth-walled, radiate conidiophores (up to 1 mm long) ending in globose vesicles (20-50mm diameter). These vesicles have biserial phialides in two rows, giving rise to chains of globose to sub-globose conidia (3- 5 mm diameter), rough-walled and pigmented. Such morphological features are uniform among isolates of rotted yam tubers and are effective diagnostic features when identifying them. Infection by *Aspergillus flavus* typically occurs through mechanical trauma during harvesting, transportation, handling, or via natural openings such as lenticels or cracks in the periderm. After gaining entry, the germinating spores generate hyphae that propagate within the periderm and parenchyma beneath. The fungus releases a cocktail of cell-wall-degrading enzymes, including pectinases, cellulases, and hemicelluloses, which break down host tissues and facilitate the acquisition of nutrients. This enzymatic activity causes the typical symptoms of dry rot so that the surface of the tuber has sunken and wrinkled brownish-black lesions with underlying tissues becoming hard, dry, and powdery. Afflicted tissues tend to detach readily from healthy tissue, the outcome being a high degree of shrinkage and loss of weight (Dania *et al.*, 2019). The ecological effectiveness of *Aspergillus flavus* in the storage of yams is also promoted by its capability to adapt to a large variety of environmental conditions and the ability to generate large amounts of conidia which are easily spread by or through air movements and handling. The fungus can also survive in soil, plant debris and storage structures which act as a source of inoculum for future infections. In large yam-producing countries like Benue,

Nasarawa, and Anambra, *Aspergillus flavus* has continued to be one of the most common species found after rot on the tubers, frequently ranging from 20 to 40 percent of the isolates based on the length of storage and storage. These two factors together (tissue degradation and mycotoxin production) result in *A. flavus* being a major issue to yam after-harvest quality and food safety. The fact that it is common in tropical storage systems highlights the necessity to apply more effective handling methods, enhanced ventilation, and specific control measures to reduce its effects on food security and monetary gains in communities that rely on yams as the main crop. The pathogenic ability of *A. flavus* was further demonstrated through pathogenicity tests on healthy yam tubers. Inoculated tubers developed characteristic symptoms of dry rot, including sunken, wrinkled and brownish black lesions. In contrast, the control tubers inoculated with sterile Potato Dextrose Agar discs remained firm and showed no visible signs of rot (Figure 2.1). The growth of *A. flavus* confirms the ability of the organism to penetrate wounded tissues and cause significant deterioration under storage conditions (Gwa and Akombo, 2016).



Figure 2.1: Pathogenicity test demonstrating *Aspergillus flavus*-induced rot and pathogenicity test control demonstrating no rot

Source: Gwa and Akombo, 2016.

2.3.3 *Rhizopus stolonifer*

The bread mold fungus (*Rhizopus stolonifer*) is one of the worst and most common pathogens that cause soft rot in stored yam tubers (*Dioscorea rotundata* Poir.). This zygomycete fungus is of the order Mucorales and is very common in the tropical and sub-tropical regions and is the cause of some of the greatest and quickest post-harvest losses in yam production systems. It is especially problematic in less well-managed storage facilities in West Africa, such as Nigeria because it is capable of causing total tuber breakdown within several days under favorable conditions (Dania *et al.*, 2019).

Rhizopus stolonifer is identified by such large aerial mycelia of extremely high growth rate, white or grey in colour which spread quickly on the substrate. In 2-4 days of incubation on the potato dextrose agar (PDA) at 25-30 °C, the mycelium turns black as a result of high production of the sporangia. The colonies are fluffy and cottony and can easily cover the whole Petri dish in 5-7 days. Colony reverse is normally pale or unpigmented. The sporulation is abundant with tall sporangiophores (up to 2 mm long) emerging on stolons which interconnect groups of sporangia. Spores are globose to sub globose (50- 300 mm diameter) and are white and blackening when they grow to form sporangia. Sporangiospores have an irregular shape of ellipsoid form, non-septate and range in length between 5-15 mm (Dania *et al.*, 2019). *Rhizopus stolonifer* infection mostly happens due to mechanical injury (cut, bruise, or piercing of the tuber with a speargrass) or openings of the body (lenticels and cracks on the tuber periderm). It is not normal to have the fungus penetrate undamaged surfaces but it uses any crack to enter the surface. The germ tubes begin to grow into vigorous hyphae when they are inside causing a rapid breakdown of the middle lamella and cell wall components due to the enzymes that they secrete which are high in pectinolytic and cellulolytic enzymes. This enzymatic maceration leads to typical soft rot symptoms: the tissues become soft, watery and translucent with a typical fermented or sour smell because of the release of volatile compounds. At

advanced stages, the whole tuber becomes collapsed in the form of a slimy disintegrated mass, with excretion of fluids, and entirely lost integrity.

Rhizopus stolonifer grows in high temperature (best at 25-35 °C) and relative humidity (>85%), which is often present in domestically used yam barns, pile storage or poorly ventilated market stalls in tropical areas. The fungus is extremely sporulating and generates huge quantities of airborne sporangiospores, which contribute to major transmission in storage facilities and between tubers. It has one of the highest growth rates amongst all the other yam rot pathogens and in the best conditions, it can fully decompose the tuber in 3-7 days of infection. This accelerated development leads to a significant number of quantitative losses in the countries producing yams, especially during the rainy season or in the storage facilities that lack proper ventilation (Dania *et al.*, 2019). *R. stolonifer* also has an economic effect, as it can infect a diverse variety of crops dominant in the tropics, such as other tubers, fruits, and vegetables, which is why the pathogen remains a highly active source of inoculum in farming settings. *Rhizopus stolonifer* is commonly obtained in Nigeria as a staple food and as a major cash crop in the principal production areas in Benue, Nasarawa, Cross River, and Enugu state where yam is a staple food crop. In damp storage environments, it is usually the predominant pathogen (20-40% of isolates in certain surveys).

2.3.4 *Penicillium* species

Commonly isolated specimens of the genus *Penicillium*, especially *Penicillium expansum* and *Penicillium purpurogenum* are listed as some of the most commonly found fungi related to dry rot in stored yam tubers. They are cosmopolitan saprophytes, which proliferate in tropical storage environments and opportunistic pathogens that use wound or damaged periderm to cause an infection. The fact that they are quite common in the parts of West Africa and Nigeria that produce yams highlights their role in post-harvest losses which are usually accompanied

by other storage moulds such as *Aspergillus* and *Fusarium species* (Gwa *et al.*, 2018). Macroscopically, the colonies of *Penicillium* on potato dextrose agar (PDA) are characteristic: velvety to powdery, blue-green to grey-green in the surface, white to pale yellow margin, and pale to yellowish reverse. It grows quite fast, developing a diameter of 30-50 mm in 7 days. The colonies have a typical musty or earthy smell. Microscopically, the genus is typified by brush-like conidiophores (penicilli) that are branched and carry metulae and phialides in verticils. The phialides give rise to long chains of globose to subglobose conidia smooth or rough-walled depending on the species. *P. expansum* is characterized by smooth conidia walls and the formation of a large quantity of blue-green spore masses, whereas *P. purpurogenum* is characterized by a reddish to purple colony reverse and rough conidia (Gwa *et al.*, 2018). *Penicillium* species infection is mainly acquired by mechanical trauma (cuts, bruises or abrasions) during harvesting, transportation, or handling. The fungus can also gain access via natural wounds or by already existing ones inflicted by insects or other disease agents. The hyphae invade the periderm, once the hyphae enter it and release pectinolytic and cellulolytic enzymes which begin to break down cell walls causing local tissue destruction. The rot occurs as dry rot: there are sunken wrinkled, discoloured (brown to dark brown) areas, which have powdery masses of conidia on the surface. Tissues lose their moisture, shrink and become separated from healthy parts, causing a serious loss of weight and making them less marketable (Gwa *et al.*, 2018; Dania *et al.*, 2019). One big problem with *Penicillium* species in stored yams is that they form mycotoxins, especially patulin. Patulin is a mycotoxin with low molecular weight that is produced predominantly by *P. expansum* when actively growing in rotted tissues. It is genotoxic, cytotoxic and immunotoxic and has been linked to gastrointestinal disorders, nausea, ulceration and possible neurotoxicity in animal research. In spite of the fact that patulin is not as stable as aflatoxins in an alkaline environment, it remains in acidic environments and can be washed into neighboring healthy tissues, contaminating more significant parts of the

tuber. Patulin can be persistent as a food safety hazard in areas where yam is a food source because in processed food products, like yam flour or chips, the toxin may be preserved during drying and milling (Gwa *et al.*, 2018; Okafor and Eni, 2018). The importance of patulin to the health of the population is reflected in the fact that regulatory agencies like the World Health Organization and Codex Alimentarius have set a maximum content of patulin in food. The effect of the species in terms of *Penicillium* improves its ecological flexibility. They can withstand a great temperature range (best 20 to 30°C) and moderate humidity and generate plentiful airborne conidia contributing to the secondary spillage in a barn, heaps, or market stall. *Penicillium* spp. are commonly recovered on dry-rotted tubers in Nigerian storage environments with high moisture and low ventilation (such as traditional barns), occasionally representing 15 to 30 percent of the isolates in a survey of Nigerian states (Benue, Nasarawa, and Anambra).

2.3.5 *Fusarium oxysporum*

Fusarium oxysporum is a common and economically relevant soil fungal pathogen that is a significant cause of post-harvest degradation of yam tubers in tropical post-harvest conditions. It is a species of the genus *Fusarium*, a filamentous fungus whose characteristic is the ability to survive in soil for long periods as chlamydospores and to infect plants through both the root system and wounds. *F. oxysporum* is commonly found in rotted tubers of yam production systems, especially in Nigeria and other West African countries, and is a cause of dry rot, vascular discoloration, and considerable decreases in tuber quality and market value.

Macroscopically, *F. oxysporum* colonies on potato dextrose agar (PDA) are cottony to floccose and have a range of colours from white to pinkish, purple, or violet colour, often acquiring a characteristic reddish-purple colour on the reverse side. The aerial growth is medium-fast at 25-30°C with the formation of aerial mycelium and having high amounts of macroconidia after

5-7 days. The fungus is microscopically characterized by sickle-shaped (fusiform) macroconidia, which are septate (3-5 septa), curved, and tapered to pointed ends, 25-50 mm in length. Root infections in the field are often the source of infection by *F. oxysporum* in the tubers of yams and the fungus enters the root system and spreads systemically via the vascular tissues. The pathogen spreads within the storage period resulting in vascular wilting and typical internal browning or streaking. The rot occurs as dry rot: the lesions of the rot are hard, dry and discoloured (brown to reddish-brown), and the external lesions are sunken, and the tuber gradually becomes smaller. In some extreme cases, the rot can develop into cavities or hollow spaces which cause significant weight loss and loss of the integrity of the tuber (Dania *et al.*, 2019). *F. oxysporum*, unlike soft rot pathogens, does not lead to rapid tissue liquefaction but causes slow desiccation and necrosis and the rot is more insidious and difficult to notice at an early stage. The ability of *F. oxysporum* to generate extensive repertoires of cell-wall-degrading enzymes, which include pectinases, cellulases, and xylanases that aid in penetration and acquisition of nutrients, increases its pathogenicity. The fungus also releases phytotoxic metabolites, which lead to the death of tissues and dysfunction of the vascularity. *F. oxysporum* is always ranked as one of the most common species in the presence of many types of tubers in Nigerian yam storage systems, with a consistent proportion of 15-30% in numerous surveys in Benue, Nasarawa, Anambra, and Enugu state. Being soil-borne and long-lasting as the chlamydospores that it produces, the pathogen causes a recurrent threat, particularly where infected plant material is employed or where the tubers are kept in an environment conducive to slow yet progressive putrefaction. *F. oxysporum* may remain silent in the production of mycotoxins including the aflatoxins and fumonisins in the tubers of yams; however, this capacity of the pathogen to induce systemic infection and progressive tissue degradation renders it a major limitation to post-harvest storage and usage (Dania *et al.*, 2019). The prevalence and persistence of *F. oxysporum* in yam-producing and storage systems have

prompted the consideration of integrated management control methods such as the application of planting materials free of inoculum, crop rotation, soil solarization, and better storage methods to minimize inoculum counts and delay disease development. In managing the losses associated with the production of yams, it is necessary to control this pathogen effectively to ensure the sustainability of production by the resource-constrained tropical farming societies.

