ISOLATION AND IDENTIFICATION OF FUNGI ASSOCIATED WITH THE DECAY OF COCOYAM (*Colocasia esculanta*) USED AS A SOUP THICKENER IN ENUGU

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APPROVAL

This research titled "**Isolation and Identification of Fungi Associated with the Decay of Cocoyam (*Colocasia esculanta*) Used as a Soup Thickener in Enugu**" has been assessed and approved by the committees of the Department of Biological Sciences and the Faculty of Natural Sciences and Environmental Studies, Godfrey Okoye University, Enugu, Nigeria.

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This is to certify that this research titled **Isolation and Identification of Fungi Associated with the Decay of Cocoyam (*Colocasia esculanta*) Used as a Soup Thickener in Enugu** was carried out by Omiete Peculiar Alabo with Registration Number GOU/U22/MCB/536 under the supervision in the department of Biological Sciences, Godfrey Okoye University Enugu Nigeria.

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DEDICATION

This project work is dedicated to God Almighty who has always been faithful to me.

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LIST OF ABBREVIATIONS / ACRONYMS

AFB1:	Aflatoxin B1
DNA:	Deoxyribonucleic Acid
HIV/AIDS:	Human Immunodeficiency Virus / Acquired Immunodeficiency Syndrome
LPCB:	Lactophenol Cotton Blue
OTA:	Ochratoxin A
PDA:	Potato Dextrose Agar
Sp.:	Species (Singular)
Spp.:	Species (Plural)
WHO:	World Health Organization
°C:	Degrees Celsius
g:	Grams
kcal:	Kilocalories
ml:	Millilitres
nm:	Nanometres
µm:	Micrometres

ABSTRACT

Postharvest fungal rot of cocoyam (*Colocasia esculenta*) poses a serious threat to the economic value of the crop especially its functional property as a primary soup thickener in Nigeria. At the same time, it poses a serious public health risk to the populace due to possible exposure to mycotoxins. This study aimed at identifying and isolating the fungi pathogens responsible for post-harvest decay of cocoyam corms sold at Abakpa Market in Enugu State. Twenty (20) pieces of rotten cocoyam samples were collected and cultured on Potato Dextrose Agar (PDA) using standard microbiological methods. Pure cultures were obtained by sub-culturing and isolates were characterised on the basis of their macroscopic (*Colonial morphology*) and microscopic features using slide culture technique and Lactophenol Cotton Blue (LPCB) staining. The results obtained showed the presence of three different genera of filamentous fungi responsible for the decay. Morphological identification of the isolated fungi showed them to be *Rhopalomyces sp., Basidiobotrys sp.* and *Scopulariopsis sp*. These spoilage organisms secrete extracellular hydrolytic enzymes that degrade the tuber's starch and mucilage matrix and cause a loss of its rheological and soup-thickening properties. The results of the study showed that *Rhopalomyces sp., Basidiobotrys sp.* and *Scopulariopsis sp.* are the major cause of cocoyam spoilage in the study area, which is contrary to the usual ones like *Aspergillus* and *Rhizopus* found in other studies. The study demonstrated that decayed cocoyam sold for use as soup thickener harbours several fungal species capable of contributing to spoilage and highlights the need for improved handling and storage practices.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

In Nigeria, there are two primary types of cocoyam: *Xanthosoma sagittifolium* (Tannia), locally known as "ede-oku", and *Colocasia esculenta* (Taro), known as "ede-uli" (Mba and Agu, 2021). On one hand, tannia tubers can be processed to make a variety of foods. They can be made into flour, chips, pepper soup, or porridge; boiled, fried, baked, roasted, or ground into a paste (Akhigbe *et al.,* 2024). While on the other, the primary use of taro is as a thickening for soups. It is applied to a certain category of soups prepared with bitter leaves (*Veronia amygdalina*) or ora leaves, stockfish, dried fish, pepper, red palm oil, meat, crayfish, salt, and fermented ogiri (*Ricinus communis*), commonly consumed with fufu in Nigeria (Mba and Agu, 2021). *Colocasia esculenta* are natural hydrocolloids; they derive their thickening properties from their gum and mucilage constituents. These are water-soluble polysaccharides also found in other plants used generally in the food industry as thickeners, emulsifiers and stabilisers (Tosif *et al.,* 2022). This thickening ability is central to the economic value of the *Colocasia esculenta* and is often threatened by fungal degradation.

Cocoyam is often regarded as a neglected crop, a crop for poor men in the Nigerian agricultural sector (Nzeh and Anionwo, 2023), because, according to Otekunrin *et al*. (2021), the majority of its consumers are low-income households. Regardless, it remains the third largest tuber crop by volume of production in Nigeria, next to yam (*Dioscorea* spp) and cassava (*Manihot esculenta*) (Otekunrin *et al.,* 2021). Its economic value is most felt during the off-season of other tubers like yam. When yam becomes scarce and expensive, people turn to it as an alternative source of food, and as such, it bridges the hunger gap created. (Nzeh and Anionwo, 2023). Beyond this, cocoyam provides a unique form of nutritional content not seen in other tuber crops. (Mato *et al.,* 2025). Nutritional analysis reveals that *Colocasia esculenta* corms

contain a crude protein content ranging from 3.38% to 9.36%, 7.79% on the average, which is higher when compared to that of Nigerian cassava, at 3.50%, and an average energy content of 76.48 kcal/100 g (Fufa and Tadese, 2023). Furthermore, the starch of cocoyam has unique structural properties; its granules are very small (3 to 20 nm in size), compared to other tubers (Mato *et al.,* 2025). This feature makes it easily digestible and suitable for infants, elderly people and people with gastrointestinal disorders (Fufa and Tadese, 2023).

The mechanism of decay in *Colocasia esculenta* is initiated by a breach of its natural periderm. The periderm is a suberised skin that microorganisms cannot easily penetrate (Abewoy, 2021); however, during field operations, such as weeding, harvesting, and post-harvest handling, rodents, nematodes, and humans can readily injure the tubers, thereby predisposing them to microbial attack (Anyanwu *et al.,* 2023). Upon invasion, the crops' high moisture content and the humidity of the storage facility promote the rapid growth and spread of the invading fungi and the consequent spoilage of the infested crop (Nkemakonam *et al.,* 2024).

Both fungal and bacterial infestations are responsible for damage to foods. Spoilage fungi fall into two categories: storage fungi and field fungi (Nkemakonam *et al.,* 2024). According to Anyanwu *et al*. (2023), field fungi can alter the structure and quality of food. They destroy tubers and corms before harvest and are typically identifiable by routine inspection. Storage fungi, on the other hand, attack the food while in storage and produce spores that eventually lead to spoilage (Nkemakonam *et al.,* 2024). Primary spoilage moulds involved are *Aspergillus tamarii*, *Aspergillus niger*, *Fusarium oxysporum*, *Fusarium solani*, and *Mucor circinelloides* (Uthman and Rashidat, 2024). These pathogens do not cause damage in the same way; the end result could be either dry rot or wet rot. In dry rot, *Penicillium oxalicum* and *Penicillium cyclopium* infections cause tubers to turn brown, harden, and dry without losing their integrity except in cases where *Serratia marcescens* invades the tissues, in which case the infected

tissues get layered by the fungal mycelia which is greenish greenish in color (Akinbo, 2019). Furthermore, *Aspergillus niger* and A. *tamari* infections cause tubers to appear brown with a yellowish border. *Penicillium oxalicum* and *Penicillium cyclopium* infections cause the brownish discoloration of tubers, it also hardens, and dries them while retaining their integrity (Akinbo, 2019). In contrast, *Rhizopus spp.S. rolsii, Rhizoctonia solani, Mucor circinelloides, amd Armillariella mellea* are the primary agents of soft rot (Akinbo, 2019). This pathogen aggressively hydrolyses the tuber tissue to produce a wet mass, characterised by extensive liquefaction and a rapid collapse of the corm's cellular structure (Anyanwu *et al.,* 2023).

This collapse is the result of a cascade of events driven by the activity of amylases, pectinases, cellulases and other cell wall-degrading enzymes secreted (Anyanwu *et al.,* 2023). During this process of decay, the protein and polysaccharide content of taro mucilage, the gel-like substance responsible for soup thickening, is lost to these fungal enzymes via hydrolysis of complex starch polymers to simple sugars (Tortoe *et al.,* 2019). Consequently, this results in the loss of its ability to dictate the rheology and texture of soup (Awoyale *et al.,* 2024).

The implication of fungal infection and spoilage of food extrapolates beyond the economic loss associated with the loss of food. It presents an additional risk of mycotoxicosis. Although boiling, which is a step in the processing of cocoyam as food, can and does destroy fungal mycelia, it is ineffective against mycotoxins, which are a by-product of their metabolism. Aflatoxins, produced by *Aspergillus flavus,* and fumonisins, produced by *Fusarium* species, are thermally stable compounds that can survive standard cooking temperatures (100°C) (Enyiukwu *et al.,* 2020; Uthman and Rashidat, 2024). Unaware individuals who try to salvage partly rotten cocoyam for their soup are, as a result, predisposed to the extreme health hazards posed by these mycotoxins, which may manifest as immunosuppression, hepatotoxicity or cancer in worst-case scenarios (Uthman and Rashidat, 2024).

1.2 Statement of Problem

The primary value of *Colocasia* corms lies in their function as a soup thickener, a characteristic attributed to their mucilage and starch content. Fungal enzymes produced by spoilage fungi infesting the crop lead to the loss of this feature. This in turn renders it less valuable economically. Some individuals, especially poor farmers and low-income consumers, may choose to salvage partially rotten ones. But the consumption of the apparently healthy portions of the tuber presents a public health risk in the form of mycotoxin poisoning. This is because fungal mycelia and mycotoxins often diffuse into tissues beyond the necrotic lesion. And since key mycotoxins such as aflatoxins and fumonisins are thermally stable and survive the boiling point of soup.

Due to the lack of data about the microbial ecology of cocoyams in Enugu State, public health policy makers may be unable to create effective strategies to prevent cocoyam infestations, combat fungi in already infested crops or draft accurate educational material for public awareness campaigns. Treatment of victims already suffering from mycotoxin poisoning will also be less efficient without proper identification of the fungal culprit. Without isolating and identifying the specific fungal profile responsible for the decay in local markets, it is almost impossible to determine the severity of the mycotoxin threat or design targeted preservation strategies. Therefore, this study is necessitated by the urgent need to identify these spoilage organisms to mitigate both the economic loss of the thickener and the health risks to the consumer.

1.3 Significance of the Study

Salvaging is a common culture amongst poor consumers. In the case of partly rotten corms, they may cut off the decaying portion with the hope of consuming the seemingly good part. This research will provide valuable information on the dangers of this practice, which can be

used by policymakers and implementers to educate the public. Secondly, as a soup thickener, *Colocasia esculenta* serves a very important role in food security and in the economy. Therefore, a good understanding of the fungi responsible for its spoilage is an important step in reinforcing this role. Furthermore, this study will bridge the information gap with valuable data from samples from select markets in Enugu State. It will help define the fungal ecology that affects *Colocasia esculenta* in the state and provide researchers with information which can be used to develop fungicides and improved storage technology.

1.4 Scope and Limitations of the Study

1.4.1 Scope

The identification of fungi responsible for the decay of cocoyam used as a soup thickener in Enugu State is the primary focus of this study. Fully decayed and partly decayed cocoyam tubers will be obtained from selected markets in Enugu State. The laboratory analysis will be limited to the isolation and identification of fungi using standard mycology analysis techniques. Sabouraud dextrose agar, a standard medium for fungal cultures, will be used to grow the fungi. After incubation, constituent fungi will be isolated and identified based on their colonial characteristics, such as size, texture, shape and growth pattern. Microscopic features such as the nature of hyphae and the morphology of their spores will also be observed. This information will be used to find the most predominant fungi that cause cocoyam spoilage in the selected locations.