2.4 Fungal Agents Associated with Potato Rot

Potato tubers are susceptible to a number of harmful fungal diseases, many of which have distinct symptoms and patterns of disease progression that impact both the field and storage stages.

2.4.1 *Phytophthora infestans*

Phytophthora infestans, the causative agent of the late blight, produces branched sporangiophores that have lemon-shaped sporangia. It results in dark, wet lesions on the leaves, stems and tubers which rapidly become brown-black. Infected tubers in storage have solid discolored rots, which later become soft and are often followed by white sporulation in humid conditions. Under good conditions, the spread of the late blight is rapid resulting in massive tissue decay and consequential bacterial infection. One of the most notorious oomycete pathogens is *Phytophthora infestans*, the cause of potato (*Solanum tuberosum* L.) and other solanaceous crops, such as tomato (*Solanum lycopersicum* L.) and eggplant (*Solanum melongena* L.): the pathogen causes late blight in potato and other plants. The historical meaning of this pathogen is significant, as it is the cause of the Irish potato famine in the middle of the 1840s that resulted in extensive starvation, migration, and social disintegration. The disaster was a central event in the history of modern plant pathology as a field as it shaped initial ideas of how plant diseases could be caused and the creation of fungicides (Yuen, 2021). *P. infestans* remains one of the most significant risks to potato production in modern

agriculture, and it can result in disastrous losses until it is properly managed, making it a valuable system to study the interaction between hosts and pathogens, resistance phenomena and large-scale disease prediction. *P. infestans* causes white, cottony sporulation on the surface of the infected tissues in a high level of humidity, which is frequently presented as a halo of the lesion of the leaves and stems in the form of a mildew. It causes dark, irregular, water-soaked lesions on potato tubers which can be superficial at first but eventually deepen and cause firm, reddish-brown internal rot. The pathogen has coenocytic hyphae and branched sporangiophores that sprout through stomata and have lemon-shaped and papillate sporangia (25-50 mm long) (microscopic). These sporangia may either germinate directly through germ tubes or produce 8-12 motile biflagellate zoospores under cool wet conditions so that secondary infections may be achieved quickly. Both of the mentioned mating types together form the oospores, which are thick-walled structures that allow the long-term existence of the genus in the soil or plant debris (Yuen, 2021). Its infection normally begins on the foliage in the growing season, with the sporangia being dispersed by wind, splash, or irrigation water. The pathogen has a lengthy period of wetness of the leaf necessary to germinate (a minimum of 10 hours at 10-18 °C) and penetrate the plant via stomata or direct infection on the epidermis. The fungus spreads systemically in the form of stolons or soilborne sporangia washed away during heavy rain, on the leaves and on stems and tubers. In tubers, infection leads to brown rot and external signs of infection appear as irregular, purplish-black lesions that sink and wrinkle and internal tissues appear reddish-brown and firm and subsequently turn soft. The rot in storage takes time, although it may accelerate when secondary bacteria invade the tuber causing foul odours and total degeneration (Azil *et al.*, 2021).

2.4.2 *Alternaria solani*

The major causal pathogen of early blight disease in potato (*Solanum tuberosum*) is *Alternaria solani*, a necrotrophic fungus that has a profound effect on the quality of foliage and tuber

during the growing season and later storage. The fungi are members of the genus *Alternaria*, a family of dematiaceous (dark-spored) fungi commonly present in agricultural settings and that cause leaf spots, blights and tuber rots in a wide range of crops. One of the most widespread and economically significant potato diseases in the world, especially in warm and humid temperatures, is early blight which can lead to significant defoliation and yield losses. On potato dextrose agar (PDA), *A. solani* colonies are dark olive-green to black in color, velvety to woolly, possess concentric growth rings, and have an excessive amount of aerial mycelium. Colonies have a typical musty smell and grow to 30-50 mm in diameter in 7 days at 25-30 °C. The fungus is microscopically characterized by large, muriform, conidia (multicellular with transverse and longitudinal septa), which are obclavate to obpyriform, tapered at the apex, and possess a beak-like extension. The conidia are single or in brief chains on short unbranched conidiophores of dark septate hyphae. The conidia are 120-296 mm in length, 12-20 mm in width with 9-11 transverse septa and several longitudinal septa. Infection by *A. solani* normally starts at lower, older leaves during the growing season whereby conidia are dispersed by wind, splash of rain, and overhead irrigation. Germination and penetration of the pathogen by the free moisture on the leaf surface are optimal at 25-30 °C and wetness of the leaf for up to 6-12 hours. Small dark spots become the first symptoms which spread out to typical target-like lesions with concentric rings of necrotic tissue and yellow halos. Lesions then combine and result in massive defoliation and early death of plants as the disease advances. Based on the foliage, the fungus expands to stems and petioles, and conidia are also carried away to infect tubers, especially via wounds, lenticels, or stolons. *A. solani* causes sunken and corky rots in potato tubers, which develop as irregular lesions with a leathery and rough surface that is black or dark brown in color. The rot is usually dry and firm with internal tissues being brown, discolored and corky. The affected regions can drag and become wrinkled, causing bullheaded symptoms of dry rot that cause a decrease in tuber weight, marketability, and processing quality

(i.e., chips or fries). In comparison to late blight rot, early blight tuber rot develops gradually without generally being accompanied by any extensive softening or secondary invasion by bacteria unless the storage conditions are too humid (Azil *et al.*, 2021). Disease gets an outlet in storage whereby infected tubers can be used as an inoculum into the next season as seed material, thus continuing the cycle of the disease. The cost of *A. solani* to the economy is high, especially in areas where the growing seasons are warm and wet, and the loss of early blight can be seen as 20-50 percent of the yield due to defoliation and smaller tubers. The pathogen also contributes to food insecurity in developing countries, such as some parts of Africa and Asia by reducing marketable yield and quality, which in many cases requires frequent fungicide application that raises production costs among the smallholder farmers. Even though aflatoxin and patulin are not some of the major mycotoxins produced by *A. solani*, it causes tuber blemishes and internal discoloration hence rejection in the fresh markets and the processing industries.

2.4.3 *Fusarium* species

The species in the genus *Fusarium*, especially *F. sambucinum* and *F. solani*, are important causes of dry rot in potato tubers. Such fungi generate chlamydospores and macroconidia. They result in dry rot accompanied by a concentric area of withered tissue and masses of coal-like spores and finally hollowing (Azil *et al.*, 2021; Tiwari *et al.*, 2020). *Fusarium* species are widespread soil-borne fungi, which attack tubers with either wounds or injuries causing internal necrosis and massive losses post-harvest. Macroscopically, *Fusarium* colonies growing on potato dextrose agar (PDA) appear cottony to woolly with colours that vary between white, pink, purple, or orange, and a pigment diffusing into the medium. It grows fast at 25-30 °C and is excessively prolific in aerial mycelium and sporodochia (spore-bearing structures). *F. solani* and *F. sambucinum* are microscopically indistinguishable, except that the curved, septate macroconidia (3-5 septa, 25-60mm long) have a foot-like cell of the basal cell and a pointed

cell of the apical cell, produced in slimy masses on sporodochia. Microconidia are numerous and oval or reniform and carried on elongated monophialides. The chlamydospores are thick-walled, globose and single or in chains, which allows the survivors to survive in soil and tuber debris long-term (Jahan, 2020). *Fusarium* species infection is usually caused by mechanical injuries done during harvesting, handling, or storage, because *Fusarium* does not penetrate intact periderm. The injured tissue is colonized by hyphae, which produce enzymes that destroy cell walls and lead to desiccation of the tissues. The rot starts off as pepper-like, small, and sunken areas that grow to concentric rings of wrinkled and dried tissue with white to pinkish mycelial proliferation. The further development of the disease is caused by internal cavities filled with the powdery spores of necrotic debris. The symptoms are also worse in tubers kept at temperature levels exceeding 15 °C and low humidity because these conditions favour the saprophytic growth of the pathogen (Tiwari *et al.*, 2020; Jahan, 2020). Pathogenicity The pathogen produces fumonisins, a family of mycotoxins that interfere with the sphingolipid metabolism which have been associated with esophageal cancer and neural tube defects, contributing to the health hazards of the pathogen. Fumonisin concentrations rise as dry rot develops in stored tubers and contaminates processed foods and causes chronic exposure in the event of tuber dependency diets (Tiwari *et al.*, 2020; Okafor and Eni, 2018). *Fusarium* spp. is commonly found on rotted tubers in Nigerian yam and potato systems, and in surveys in states such as Benue and Nasarawa, could often take 10-25 percent of pathogens (Gwa and Akombo, 2016). The economic effect involves lowering of tuber weight, market rejection as well as loss of seed quality and this aggravates food insecurity in developing areas. Treatment includes reduction of the wound, curing at 15-20 °C in 10-14 days to facilitate suberization and fungicides such as thiabendazole. Antagonist treatment of infection with biological control using *Trichoderma* spp. has potential (Jahan, 2020).

2.5 Factors Promoting Fungal Growth in Tubers

In stored yams and potato tubers, a number of biotic and abiotic variables work in concert to encourage fungal growth and subsequent spoiling. These elements promote mycelia growth, spore germination and the enzymatic breakdown of tuber tissues which eventually speeds up post-harvest losses.

2.5.1 High moisture content and relative humidity

Among the most important causes of fungal infection and degradation are the tubers' high moisture content and the storage environment's high relative humidity. For fungal spore germination and subsequent hyphal expansion, tubers with internal moisture contents greater than 65% - 70% offer the perfect watery media. In addition to maintaining spore viability, relative humidity levels of 75% - 85% dramatically increase enzymatic activity, including pectinases and cellulases released by pathogens, resulting in rapid tissue maceration and breakdown (Sameza *et al.*, 2016). Long-term exposure to such humid circumstances accelerates the onset of soft rot in traditional yam barns seen in Nigeria and other tropical countries, especially for diseases like *Rhizopus stolonifer* and *Mucor* spp., leading to full tuber liquefaction in a matter of days to weeks.

2.5.2 Mechanical injuries

Fungal spores and mycelia primarily enter through mechanical injuries sustained during harvesting, transit, handling or storage. When the tubers' protective periderm layer is compromised by wounds like cuts from sharp objects, bruises from rough handling or piercing from spear grass (*Imperata cylindrica*) rhizomes, opportunistic pathogens can enter the tuber directly. Once these wounds are broken, fungi including *Aspergillus flavus*, *Fusarium oxysporum* and *Rhizopus stolonifer*, can quickly colonize the injured tissues and start the rot process (Aidoo *et al.*, 2020). Such injuries greatly raise infection rates in Nigerian storage

systems, where hand harvesting and transportation are typical. These injuries frequently result in isolated lesions that eventually spread into large dry or soft rots. The size and depth of the lesion directly affect how severe the rot is; deeper wounds allow for faster interior spreads and more tissue loss.