1.4.2 Limitations of the Study

The restriction of this study to select marketplaces in Enugu State is a major limitation to this study. This is because findings may not fully represent the fungal ecology of cocoyam in Enugu State as a whole. This limits the generalisability of the study. The use of cultural and morphological characteristics and microscopic features in the identification of fungal species

is another limitation to the study. While advanced molecular techniques that are based on DNA analysis are the gold standard, they cannot be carried out due to lack of funding.

Environmental factors like storage duration, temperature, and humidity influence the growth of fungi. However, measurement of these conditions is outside the scope of this study, and as such presents a limitation. These limitations notwithstanding, the study is expected to provide useful insights into the major fungi responsible for cocoyam spoilage in Enugu metropolis.

1.5 Research Hypothesis

i. **Null Hypothesis:**

There is no significant fungal association with the post-harvest decay of *Colocasia esculenta sold* in markets in Enugu State.

ii. **Alternative Hypothesis:**

Fungal species are a significant cause of post-harvest decay of *Colocasia esculenta* corms sold in markets in Enugu State.

1.6 Research Questions

Below are the questions to answer in this study:

1. What specific fungal pathogens cause post-harvest decay of *Colocasia esculenta* (cocoyam) corms in Enugu State markets?
2. What are the characteristics of the fungal isolates responsible for the spoilage? Both micro and macroscopic

1.7 Aims and Objectives

1.7.1 Aim: Isolation And Identification Of Fungi Associated With The Decay Of Cocoyam (*Colocasia esculanta*) Used As A Soup Thickner In Enugu

1.7.2 Objectives:

i. To Isolate fungal pathogens responsible for the post-harvest decay of *Colocasia esculenta* corms obtained from markets in Enugu State.

ii. Characterise and identify the fungal isolates to the species level based on their macroscopic (cultural morphology) and microscopic features.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Colocasia esculenta (Cocoyam)

In many regions of the world, *Colocasia esculenta* (L.) Schott, also known as taro, and *Xanthosoma sagittifolium* (L.) Schott, also known as tannia, are two perennial edible aroid species that are herbaceous and members of the monocotyledonous Araceae family and are referred to as cocoyams (Emmanuel *et al.,* 2024). Before the columbian crop trade started between the old world and the Americans, *Colocasia esculenta* (L.) Schott dominated the food crop market (Ahmed *et al.,* 2020). According to evolutionary geography, wild Colocasia species are grow in Southeast Asia, which is also thought to be the region of origin of C. *esculenta* species (Ahmed *et al.,* 2020).

Xanthosoma and Colocasia can be differentiated using the junction between the petiole and the leaf lamina (Arogundade and Adedeji, 2019). Unlike what can be seen on the leaves of the later, the leaves of the former are not peltate. Instead they have a V-notch in the top area that extends to the point where the leaf attaches its petiole to the blade (Arogundade and Adedeji, 2019). Additionally, microscopic examination shows that the two Colocasia types have papillae on their adaxial surfaces, whereas the Xanthosoma taxa lack these features (Arogundade and Adedeji, 2019).

Nigeria takses the sport as the largest producer and supplier of cocoyam to the global market in terms of agronomic significance, with a production rate of 5.49 million metric tonnes annually in the country's middle and southern belt zones (Fakunle *et al.,* 2025). This amount is equal to 72.2% of all produced in West Africa and 45.9% of global production (Fakunle *et al.,* 2025). Given that the root crop is comparatively less expensive than yam and hence more accessible, it follows that there can never be a shortage of demand for it (Inegbedion, 2025).

And since they are higher in starch than cassava and yam, corms and tubers are therefore vital to food security (Akaza *et al.,* 2023). Cocoyam is often referred to as a women's crop in Nigeria, specifically in the southeastern part. This is because the crop is usually planted by rural women farmers who often plant it on their husband's yam mounds (Okolo-Obasi *et al.,* 2025). Regardless of its sociocultural connotation, it is a vital component of the country's food security setup (Inegbedion, 2025).

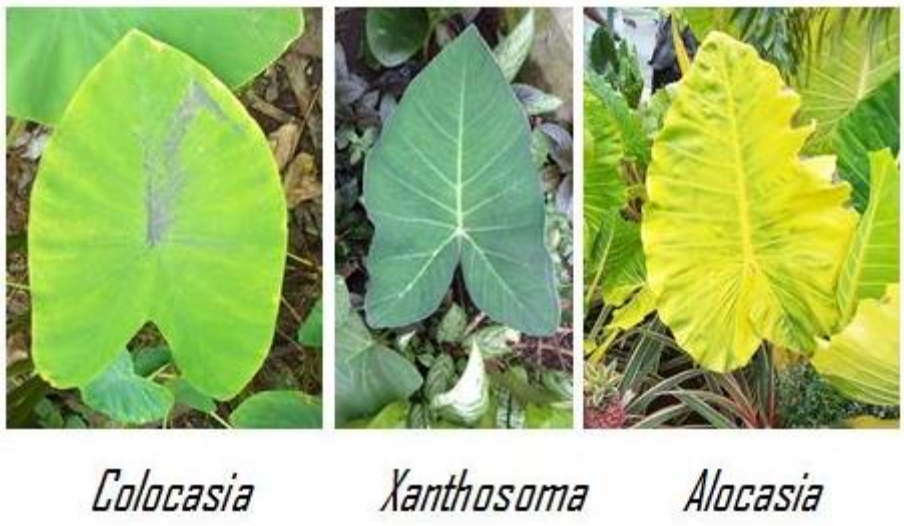

Figure 2.1 Differentiation of cocoyam based on leaf structure (efloraofindia.com)

2.2 Nutritional and Functional Properties

Colocasia esculenta is valuable nutritionally because it is one of the top six crops of root and tuber origin on earth, with a variety of uses in food, feed, medicine, and industry (Emmanuel *et al.,* 2024). The corm contains a large amount of carbohydrates and also minerals, however, when compared to yam and cassava, it has more protein, phosphorus, vitamins, and easily digested starch (Fakunle *et al.,* 2025). Cocoym consists of 1.4-3.0% of vitamins, minerals, proteins combined (Fru and Vange, 2023). Its carbohydrates constituents on the other hand make up about 13–29% (Fru and Vange, 2023). On a dry weight basis, it has roughly 11% protein (Mubarak *et al.,* 2021). In terms of energy provision, C. esculenta produced 135 kcal

per 100 g and had a greater carbohydrate content than potatoes (Mubarak *et al.,* 2021). When compared to yam and cassava, cocoyam emerges with higher nutritinal value, ease of digestion and its content of vital minerals. Its mineral contents are, among many others, calcium, magnesium, and phosphorus (Inegbedion, 2025).

Cocoyam, C. *esculenta* in particular, serves as a soup thickener because of its constituent 85-87% dry mass of starch (Mubarak *et al.,* 2021). However, it contains about 19% of mucilage, which contributes to this property (Tosif *et al.,* 2022). Mucilage is a product of a complex interaction between carbohydrates (xylose, glucose, galactose, mannose, including arabinose) and proteins (Tosif *et al.,* 2022). Because of this particular characteristic, it is a preferred raw material in enterprises that produce goods like sausage binder and salad cream (Okolo-Obasi *et al.,* 2025). In addition to its rheological qualities, the crop has a number of health benefits, most notably that its low starch content makes it a better option for diabetics than other root crops like yam and cassava (Okolo-Obasi *et al.,* 2025). Furthermore, because its starch granules are small, about 3-18 μm (Mubarak *et al.,* 2021), it is very beneficial for diabetics, those with digestive diseases, the elderly, and children with allergies (Fakunle *et al.,* 2025).

2.3 Storage

Fresh tubers are extremely perishable and easily spoil after harvest. This occurs mainly due to the lytic action of enzymes, proliferation of microbes (fungi or bacteria), structural damage, and bad condition of storage. This susceptibility is often described as a vulnerability paradox caused by the tuber's basic biology (Raju, 2021). The loss of moisture during poor storage causes structural alterations that have a detrimental impact on the tuber's textural, mechanical, chemical, and nutritional composition, and also compromises its structural integrity (Ijabo *et al.,* 2019).

Furthermore, exposure to ethylene can result in modifications to cell wall components which can contribute to tuber texture changes and oxidative damage brought on by an increased susceptibility to infections (Raju, 2021). In addition to these physiological factors, the harvesting process is frequently the main source of damage (David-Chukwu *et al.,* 2021). This is because corms are typically vulnerable to physical harm during harvesting, and these mechanical injuries result in significant post-harvest losses by facilitating the entry of spoilage microorganisms.

Poor storage, on the other hand, compounds this. Traditional storage techniques such as pit storage and heaping of the cocoyam under shade have been explored in Nigeria and have been shown to be inefficient (David-Chukwu *et al.,* 2021), because exposure to high temperatures in storage in systems like these impact the pace of degeneration of cocoyam corms. This happens due to the fact that the rate of metabolic activities increases in the corm (Fru and Vange, 2023). In Nigeria, microbial action is the primary cause of cocoyam corm deterioration and loss during storage, with an estimated 40–50% loss due to these physiological and storage issues that ultimately open the door for infection (Fru and Vange, 2023).

2.4 Fungal Spoilage of Cocoyam

The deterioration of cocoyam corms is a major agricultural challenge in Nigeria that is primarily caused by biological agents. Loss due to spoilage after harvesting of crops that are classified as root and tuber have historically been a problem of storage that farmers face. Fru and Vange (2023) estimate that over 40% of such harvests are lost due to decay (Fru and Vange, 2023). This decay process is classified according to the timing of infection. Field fungi attack corms in the farm field prior to harvest and can alter the structure and quality of produce to produce damaged tubers and corms (Muonyelu *et al.,* 2024), whereas storage fungi damage

corm stored under poor storage conditions in facilitis and typically do not occur at a serious level before harvest (Muonyelu *et al.,* 2024).

This spoiling has been linked to a wide variety of mycoflora. *Penicillium digitatum, Aspergillus flavus, Erwinia carotovora and Botryodiplodia theobromae* are the main microorganism species that have been linked to cocoyam rot in Southern Nigeria (Akomah-Abadaike and Didia, 2024). However, seven other fungal species including, *Mucor racemosus and Aspergillus niger Also, Fusarium oxysporium, Aspergillus flavus, Rhizopus stolonifer, Penicillium expansum and Rhizopus oryzae* have been isolated and identified as spoilage agents of cocoyam (Mubarak *et al.,* 2021). Certain infections are more common than others, *Fusarium oxysporum* and *Rhizopus stolonifer*, for instance, Muonyelu *et al*. (2024), found in their study that were 100% prevalent. Furthermore, *Aspergillus flavus* (60.0%) induced the most extensive rot, followed by *Rhizopus stolonifer* (73.33%) and Bipolaris sp. (73.3%), according to pathogenicity testing (Fru and Vange, 2023).

The mechanism and pattern of spoilage are determined by the invading pathogen. Fungi target the cell wall polymers in the invaded cocoyam and digest it with enzymes, which leads to the damage of the crop (Akomah-Abadaike and Didia, 2024). Spoilage caused by *Aspergillus niger* weakens interior tissues and yields the growth of a black spore mass over the infected area (Mubarak *et al.,* 2021). This degradation is not the same as bacterial infections, which can cause the entire corm to rot. In the former case, the symptoms are typically a soft, watery rot of individual scales (Opara and Okpara, 2020). These organisms are soil saprobes that break down plant lignocelluloses using a variety of hydrolytic and oxidative enzymes (Muonyelu *et al.,* 2024).

2.5 Mechanisms of Fungal Decay

The main way that fungi cause cocoyam to deteriorate is by secreting extracellular lytic enzymes that penetrate the tuber's structural barriers. A variety of enzymes capable of breaking down the cell-walls of the corm are produced by saprophytic fungi which are phytopathogenic in nature. These enzymes are believed to be vital to pathogenicity functions such as penetration, degradation, and symptom development (Bretträger *et al.,* 2024). After making contact, the pathogen can actively enter the tissues and feed on its nutrients. The process if unchecked depolymerises the primary structural elements of the cell wall with the help of secreted hydrolytic enzymes (Bretträger *et al.,* 2024).