2.5.3 Poor ventilation in traditional storage systems

Weak ventilation in traditional storage structures including yam barns, heaps or the tightly stacked market stalls is a key factor and greatly contributes to fungal proliferation and the rapid post-harvest rot of yam (*Dioscorea rotundata* Poir.) and potato (*Solanum tuberosum* L.) tubers. Poor air circulation conserves respiratory heat and moisture used by the tubers resulting in a localized area of high relative humidity (usually more than 85-90%) and low oxygen concentration. These low-oxygen, high-moisture microenvironments provide excellent conditions to support the growth of soft rot pathogenic microbes, especially facultative anaerobes and fungi like *Rhizopus stolonifer*, *Mucor* spp., and some species of *Fusarium* which thrive in these types of anaerobic environments. Traditional yam barns often have large piles, heaps, or boxy dwellings in the rural regions of Nigeria and all over the West African continent, which contain tens of thousands of tubers, often stacked tightly together, creating a restricted airflow. This design offers some level of shading and protection against direct sunlight, but highly limits natural ventilation, and this results in condensation of tuber surfaces and extended exposure to high humidity. The following wet environment boosts enzymatic reactions by soft rot pathogens that secrete pectinases and cellulases that break down cell walls and cause tissue maceration, watery decay, unattractive smells, and complete liquefaction of tubers in the course of a few days to weeks (Dania *et al.*, 2019). Nigerian research on storage systems has revealed that when the barns are poorly ventilated, they may lead to losses of 30-50 percent in the first three months of the storage, and in such situations, the soft rot pathogens prevail. The issue is only enhanced with the closely-packed piles or heaps of yams where internal heat due to

respiration is not allowed to disperse causing temperature gradients resulting in an additional rise in humidity and condensation. In this type of system, the outer tubers are relatively dry, whereas the inner layers are subjected to anaerobic stress, preferring the growth of *Rhizopus* and *Mucor* species, which have become adapted to low-oxygen conditions. These pathogens generate vast aerial masses (mycelia and sporangia) that cause the release of huge amounts of airborne spores that grow quickly in the storage facility and cause secondary infections on other tubers. The lack of ventilation would also increase the length of the fungal propagules on storage surfaces, tools, and debris, thus acting as a continuous source of inoculum to follow-up batches of tubers. The same happens in traditional potato storage where clamps, pits or poorly ventilated sheds are used in areas where there are no modern cold storage facilities. The resulting moisture and carbon dioxide traps sprouts; the low levels of carbon dioxide encourage soft rot pathogens, like *Erwinia* spp. (bacteria) and fungus, like *Rhizopus* and *Fusarium*, resulting in rapid decay in wet areas (Tiwari *et al.*, 2020). The poor ventilation of the storage facilities in developing countries where the majority of potato and yam storage systems depend on ambient conditions is one of the primary causes of post-harvest losses estimated at 20-50% in the world (Abewoy, 2018). The excessive moisture can be greatly reduced by means of improved air circulation by a few simple adjustments: raised platforms, perforated floors, increased spacing between tubers, or natural opening of the air to the outside etc. Research has shown that ventilation in yam barns can decrease cases of soft rot by 40-60 percent and increase the storage time by several months. These minimalist interventions are more needed in rural African societies that are resource-constrained yet they still stick to their traditional means of storing goods despite their security shortcomings. The overwhelming contribution of poor ventilation to the growth of fungal rot is a matter that needs more understanding and implementation of better storage designs. Even non-complex structural modifications coupled with effective food handling

practices can help to greatly reduce the losses and improve food security in areas where tuber is a major threat.

2.5.4 Contaminated surfaces, tools and materials

Fungal spores can spread from infected to healthy tubers through contaminated surfaces, tools and materials, starting a new infection cycle. Spores and mycelial fragments from previously sick tubers are frequently found on harvesting knives, baskets, transport vehicles and even the floors or walls of storage barns. Pathogens such as *Aspergillus niger*, *Penicillium* spp., and *Fusarium* spp. are easily transferred when these contaminated materials come into contact with healthy tubers especially those with slight wounds, resulting in cross-contamination and extensive rot. This mechanism of transmission greatly accelerates the spread of storage rots, especially during the peak harvest period in traditional Nigerian markets and farms where cleanliness controls may be inadequate.

2.5.5 Storage temperature

Fungal metabolic activity and sporulation rate are significantly influenced by storage temperature. The majority of storage fungi linked to yams and potato tubers, such as *Aspergillus*, *Penicillium*, *Rhizopus* and *Fusarium* species, have been found to thrive best at temperatures between 25 and 30 °C. Mycelial development and sporulation happen quickly within this region, allowing infections to quickly infiltrate injured tissues and cause widespread rot symptoms (Pastukhov *et al.*, 2021). Temperature variation, especially in uncontrolled storage settings, results in condensation on tuber surfaces which raises local humidity and creates the perfect microclimates for spore germination and infection. Temperature control is essential for reducing fungal growth in tropical areas like Nigeria, where ambient temperature regularly falls within or surpasses this ideal range during storage.

2.6 Mycotoxins Produced by Storage Fungi

A range of mycotoxins are toxic secondary metabolites that contaminate produce and pose a major danger to human and animal health which are produced by storage fungi linked to potato and yam tubers. These substances frequently endure in processed goods like flour, chips or cooked tubers even after fungal growth is stopped.

2.6.1 Aflatoxins

Among the most powerful and extensively researched mycotoxins produced by storage fungi in tubers are aflatoxins especially aflatoxin B1. *Aspergillus flavus* and *Aspergillus niger*, two of the most common infections found in rotten yam tubers in tropical storage systems, are the main producers of them. International health authorities consider aflatoxin B1 to be a strong carcinogen and a highly hepatotoxic substance that damages the liver. Additionally, it has immunosuppressive properties that make people more vulnerable to infections and other illnesses. Aflatoxins build up in tainted yam tubers when they are stored for an extended period of time in warm, humid circumstances, making the fruit unhealthy to eat even if there isn't much obvious rot (Okafor and Eni, 2018).

2.6.2 Ochratoxins

Another class of mycotoxins frequently linked to stored tubers are ochratoxins which are primarily generated by specific species of *Aspergillus* and *Penicillium*. These substances have been linked to possible teratogenic and carcinogenic effects in addition to causing kidney damage (nephrotoxicity). When conditions are favorable for mycelial growth and toxin production, *Penicillium* species including *Penicillium expansum* and *Penicillium purpurogenum*, as well as some *Aspergillus* strains, create ochratoxins in the yam and potato storage environment. The toxins build up in the tubers' tissues especially in dry rot lesion and can spread to processed goods, endangering consumers' long-term health. Ochratoxins are

especially dangerous in areas with little food safety monitoring because of their stability throughout processing and heating.

2.6.3 Fumonisin

Mycotoxins known as fumonisins are mostly produced by *Fusarium* species such as *Fusarium oxysporum*, *Fusarium sambucinum* and *Fusarium solani* which are commonly isolated from rotten potato and yam tubers. By blocking ceramide synthase, these substances interfere with the metabolism of sphingolipids, causing tissue injury and cellular malfunction. Neuronal tube abnormalities in animal models and esophageal cancer in people have been closely associated with fumonisins. Fumonisin synthesis in stored tubers contaminates both fresh and processed goods as dry rot progresses, particularly in warm temperatures and moderate moisture (Tiwari *et al.*, 2020).

2.7 Control and Prevention of Fungal Rots

Chemical, biological and cultural methods must be combined for fungal rots to be effectively managed. Synthetic fungicides like mancozeb which offer 100% inhibition in vitro but carry concerns of resistance development, environmental pollution and toxicity to non-target organisms, are used in chemical control (Dania *et al.*, 2019). Antagonistic microorganisms are used in biological control. Through competition, antibiosis and mycoparasitism, *Trichoderma harzianum* suppresses pathogens' growth by up to 69%. Although their effectiveness vary depending on the environment, bacterial agents like *Bacillus subtilis* generate antifungal chemicals. Sustainability and low toxicity are benefits; slower action and sensitivity to abiotic variables are drawbacks (Mabou *et al.*, 2020). Curing, ventilation and temperature control are examples of better storage techniques that lower moisture and prevent growth (Abewoy, 2018). As preventive methods, sorting and cautious handling eliminate diseased tubers and reduce

wounds. The most effective and long-lasting control is provided by integrated solutions that combine these methods (Aidoo *et al.*, 2020).

2.8 Management and Control of Yam and Potato

For fungal rots in potatoes and yams to be sustainably controlled, an integrated disease management (IDM) strategy is necessary. Crop rotation, disease-free planting materials and resistant cultivars are examples of cultural techniques that lower initial inoculum levels (Mignouna *et al.*, 2021). Chemical interventions offer quick suppression but in order to postpone resistance, they should be used sparingly and alternately. During storage, post-harvest procedures include curing, appropriate ventilation, temperature control and the use of natural extracts to stop the spread of disease (Abewoy, 2018).

2.9 Economic Consequences of Fungal Rots in Nigeria.

Dioscorea rotundata and *Solanum tuberosum* tubers cause significant economic losses in Nigerian agriculture depending on these products are the core of food security, nutrition, livelihoods and food supply chains at the national level. The economic losses caused by fungal pathogens are estimated at 20 to 50% in stored yams in Nigeria, which amounts to over \$500 million each year as a result of the lower market share, loss of seed quality, waste and higher transportation costs to replace fungal-infested tubers. Production of potatoes, which is not as massive as that of yams, also encounters issues with ambient storage systems, which entail losses of 30 to 40% to shortages in supply, which drives up market prices by 20 to 30% during off-seasons and during peak demand (Wang and Wang, 2019). Such losses go beyond direct financial costs to influence the economy of the rural areas where trading of yams and potatoes supports livelihood in harvesting, transportation, processing and marketing. Fungal rots in key production regions like Cross River, Benue, Nasarawa, and Enugu state lower household income because they restrict the potential of exporting products, destabilize domestic markets,

and cause poverty cycles amongst smallholder farmers who use the products as a subsistence crop and source of cash earnings (Mignouna *et al.*, 2021). Economic consequences are further enhanced by mycotoxin contamination, especially aflatoxins of *Aspergillus flavus* which makes contaminated yam flour or chips unsafe to consume causing market rejection, health-related costs, and long-term costs to the health of the population due to liver disease and immunosuppression induced by tuber dependence (Okafor and Eni, 2018; Gwa *et al.*, 2018). On the national level, these losses are the source of food insecurity and greater reliance on imports of staple foods which affect the foreign exchange reserves and farming budgets. Since the year 2020-2023, the annual production of yam in Nigeria was estimated to be 38-40 million metric tonnes, which means that the fungal rots and other post-harvest losses decreased the effective availability of yam by up to 40 percent, which increases the seasonal price volatility and causes households with low income to have a lower access to produce (FAO, 2022). Equally, storage losses of potatoes jeopardize the intensification of production as a diversification crop on highland regions to benefit dietary variety and income production. These effects can be reduced through integrated management strategies, which may save 15-25 percent of the production value due to the reduction of rot incidence and longer shelf life, such as an improved approach to ventilating storage facilities, a biological control using *Trichoderma harzianum* and a natural plant extract (Abewoy, 2018). It is thus necessary to address the economic impacts of fungal rots to attain sustainable production of tubers, improve rural livelihoods and food security in Nigeria.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site and Location

The study was done in the Microbiology Laboratory of Godfrey Okoye University, Enugu, Nigeria. The samples of diseased Yams (*Dioscorea rotundata* Poir.) and Potatoes (*Solanum tuberosum* L.) tubers were processed in the microbiology laboratory of Godfrey Okoye University, Thinkers Corner, Enugu, Enugu East Local Government Area.