α-amylase along with α-glucosidase and glucoamylase, are among the several starch-hydrolytic enzymes secreted by filamentous fungi (Wang *et al.,* 2020). Using these, they target the starch reserves, the main functional component for thickening soup, after breaking through the cell wall. The α-glycosidic bond in starch is selectively broken by a class of hydrolases known as amylolytic-acting enzymes, which are involved in the biochemical process (Bretträger *et al.,* 2024). In particular, the 1,4-glycosidic bonds in starch are broken down by α-amylase into oligasaccharides such as maltose and glucose (Wang *et al.,* 2020). Consequently, this loss of starch structure gives away its viscous properties and turns high-molecular-weight polymers in the crop into soluble nutrients (Bretträger *et al.,* 2024).

For example, certain Aspergillus species, such as Aspergillus niger, may generate and secrete a lot of amylase, which can convert starch to glucose (Wang *et al.,* 2020). Furthermore, these fungal enzymes can hydrolyse 1,3-1,4-β-glucan (Bretträger *et al.,* 2024). Since enhanced amylolytic enzymes are known to breakdown starch which inturn reduces the viscousity of Taro, the immediate result of this enzymatic activity is a loss of functional quality (Bretträger *et al.,* 2024).

2.6 Public Health Implications (Mycotoxins)

mycotoxins According to Zhang *et al*. (2023), are substances classified as secondary metabolites produced by fungi. They are produced when the plant is still in the field, during harvest, or storage stage. Consequently, the microbiological deterioration of cocoyam is a threat to food safety and also a source of economic loss (Omotayo *et al.,* 2019). *Aspergillus flavus, also Aspergillus niger* and Penicillium including *Mucor sp.* are among the specific organisms linked to cocoyam rotting that commonly create these harmful substances (Akomah-Abadaike and Didia, 2024).

A common misunderstanding among locals is that cooking partially spoilt cocoyam gets rid of spoilage, fungi, bacteria and their metabolites. However, due to the ability of aflatoxins and other toxic substances produced by the spoilage fungi, cooking via standard heating techniques does not remove the danger posed by them (Peng *et al.,* 2023). Aflatoxins from *Aspergillus flavus,* for instance, are generally stable at dry heat up to 260°C (Dhanshetty *et al.,* 2021). Also, OTA Ochratoxin A is a chemically very stable substance and is also present in processed food products even after boiling (Ráduly *et al.,* 2020). Accordingly, mycotoxin exposure, which primarily happens by ingestion, causes a number of illnesses that could ultimately lead to death (Omotayo *et al.,* 2019).

Consuming these toxic substances has serious and systemic pathological effects. AFB1 Aflatoxin B1 is a very toxic toxin, ten times more lethal than potassium cyanide. For this reason, the International Agency for Research on Cancer classified it as a class I carcinogen (Peng *et al.,* 2023). In addition to cancer, chronic aflatoxin poisoning affects the bone marrow's ability to duplicate DNA, which results in low leukocyte counts and immunodeficiency (Ráduly *et al.,* 2020). Also, mycotoxins have been shown to reduce antioxidant enzymes, which results in

tissue toxicity and carcinogenicity of the liver and kidney, which are the primary organs for their metabolism (Omotayo *et al.,* 2019).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research Design

This project is designed as a laboratory-based experimental research. It involves the isolation and characterization of fungal pathogens present in decaying *Colocasia esculenta* corms in a systematic way using standardized microbiological techniques. Colonial and microscopic characteristics was used for identification.

3.2 Study Area and Population

3.2.1 Study Area

The research was conducted in Godfrey Okoye university Enugu State. Abakpa market was the source of completely spoilt or partially spoilt corm for this project.

3.2.2 Population of the Study

The population for this study consists of rotting corms of cocoyam, specifically the variety *Colocasia esculenta* (L.) Schott (Taro).

3.3 Sampling Techniques

A purposive and random sampling technique was employed. At Abakpa market, corms of *Colocasia esculenta* which are visibly spoilt were identified with the existence of a distinct rot and randomly selected. Purchases were subsequently made from different vendors to ensure that a diverse representation of the fungi is captured. Following this, these samples were laced in sterile polyethylene bags, sealed, labelled appropriately, and transported immediately to the laboratory for processing.

3.4 Media and Reagent Preparation

3.4.1 Preparation of Potato Dextrose Agar (PDA)

Potato Dextrose Agar (PDA) was the culture media used for this experiment. It was prepared according to the manufacturer's instructions. 15.6 grams of PDA powder was dissolved in 400ml of distilled water.

During production, the media was sterilized with an autoclave at 121°C for 15 minutes. This action is aimed at preventing contaminations in the future and to ensure that only fungal growth is observed. Following this, the media was transferred into sterilized petri dishes and allowed to solidify.

3.5 Sample Preparation and Processing

The initial step was sample preparation. Here, sterile swab sticks were used to take swab samples from the decayed surfaces of the cocoyam. Additionally, a sterile scalpel was used to cut little pieces of the actively decaying cocoyam tissues. These were then inoculated into the culture media and kept at room temperature (25–30°C) for a period of 3 to 7 days to permit the fungi to grow.

3.6 Preparation of Pure Culture

After the culture step above, fungal colonies were then selected and sub cultured in fresh plates of PDA based on their macroscopic and microscopic characteristics. A total of 14.04g PDA was weighted and dissolved in 360 mls of distilled water according to the manufacturers guide and autoclaved for 15mins at 121 degree Celsius. After cooling 15mls of the medium was transferred to 20 bijou bottles respectively. Each colony was sub cultured into the labeled bijou bottles respectively and allowed to grow for 5-7 days.

3.7 Slide culture and identification of Fungal Isolates

The isolates were identified using the slide culture method in accordance with the approach of Raju *et al*. (2021). A clean petri dish with filter paper, a bent glass rod, and a clean glass slide

was filled with approximately 10 cm of previously prepared PDA medium that had been cut with a sterile scapel. Using a sterile wire loop, the fungal colonies from the slant culture were carefully inoculated onto the corners of each agar block, which was then covered with a coverslip. To create a moist and humid environment for fungal growth, 2 mm of distilled water was added to the filter paper. The medium was cut into small squares using a sterile scapel. Sterile slides were placed in a sterile petri dish with filter paper The setup was allowed to grow at room temperature for 7-14 days.

3.8 Identification of Fungal Isolates

Isolated fungal colonies were identified using morphological characteristics. The colonies were identified based on their color, texture and growth pattern of their mycelia. Some of these tests included the use of lactophenol cotton blue staining and germ tube tests. A drop of LPCB stain was placed on a clean microscopic slide, and a small portion of the fungal isolate was teased into the stain using a sterile inoculating needle. A coverslip was placed over it, and it was observed under the microscope. The stain helped to amplify the structures of the fungi. This made it possible for the hyphae, conidia and spores on the culture plate to be clearly observed and used to aid the identification process.

3.9 Laboratory Safety and Handling

All laboratory procedures was carried out using appropriate personal protective equipments such as laboratory coats, gloves, and facemasks. This was ensured that the researcher does not inhale fungal spores, their toxins or other bacterial agents capable of compromising their health or that of others. All glasswares and heat stable equipment were sterilised by autoclaving before and after use. Standard operating proceedures was adhered to strictly to guarantee quality of findings.

CHAPTER FOUR

RESULTS

4.1 Isolation and Identification of Fungal Isolates

Several different fungal growths were produced when the decomposing *Colocasia esculenta* (cocoyam) samples were cultivated on Potato Dextrose Agar (PDA), from which pure cultures were effectively obtained. These morphological characteristics led to the identification of three different fungal genera: *Rhopalomyces sp., Basidiobotrys sp.,* and *Scopulariopsis sp*. Table 4.1 provides a summary of the particular morphological traits that were utilised to identify them, and Figures 4.1 to 4.3 provide visual representations of these traits.

The fungal isolates in table 4.1 were identified as *Rhopalomyces sp., Basidiobotrys sp.,* and *Scopulariopsis sp.* based on colony morphology on PDA and microscopic analysis in lactophenol cotton blue.

Table 4.1 Macroscopic and Microscopic Characteristics of Fungal Isolates from Decayed Cocoyam

Isolate Code	Macroscopic Appearance on PDA	Microscopic Characteristics (LPCB Stain)	Identified Fungi
F1	Colonies are thin, white, and spreading.	Simple, erect conidiophore ending in an enlarged terminal ampulla/head bearing large, pigmented, unicellular conidia	*Rhopalomyces* sp.
F2	powdery, greyish-brown, with a darker reverse	Dark, elongate-clavate conidiophores with an enlarged apex; conidia are produced on short denticles/pegs, and are hyaline, ellipsoid, and one-celled.	*Basidiobotrys* sp.
F3	Powdery, light brown,	Septate hyphae with branched bearing annellides; conidia are formed in chains by basipetal succession and are ovoid/ellipsoidal, with a flattened or truncate base	*Scopulariopsis* sp.

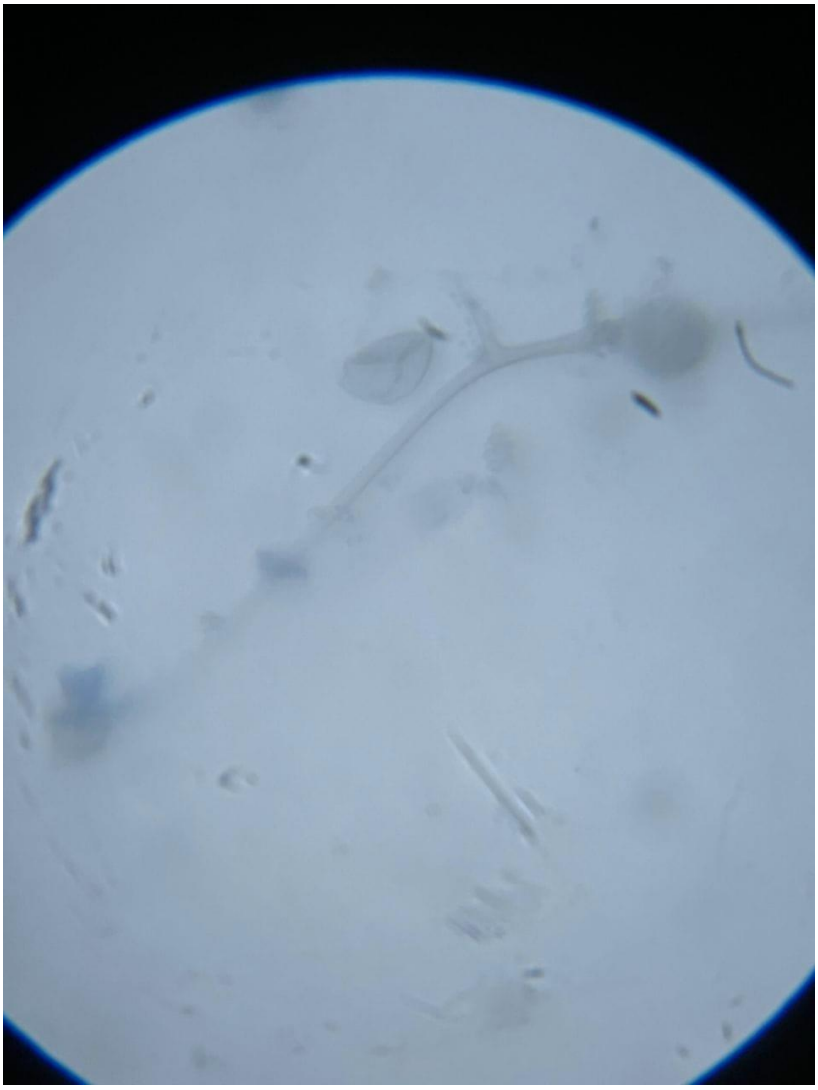

Figure 4.1. Morphological features of *Rhopalomyces sp*. (Isolate F1)

Microscopic view showing a simple, erect conidiophore ending in an enlarged terminal ampulla/head bearing large, pigmented, unicellular conidia (LPCB stain, x100 magnification).