3.1.1 Sample collection

Sample collection was done according to the method of Gwa and Nwankiti (2018). A total of five diseased yam tubers and five diseased potato tubers with evident symptoms of rot (dry, soft, or wet rot) were randomly picked in the various stalls in Abakpa Market. The samples were put in sterile polyethylene bags, labeled with the type of crop and taken straight to the lab to process within 2 hours after collection.

3.2 Preparation of Culture Media

According to the manufacturer's instructions, a total of 15.6g of Potato Dextrose Agar was dissolved in 400 ml of distilled water. The mixture was stirred until all dissolved. The flask was plugged with cotton wool and sterilized in an autoclave for 20 minutes to sterilize it. The flask was then left to cool down to a temperature of about 45°C after being sterilized. Fifteen (15) mls of the molten medium was poured into 20 sterile Petri dishes and left to solidify at room temperature.

3.3 Isolation of Fungal Pathogens

Two inoculation methods were employed for the isolation of fungal pathogens, which were the swab technique and the cutting technique. For the swab technique, a sterile cotton swab dampened in sterile distilled water was used to swab the surface of the rotten portion of each tuber. The swab was then streaked in a zig-zag pattern on the potato dextrose agar plate. These plates were labelled YS1–YS5 (Yam Swab) and PS1–PS5 (Potato Swab). For the cutting technique, small portions of the rotted tissue were removed using a sterile scalpel. Two small pieces (5 mm) from the advancing margin of the rot were placed on opposite sides of each potato dextrose agar plate. These plates were labelled YC1–YC5 (Yam Cut) and PC1–PC5 (Potato Cut). All plates were covered with aluminum foil to prevent airborne contamination and incubated at room temperature (25°C) for 3-5 days.

3.4 Subculturing of Fungal Isolates

Once visible fungal growth was observed on the 20 Potato Dextrose Agar plates, the representative fungal colonies were sub-cultured into fresh 20 bijou bottles containing Potato Dextrose Agar slants. The inoculating wire loop was flamed until red-hot and left to cool for 10 seconds. A small piece of mycelium was painstakingly removed from the edge of a clean colony on the original plate using the sterile loop and streaked in a zigzag fashion on the slanting Potato Dextrose Agar surface inside the bijou bottle. The sub-cultured bijou bottles were left at room temperature of (37°) in the incubator to further grow and develop pure isolates.

3.5 Morphological Identification of Fungi

The fungal colonies on the original Potato Dextrose Agar plates and the sub-cultured bijou slants were observed macroscopically for colour, texture (powdery, hairlike, wet, etc.), mode of growth and smell. Each of the labelled isolates was carefully observed. Each isolate was

viewed microscopically, and a drop of a tiny piece of mycelium of each isolate was placed on a clean glass slide in a drop of lactophenol cotton blue stain. A cover slip was placed on it and examined under a compound microscope with 10x and 40x magnifications. The hyphal structure, septation, the shape of spores, size, and arrangement were observed and noted.

3.6 Identification of Fungal Isolates using the Slide Culture Technique

The slide culture technique was carried out according to the method of Reiss *et al* (2012) and Agu and Chidozie (2021). Slide culture is a quick technique for making fungal colonies for analysis and identification. Fifteen milliliters of Potato Dextrose Agar was added to a sterile petri dish and was left to solidify. It was sliced into agar blocks with a sterile scalpel and a piece of sterile filter paper was placed at the bottom of five Petri dishes. A sterile glass slide was placed on the filter paper and the setup was raised by adding another sterile glass slide in a cross position on top of the first slide. Each of the five different fungi in the bijou bottles was inoculated on its own agar block using a sterile wire loop. Upon inoculation, the inoculated block was covered by a second sterile glass slide with the purpose of developing a sandwich. The Petri dishes were incubated at room temperature, sealed, and incubated for 3-5 days. The upper glass slide was then removed with sterilized forceps after incubation. A drop of lactophenol cotton blue was put on a grease-free slide. The agar block was dropped in disinfectant and a sterilized cover slip was applied to the slide. This staining was done to improve the visibility of fungal structures and help in identifying the fungal pathogens. Microscopic examination of the glass slides was then done to identify the typical shape and structure of the spores and the hyphae.

3.7 Pathogenicity Test

The identified pure isolates were used for pathogenicity tests on healthy yam and potato tubers to demonstrate Koch's postulates. Surface sterilization was then performed, followed by

wounding with a sterile cork borer (5 mm deep). One yam tuber was inoculated with pure culture and incubated in moist chambers at 25°C for 7-14 days. Only sterile Potato Dextrose Agar blocks were used as control tubers. The Rot growth was tracked and evaluated on a daily basis. Re-isolation of the organism that caused the lesion was conducted to ensure that the advancing margin of the lesion was not caused by other organisms.

CHAPTER FOUR

RESULTS

4.1 Colony Morphology

The total causative organisms obtained from rotten Yams were four (4), while seven (7) organisms were obtained from rotten Potatoes. The appearances of the fungal isolates were shown in figures 2, 3, 4, 5 and 6. **Figure 2:** *Aspergillus fumigatus* showed a discolored greenish-black color and microscopic observation showed septate hyphae that ended in a swollen vesicle and were single-celled phialides. **Figure 3:** *Candida tropicalis*, which exhibited a wet and slimy appearance. Microscopically, it showed oval to elongated budding yeast cells with no true septate hyphae. **Figure 4:** *Chrysomilia sitophila* displayed a white fluffy growth on the tubers and under the microscope, septate hyphae were seen to generate a lot of cylindrical arthrospores that appeared in chains. **Figure 5:** *Aspergillus niger* showed heavy black powdery colonies which were in line with black dry rot and microscopic characteristics were septate hyphae with long conidiophores terminated with large globose vesicles having biserial phialides. **Figure 6:** *Candida krusei* displayed colonies that were moist and with yeast-like features and microscopic observation revealed long budding yeast cells with short pseudohyphae. **Figure 7:** *Aspergillus clavatus*, colonies appeared bluish green with velvety textures. Microscopic view showed septate hyphae with long conidiophores and club-shaped vesicles. **Figure 8:** *Trichophyton soudanense* produced white to cream-coloured colonies with powdery to suede-like texture. Microscopically, septate hyphae with abundant macroconidia and microconidia were seen. **Figure 9:** *Candida utilis* showed creamy-white, smooth and moist colonies. Microscopic examination revealed oval budding yeast cells. **Figure 10:** *Candida famata*, macroscopically, colonies were white to cream, smooth and pasty. It showed round to oval budding yeast cells under the microscope. **Figure 11:** *Trichophyton mentagrophytes*

produced white to cream powdery colonies. Microscopically, septate hyphae with spiral hyphae and numerous microconidia were observed. **Table 1** shows the distribution of fungal isolates in yams and potatoes. The macroscopic and microscopic features of the isolates are shown in Table 2.

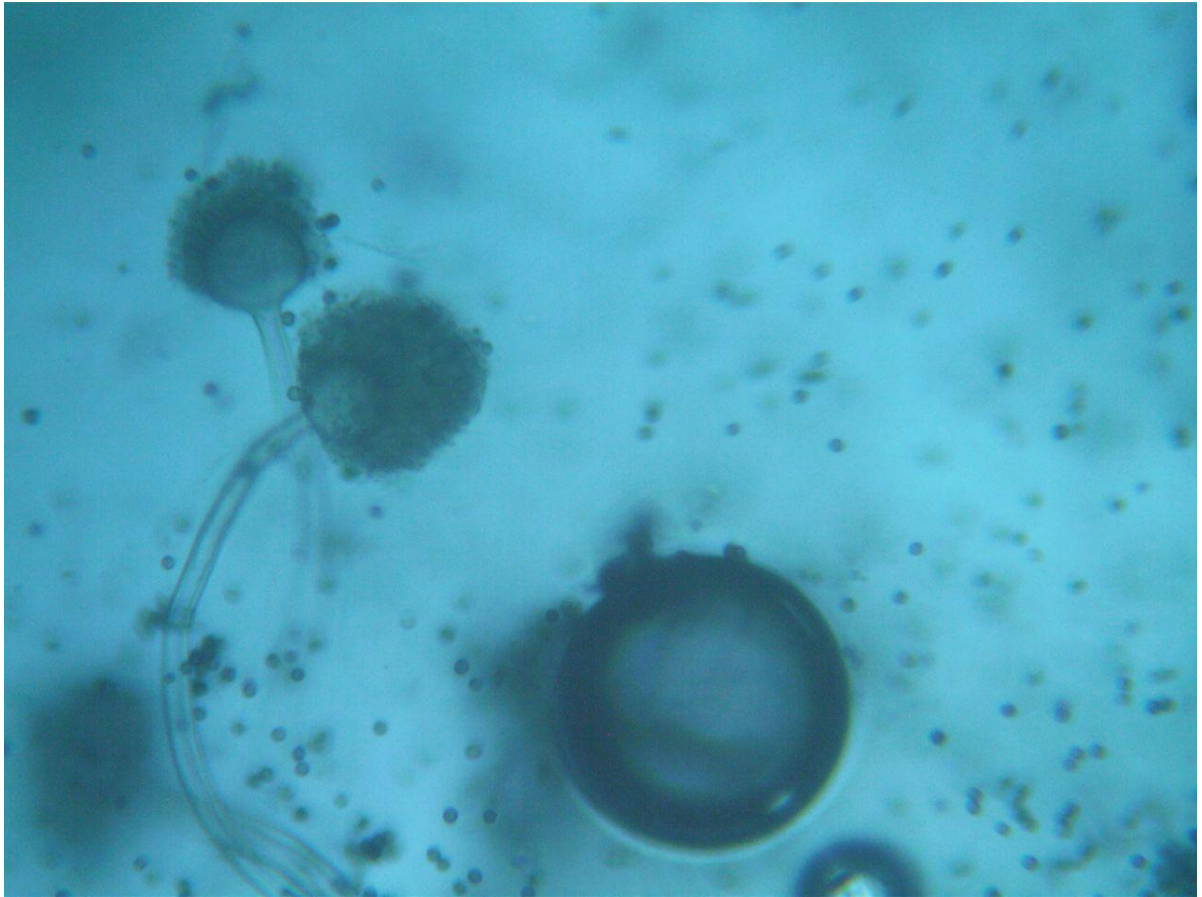


Fig 2: *Aspergillus fumigatus*

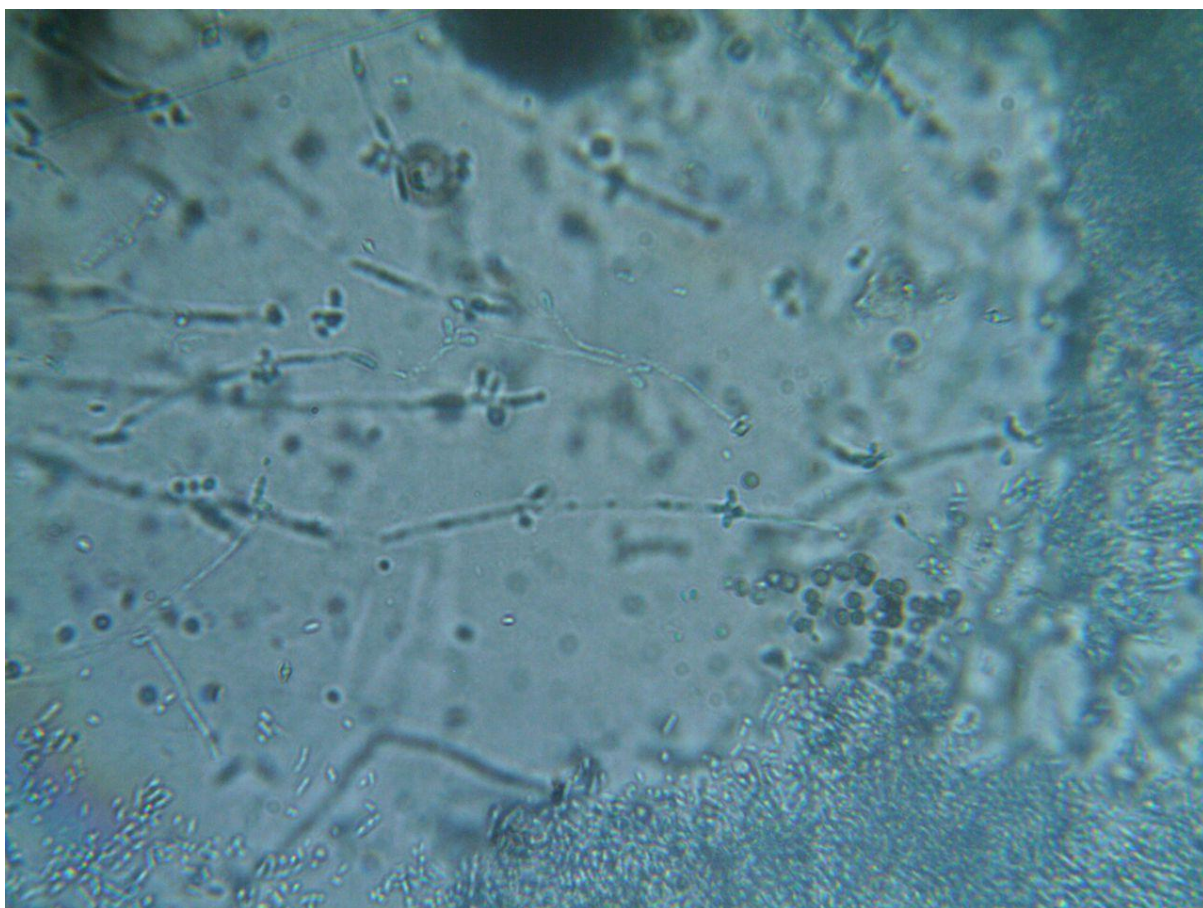


Fig 3: *Candida tropicalis*

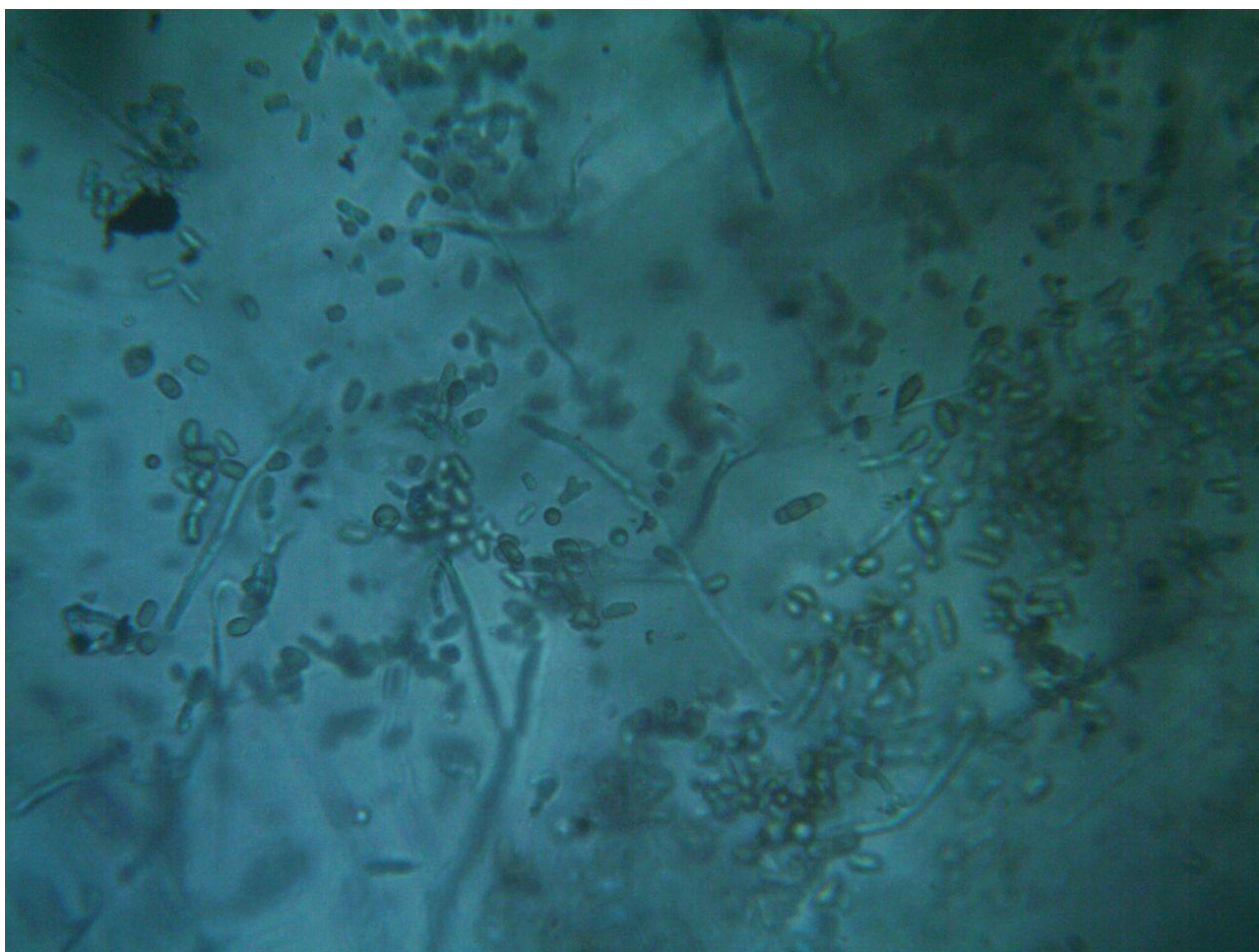


Fig 4: *Chrysonilia sitophila*

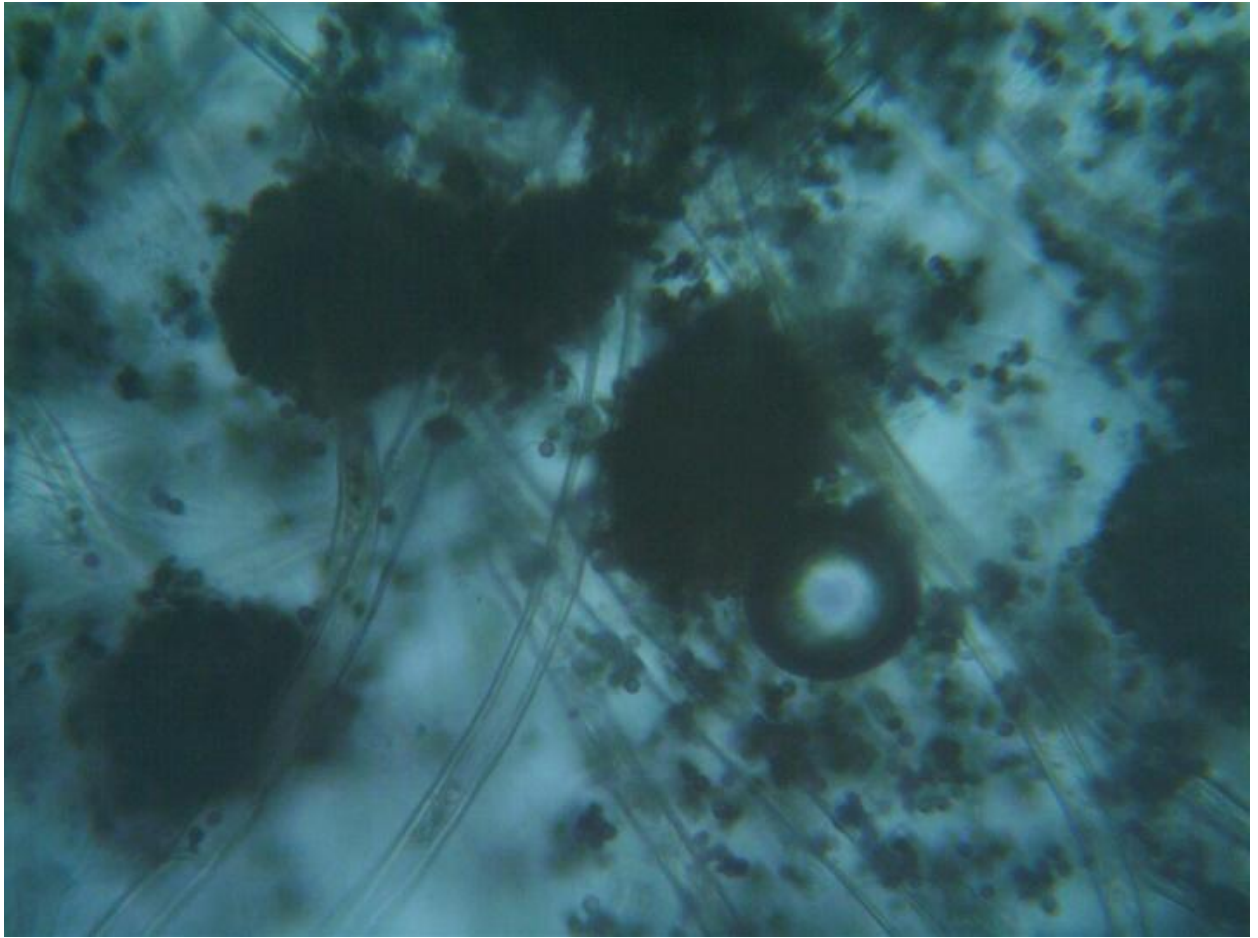


Fig 5: *Aspergillus niger*

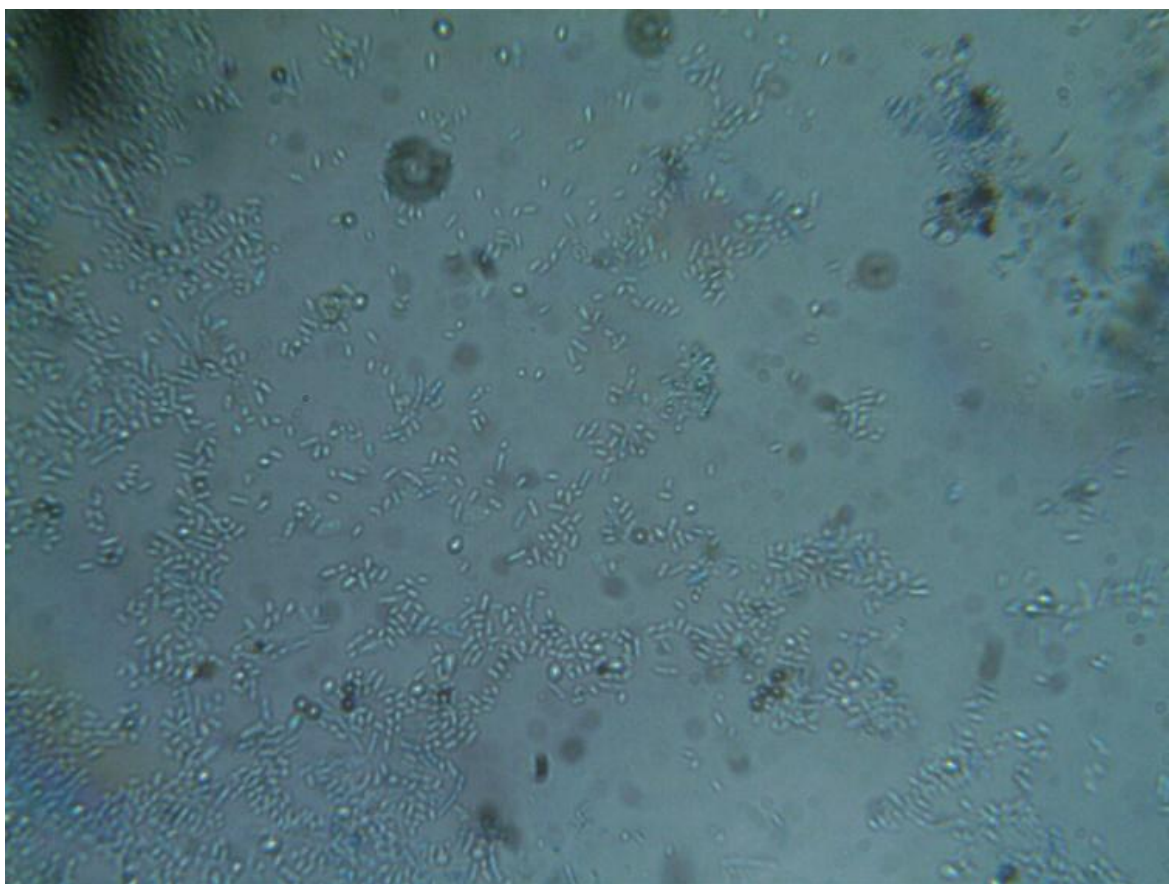


Fig 6: *Candida krusei*

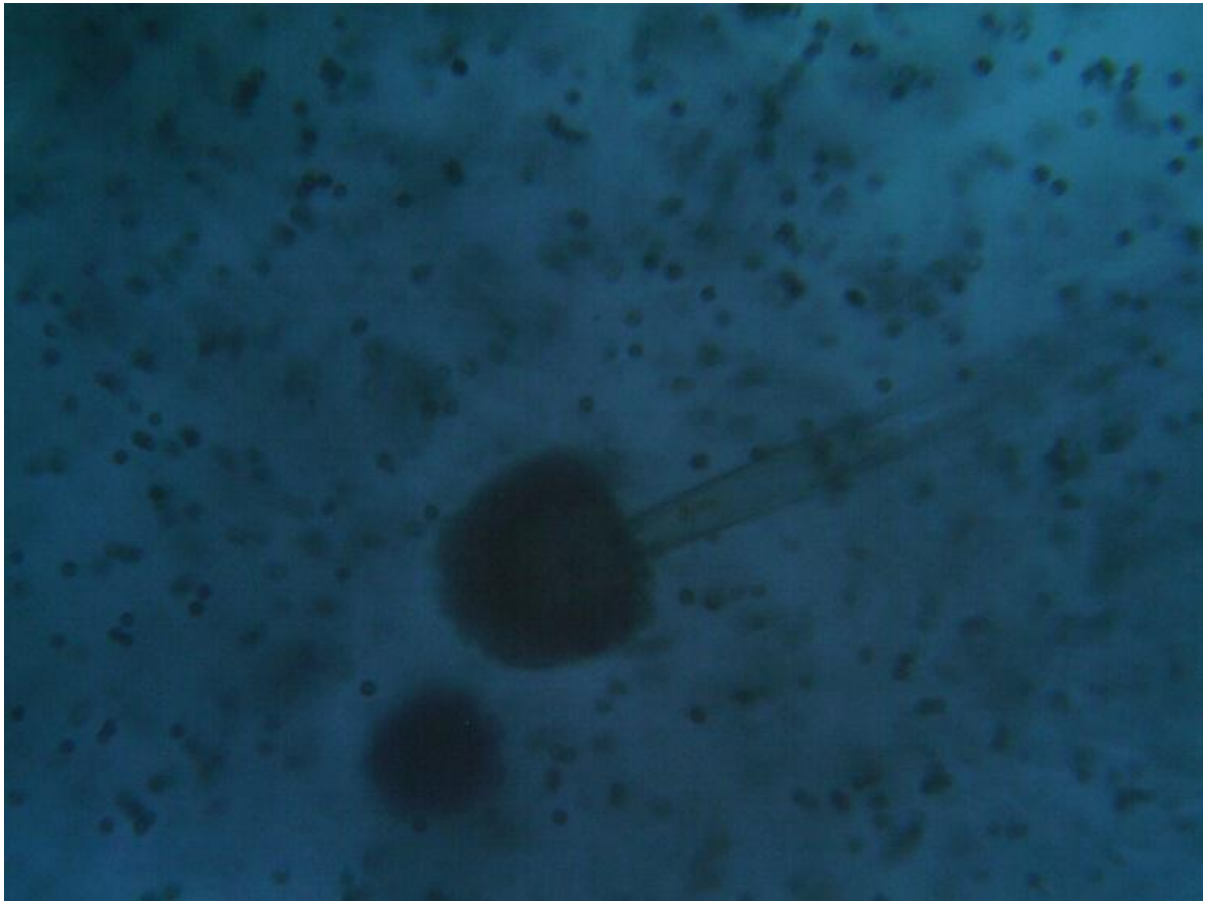


Fig 7: *Aspergillus clavatus*

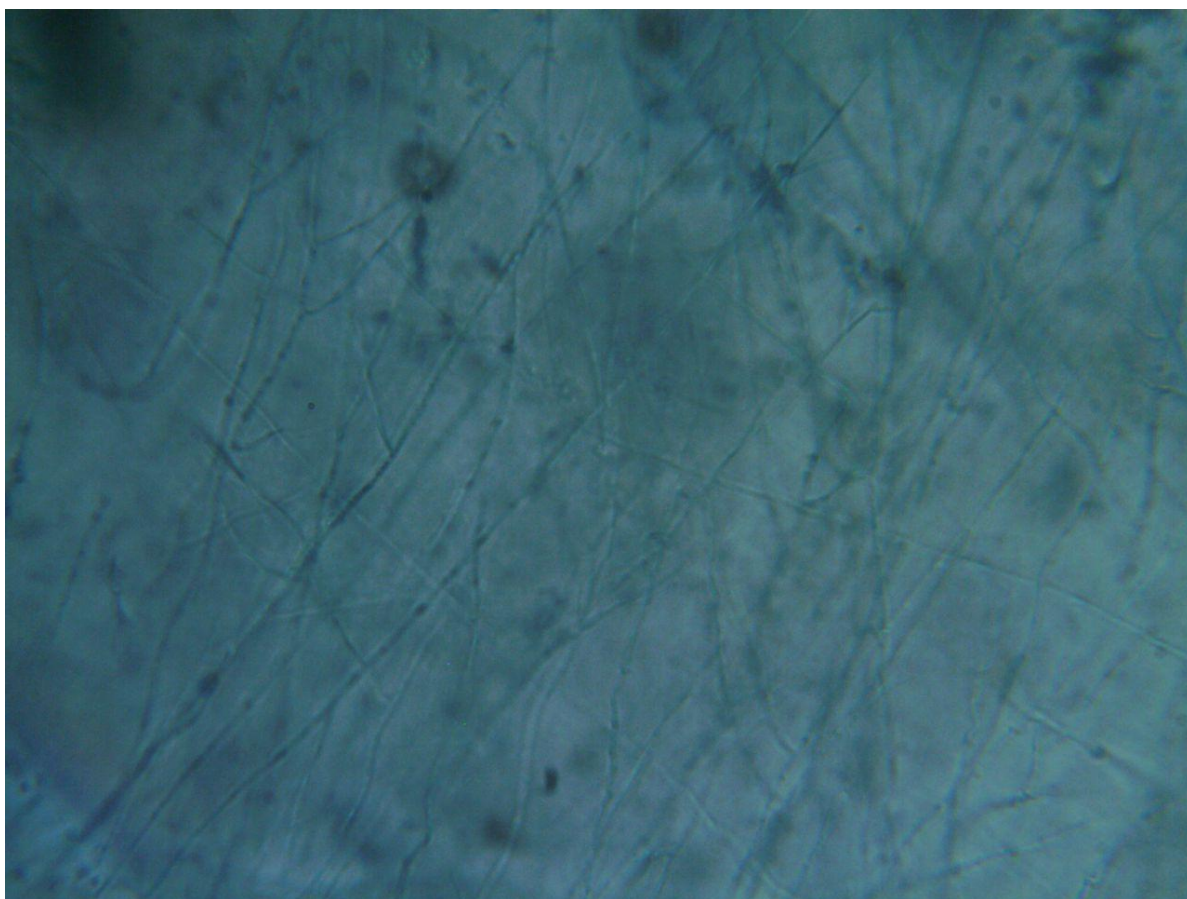


Fig 8: *Trichophyton soudanense*

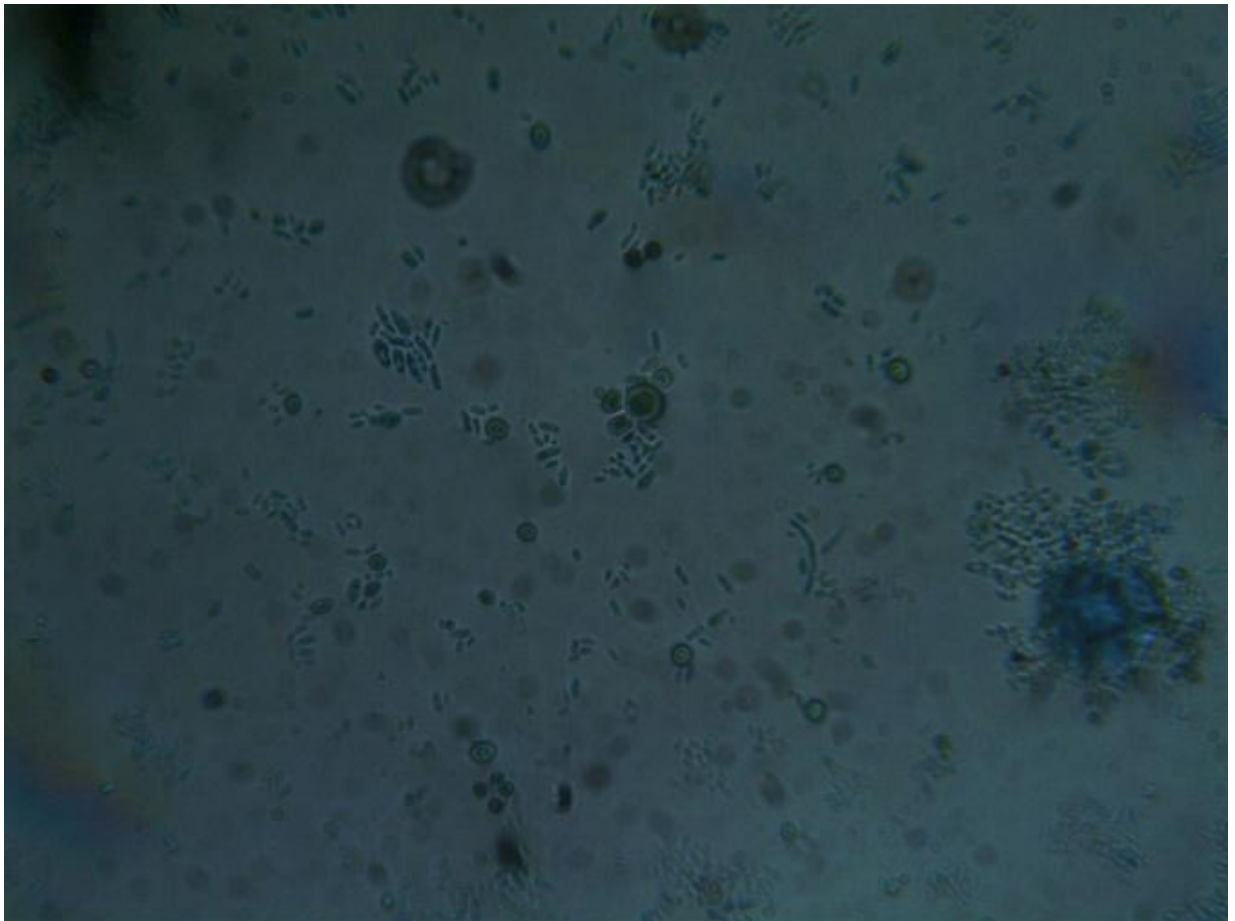


Fig 9: *Candida utilis*

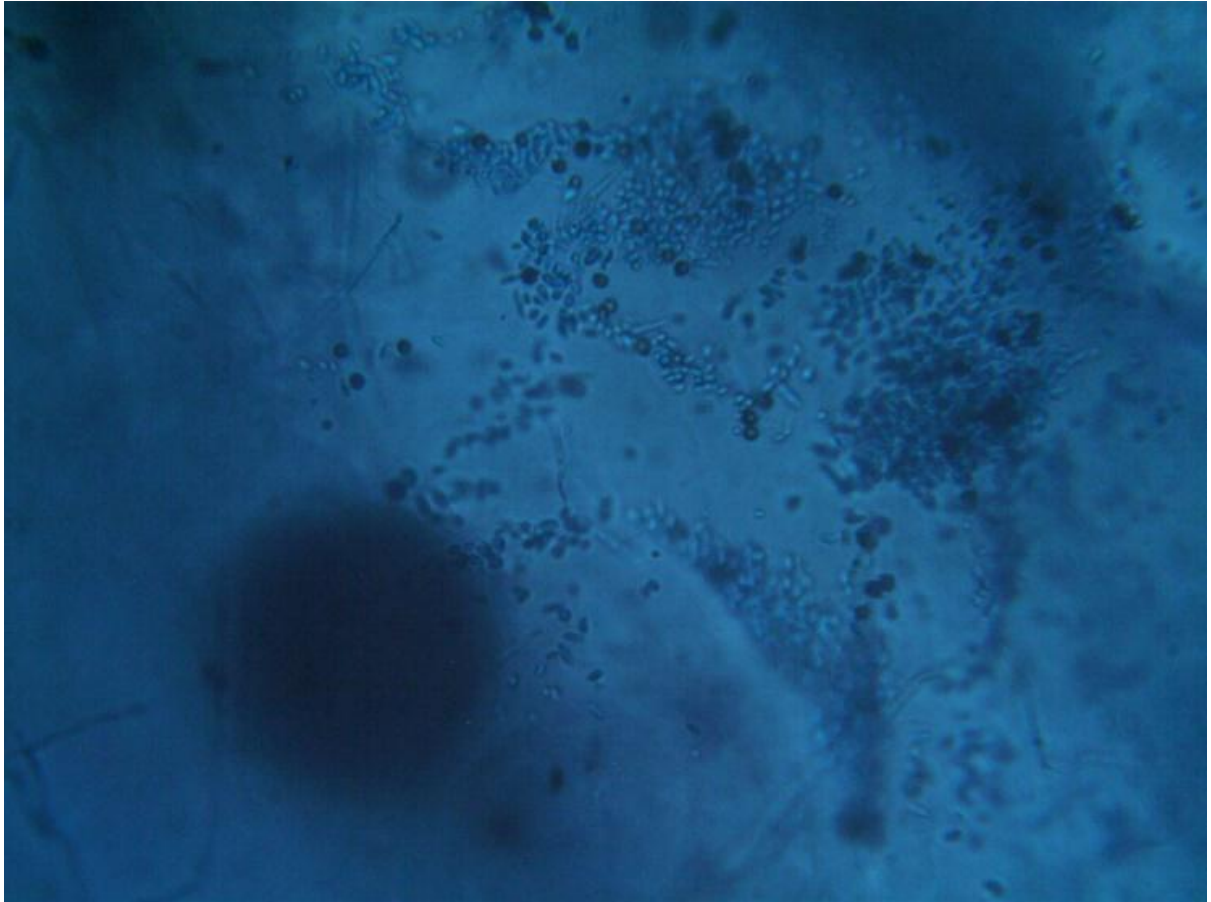


Fig 10: *Candida famata*

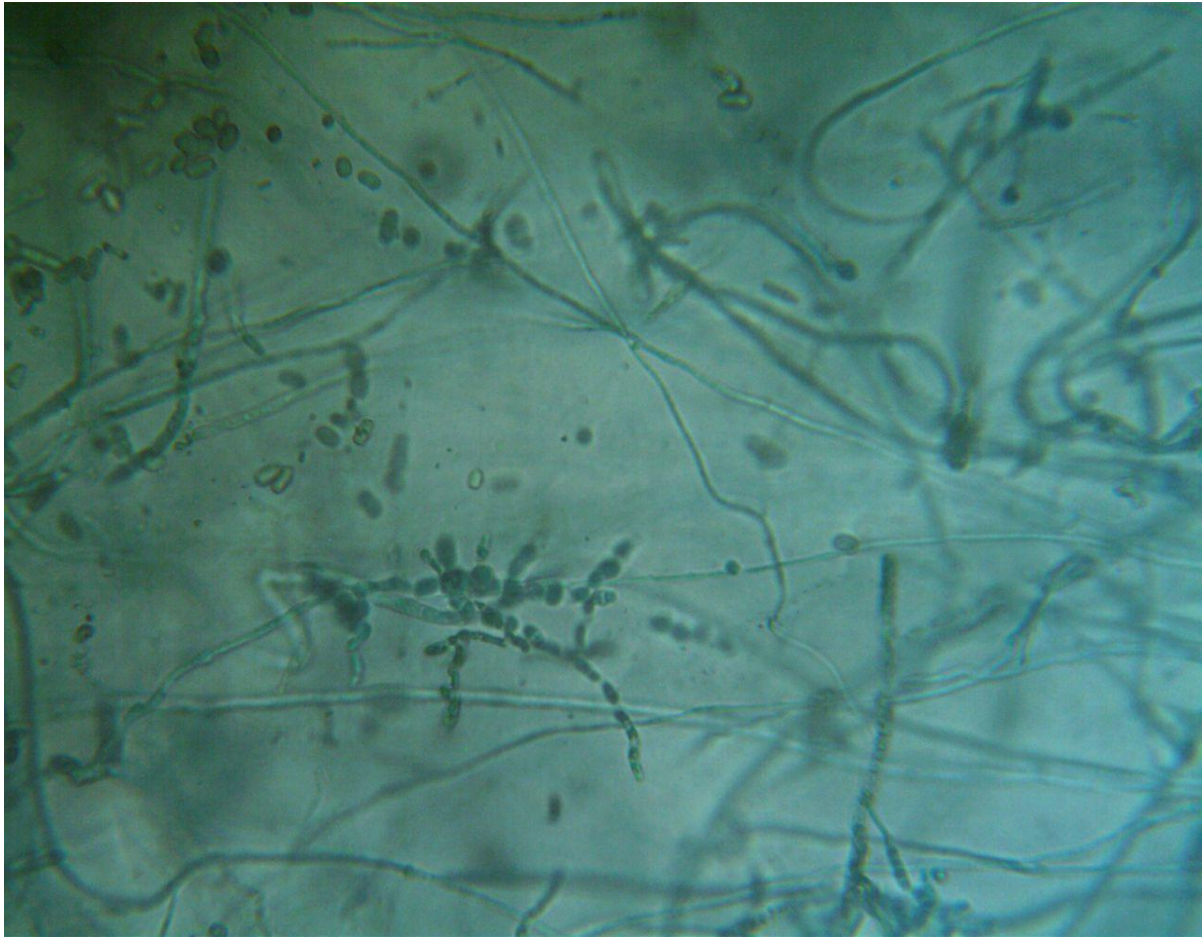


Fig 11: *Trichophyton mentagrophytes*

4.2: The Distribution of the Isolated Fungi

The results presented showed that a total of ten (10) different fungal species were isolated from the rotten yams and potato tubers. Four (4) fungal species were recovered from yam tubers while seven (7) species were isolated from potato tubers. *Aspergillus niger* was the only species isolated from both yams and potato tubers, indicating its wide adaptability and high prevalence across the two