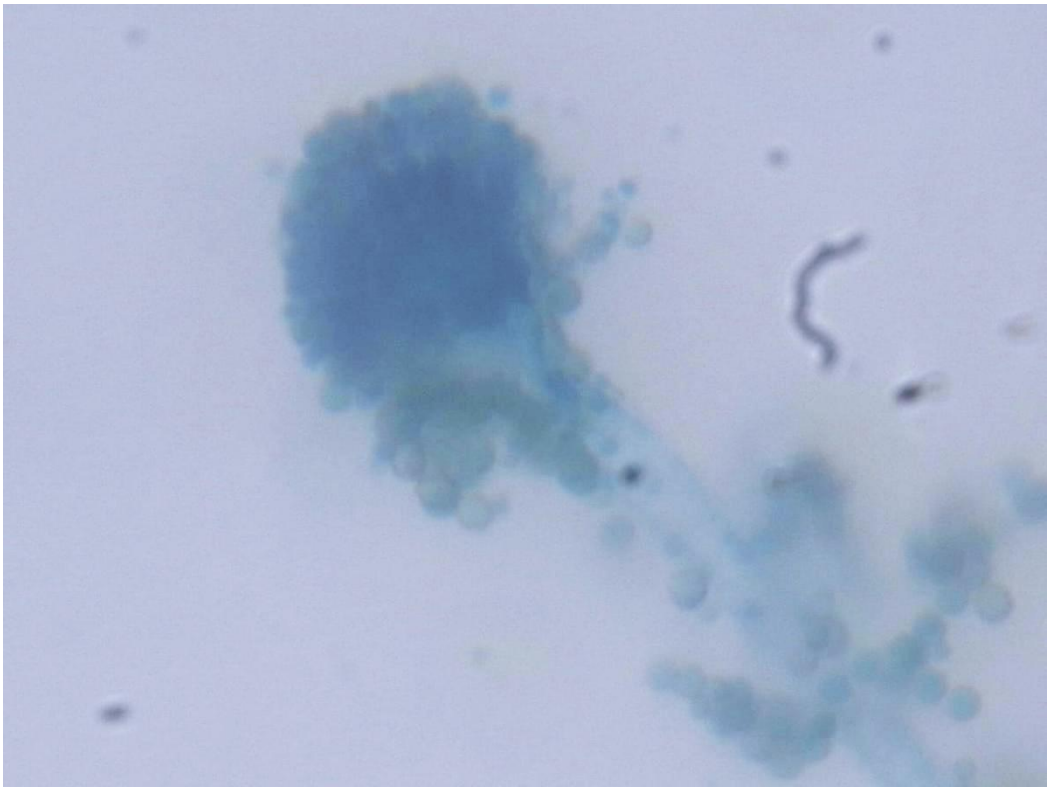

Figure 4.2. Morphological features of *Basidiobotrys* sp. (Isolate F2)

Microscopic view showing dark, elongate-clavate conidiophores with an enlarged apex, producing hyaline, ellipsoid, one-celled conidia on short denticles/pegs (LPCB stain, x100 magnification).

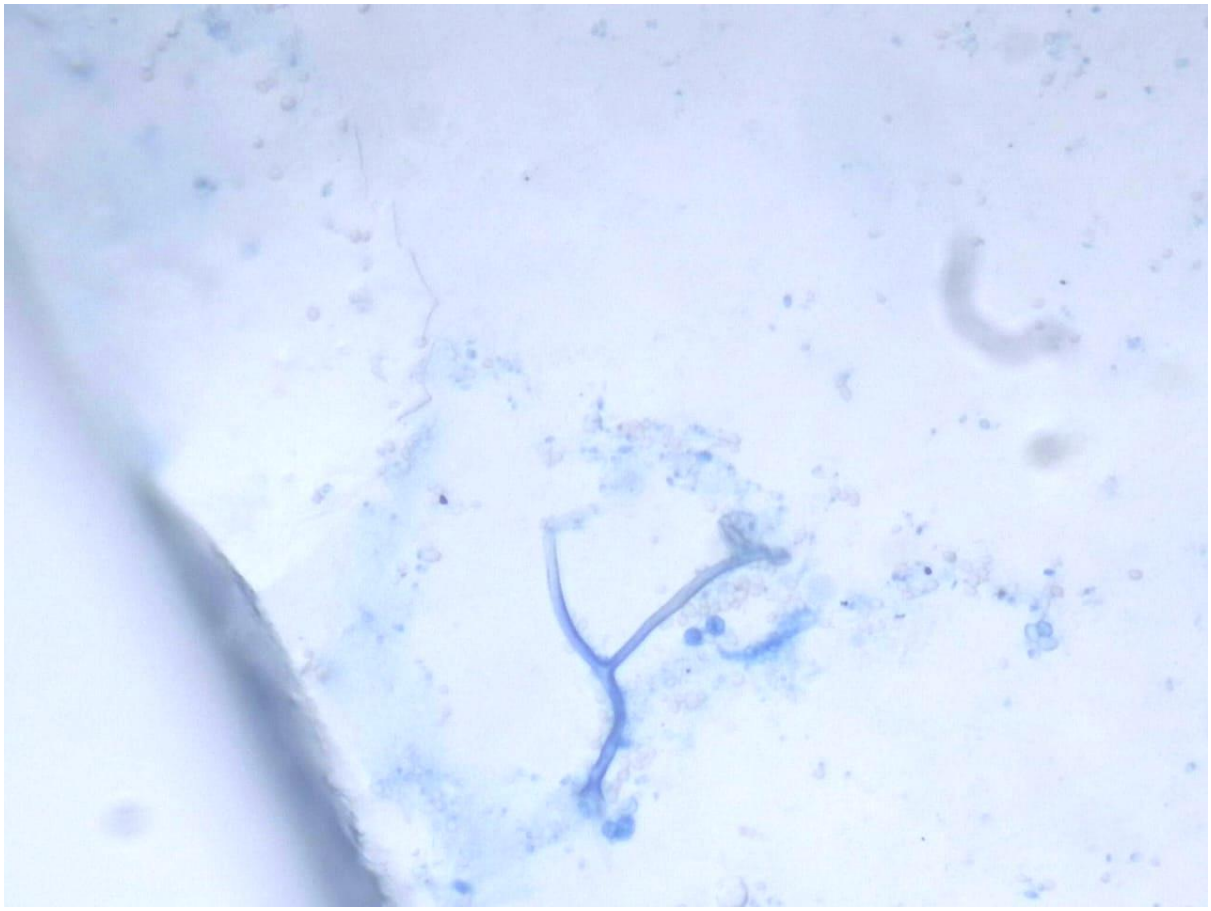

Figure 4.34 Morphological features of *Scopulariopsis sp.* (Isolate F3)

Microscopic view showing branched septate hyphae bearing annellides, forming chains of ovoid/ellipsoidal conidia with a flattened or truncate base by basipetal succession (LPCB stain, x40 magnification).