crops. The yam tubers harbored mainly *Aspergillus* species (*A. clavatus*, *A. fumigatus*, and *A. niger*) and one dermatophyte (*Trichophyton soudanense*). On the other hand, potato tubers showed higher fungal diversity, dominated by *Candida* species (*C. krusei*, *C. tropicalis*, *C. utilis*, *C. famata*), *Chrysonilia sitophila*, *A. niger* and *Trichophyton mentagrophytes*.

This distribution suggests that potato tubers may be more susceptible to a wider range of fungal contaminants under market conditions compared to yam tubers. The exclusive presence of certain *Candida* species and *Trichophyton* species in potato samples may be linked to higher moisture content or greater surface contamination in potatoes at the time of collection.

Table 4.1: The distribution of the isolated fungi

Fungal isolate	Yam	Potato	total occurrence	percentage
<i>Aspergillus clavatus</i>	+	-	1	10%
<i>Aspergillus fumigatus</i>	+	-	1	10%
<i>Aspergillus niger</i>	+	+	2	20%
<i>Trichophyton soudanense</i>	+	-	1	10%
<i>Candida krusei</i>	-	+	1	10%
<i>Candida tropicalis</i>	-	+	1	10%
<i>Candida utilis</i>	-	+	1	10%
<i>Candida famata</i>	-	+	1	10%
<i>Chrysonilia sitophila</i>	-	+	1	10%
<i>Trichophyton mentagrophytes</i>	-	+	1	10%

Key:

+ = Present

- = Absent

4.3 Morphology and Characteristics of Fungal Isolates

The macroscopic and microscopic morphological characteristics of the ten fungal isolates recovered from rotten yams and potato tubers were shown in Table 2. The isolates showed distinct colonial features ranging from powdery and dry (*Aspergillus* spp.) to moist and creamy (*Candida* spp.). Microscopically, the *Aspergillus* species were characterized by septate hyphae with prominent conidiophores and vesicles, while *Candida* species were identified as yeasts with budding cells and occasional pseudohyphae. *Trichophyton* species exhibited septate hyphae with typical dermatophyte conidia. *Aspergillus niger*, *A. fumigatus*, *A. clavatus*, and *Chrysosporium sitophila* were classified as highly pathogenic because they caused extensive and rapid tissue degradation. The *Candida* and *Trichophyton* species were moderately pathogenic, producing slower and less aggressive rot symptoms. These morphological features were consistent with standard mycological descriptions and were used for the preliminary identification of the isolates.

Table 4.2: Morphology and Characteristics of Fungal Isolates

Isolate	Macroscopic Appearance	Microscopic Features	Hyphal structure	Spore/Conidia Characteristics	Type of rot caused	Pathogenicity level
<i>Aspergillus fumigatus</i>	Dark green to greenish-grey, velvety, dry	Long conidiophores ending in swollen vesicles	Septate	Round, smooth conidia in long chains (2.5-3.5 μ m)	Dry rot	Highly pathogenic
<i>Candida tropicalis</i>	Creamy white to milkish-white, smooth, wet	Oval to elongated budding yeast cells with short pseudohyphae	Absent (yeast)	Budding cells (3-6mm x 2-4mm)	Soft/wet rot	Moderately pathogenic
<i>Chrysionilia sitophila</i>	White to pale pink, fluffy cottony aerial mycelia	Septate hyphae producing cylindrical to oval arthrospores in chains	Septate	Cylindrical to oval arthrospores (4-8mm)	Soft rot	Highly pathogenic
<i>Aspergillus niger</i>	Black to dark brown, heavy, powdery, dry	Long conidiophores ending in large globose vesicles	Septate	Round, dark brown to black conidia in chains (3-5mm)	Black dry rot (most dominant)	Highly pathogenic
<i>Candida krusei</i>	Creamy to pale pink, slightly dry	Elongated budding yeast cells with short pseudohyphae	Absent (yeast)	Elongated budding cells (4-7mm x 2-4mm)	Wet rot	Moderately pathogenic
<i>Aspergillus clavatus</i>	Bluish-green to grey-green, velvety	Long conidiophores ending in club-shaped vesicles	septate	Round to elliptical conidia in chains (3-4.5mm)	Dry rot	Highly pathogenic

<i>Trichophyton soudanense</i>	White to cream, powdery to suede-like	Septate hyphae with numerous microconidia	septate	Abundant pyriform to clavate microconidia	Dry rot	Moderately pathogenic
<i>Candida utilis</i>	Creamy-white, smooth and moist	Oval budding yeast cells	Absent (yeast)	Oval to round budding cells (3-7mm)	Soft/wet rot	Moderately pathogenic
<i>Candida famata</i>	White to cream, smooth and pasty	Round to oval budding yeast cells	Absent	Round to oval budding cells	Soft/wet rot	Moderately pathogenic
<i>Trichophyton mentagrophytes</i>	White to cream, powdery with cottony aerial hyphae	Septate hyphae with spiral coils	septate	Numerous round microconidia, cigar-shaped macroconidia	Dry rot	Moderately pathogenic

4.4 Pathogenicity Test

The pathogenicity test was carried out on healthy yam and potato tubers to confirm the ability of the isolated fungi to cause rot and to fulfil Koch's postulates.

All ten (10) fungal isolates proved pathogenic to both yam and potato tubers. Characteristic symptoms of rot similar to those observed on the originally collected diseased tubers developed between 7 and 14 days after inoculation (Figure 12).

Aspergillus niger, *Aspergillus fumigatus*, *Aspergillus clavatus*, and *Chrysosporium sitophila* were highly pathogenic, causing extensive dry rot with sunken, wrinkled, and blackened lesions, especially on yam tubers. *Candida* species (*C. tropicalis*, *C. krusei*, *C. utilis*, and *C. famata*) primarily induced soft to wet rot characterized by watery lesions and foul odour. *Trichophyton soudanense* and *Trichophyton mentagrophytes* caused moderate dry rot with relatively slower progression.

Control tubers inoculated with sterile Potato Dextrose Agar discs remained firm with no visible symptoms of rot throughout the incubation period (Figure 13). The same fungi used for inoculation were successfully re-isolated from the advancing margins of the rotted tissues in the inoculated tubers, thereby satisfying Koch's postulates.

These results confirm that all the isolated fungi are capable of causing post-harvest rot in yam and potato tubers under storage conditions.



Figure 12: Pathogenicity test results on yam tubers



Figure 13: Control (sterile potato dextrose agar)



Figure 14: Pathogenicity test results on potato tubers



Figure 15: Control (Sterile potato dextrose agar)

CHAPTER FIVE

DISCUSSION

5.1 Discussion

The occurrence of ten (10) fungal species in rotten yam (*Dioscorea rotundata*) and potato (*Solanum tuberosum*) tubers from Abakpa Market indicates high fungal contamination and post-harvest deterioration of these important food crops in the study area, Enugu State, Nigeria. This result is in line with the aim of the study which was to isolate the causative agents of the yam and potato tuber rots and establish their pathogenicity.

Aspergillus niger was the most predominant and isolated fungus from both yams and potato tubers. This is in agreement with earlier research that was conducted in Nigeria and other West African countries. Gwa and Nwankiti (2018) also reported that among the yam and potato storage fungi *Aspergillus niger* was one of the most aggressive and frequently encountered. This dominance is a result of its fast spore formation, its ability to survive in a wide range of environments and high enzymatic activity, which allows it to break down tissues quickly (pectinase and cellulases). The abundance of *Aspergillus niger* in this study further corroborates the results of Ekpenyong *et al.* (2024) and Dania *et al.* (2019), who reported the high prevalence of *Aspergillus niger* in poorly ventilated market and storage areas of Nigeria.

The isolation of other species of *Aspergillus* such as *A. fumigatus*, *A. clavatus* and dermatophytes namely, *Trichophyton soudanense* and *T. mentagrophytes*, from yam tubers also suggests that mechanical damage during harvesting, transport and market handling is an important entry point for other fungal pathogens. This finding is consistent with the findings of Aidoo *et al.* (2020), who emphasized the importance of wounding in yam tubers by spear grass in making them susceptible to fungal infection. However, fungal diversity was higher in potato tubers, dominated by *Candida* species (*C. tropicalis*, *C. krusei*, *C. utilis*, and *C. famata*),

Chrysonilia sitophila and *Trichophyton mentagrophytes*. The higher moisture content and softer texture of potato tubers could be associated with the increased diversity, as reported by Tiwari *et al.* (2020), which is favorable to colonisation by moulds and yeasts.

An important finding of this study was that all ten isolates were successfully tested for pathogenicity. All fungi caused typical rot symptoms on healthy tubers in 7-14 days and were re-isolated from the lesions, thus satisfying Koch's postulates. This indicates that the fungi were not just saprophytic and were actually active agents of the post-harvest rot. The present results are similar to those of Paul *et al.* (2021) and Azil *et al.* (2021) who also demonstrated pathogenicity of *Aspergillus* and *Fusarium* species on tubers. The use of both swab and cutting inoculation methods was successful in the removal of surface and internal fungi. Household food availability, market value, and the livelihood of small holder farmers are all under threat from high post-harvest losses from fungal rot. The predominance of potentially mycotoxigenic species of the genus *Aspergillus* also poses a public health risk because of the potential for contamination with aflatoxin and ochratoxin.

This study has some limitations because samples were collected from only source, Abakpa Market. More detailed data on fungal diversity and distribution would have been obtained from more extended sampling from a variety of markets and seasons. In addition, molecular identification (such as the ITS sequence) was not done, which would have provided more species confirmation than morphology.

In general, the findings of this study help build the body of knowledge on post-harvest fungal pathogens of tubers in Nigeria and serve as a basis for devising specific management measures to minimize post-harvest losses.

CONCLUSION

The present study has demonstrated that rotten yams and potato tubers sold in Abakpa Market harbor a wide variety of pathogenic fungi. A total of ten fungal species were isolated and identified, with four species from yams and six species from potatoes. *Aspergillus niger* was the most prevalent and aggressive pathogen recovered from both crops. All the isolated fungi proved pathogenic and were able to reproduce typical rot symptoms on healthy tubers, thereby fulfilling Koch's postulates. These findings confirm that fungal infection is a major cause of post-harvest losses and quality deterioration of yams and potatoes in the study area. The high occurrence of potentially mycotoxigenic *Aspergillus* species also raises concern about possible health risks to consumers.

RECOMMENDATION

Control of fungal rot in yams and potatoes requires an integrated approach. Farmers, traders and marketers should be educated on proper handling practices to minimize mechanical injuries during harvesting, transportation and display in markets. The use of improved storage structures with adequate ventilation and regular inspection of tubers is strongly recommended to reduce moisture accumulation and fungal proliferation.

Market authorities should enforce good hygienic practices and avoid displaying tubers directly on the ground. Further research should be conducted on the use of botanical extracts and biocontrol agents as safe and affordable alternatives for the management of these fungal pathogens. Regular monitoring of fungal contamination and mycotoxin levels in major markets across Enugu State is also recommended to safeguard public health and reduce post-harvest losses.

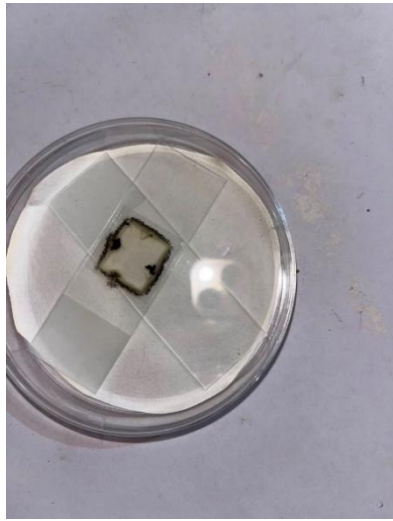
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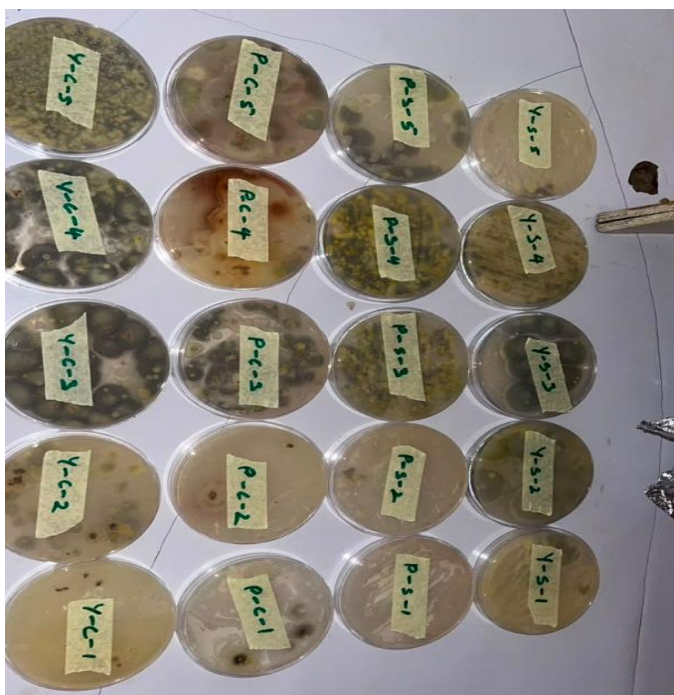
APPENDIX



Appendix 1: Picture showing slide culture technique of fungal growth on agar block for microscopic identification



Appendix 2: Picture showing laboratory observation of fungal culture during identification



Appendix 3: Picture showing primary isolation plates of fungal colonies from yam and potato



Appendix 4:

Picture showing pure isolates of fungi subcultured and maintained in bijou