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

Filamentous fungi including *Scopulariopsis* sp., *Basidiobotrys* sp. and *Rhopalomyces* sp. were identified from the decaying cocoyam studied where they may be agents responsible for the biodegradation of the tubers involved. These findings are contrary to that of Azubuike *et al*. (2023) who isolated *Aspergillus niger, Rhizopus stolonifer, Mucor circinelloides, Penicillium expansum and Fusarium oxysporum* from decayed cocoyam cormels in Oji River, Enugu State. Similarly, it doesn't follow pattern of the findings of Mubarak *et al*. (2021) who reported *Aspergillus niger, Mucor racemosus, Aspergillus flavus, Fusarium oxysporum, Rhizopus stolonifer, Rhizopus oryzae, and Penicillium expansum* as the major spoilage fungi of cocoyam in Kebbi State. The differences in fungal types observed in this study compared to these earlier reports may be due to differences in geographical location, environmental conditions, harvesting methods, storage practices and sources of contamination, all of which have been shown to influence the composition of spoilage microflora associated with food commodities (Karanth *et al.,* 2023).

Post-harvest handling is generally considered to be important in determining the pattern of fungal spoilage. Physical injury sustained during harvesting, transportation and storage creates entry points for spoilage organisms to invade cocoyam tissues (Azubuike *et al.,* 2023). According to Raju (2023), mechanical damage hampers the protective outer layers of tubers and hastens microbial colonisation and deterioration. Therefore, the fungi isolated in this study may have been introduced via breakage points sustained during harvesting or handling, and then favourable storage conditions may have promoted their growth and spread.

The isolation of *Scopulariopsis sp.* in this study is not an anomaly as members of this genus have been identified as food associated spoilage fungi. Scopulariopsis species were listed by

Rolland *et al*. (2024) in a database of food spoilage fungi, reflecting their occurrence in food and storage environments. Similarly, *Rhopalomyces elegans* has been isolated from soil habitats (Durán *et al.,* 2019) and the genus has been recorded as a nematode-associated fungus that is commonly found in soil habitats (Rämä and Quandt 2021). This means that the Rhopalomyces isolated in this study could have been introduced by soil contamination during harvest, especially if the tubers were collected directly from the field and handled without proper cleaning. Cocoyam is grown underground, so contact with fungi in the soil is inevitable, and any injury to the periderm provides an entry point for fungi (Azubuike *et al.,* 2023). In this respect too, the isolation of *Basidiobotrys sp.* indicates a possible association with plant or environmental contaminants. The occurrence of Basidiobotrys in fungi associated with plant materials was reported by Sir and Ceriani Nakamurakae (2024), indicating members of the genus can be found on decaying organic substrates. Therefore findings of this study suggests that fungal biodeterioration of cocoyam in Abakpa Market may have resulted from a combination of harvesting injuries, soil contamination, inadequate sanitation, and storage conditions favourable to fungal growth. Such determinants have been identified as the most important factors for post-harvest losses in root and tuber crops (Azubuike *et al.,* 2023; Raju, 2023).

The quality and economic value of cocoyam is affected by the deterioration caused by these fungi as they affect the texture, appearance and functioning properties of cocoyam as a soup thickener. Moreover, the growth of mould on edible tubers is a food safety issue as many of the fungi associated with tuber spoilage are capable of producing biologically active secondary metabolites that could pose health hazards to consumers (World Health Organization, 2023). From this, the importance of proper harvesting, handling, sanitation and storage practices in reducing fungal contamination and extending the shelf life of cocoyam becomes clear.

Furthermore, Among these isolates, Scopulariopsis is of particular public health concern because it is recognized as an opportunistic fungal pathogen capable of causing pulmonary disease, superficial and invasive infections, especially in immunocompromised individuals (Pérez-Cantero and Guarro, 2020). Environmental molds have been identified by public health authorities as sources of invasive fungal infections which can gain access to the human body through inhalation of spores or traumatic inoculation into tissues, particularly among susceptible populations (CDC, 2024). In contrast, no evidence was identified from current medical literature indicating that Rhopalomyces or Basidiobotrys are established human pathogens. Their significance in the present study therefore is based on the role they play as spoilage agents for cocoyam. There is also an increasing global concern regarding antifungal resistance and emerging fungal pathogens, which helps make the point on the need for surveillance of pathogenic environmental fungi (WHO, 2022; Fisher and Denning, 2023). Moreover, the isolation of spoilage-associated fungi from decomposing cocoyam tubers suggests that the commodity had already reached a deteriorated stage where food quality and potential safety is bad, even though this study did not chemically evaluate mycotoxins. As a result, the existence of these fungi should be viewed as both an agricultural and financial issue as well as a public health issue that warrants better handling, storage, consumer education, and rejection of tubers that are obviously rotting (Karanth *et al.,* 2023; WHO, 2023).

5.2 Conclusion

This study was conducted to isolate and identify the fungal pathogens associated with post-harvest decay of cocoyam (*Colocasia esculenta*) used as soup thickener and sold at Abakpa Market in Enugu State. Its objectives were achieved through cultural and morphological analyses of fungal isolates from decayed cocoyam samples. Three filamentous genera of fungi, namely, *Scopulariopsis sp., Rhopalomyces sp.* and *Basidiobotrys sp.* were associated with the deterioration of the tubers. Extracellular enzymes secreted by these fungi can degrade starch

and other structural polysaccharides, which may affect the texture, consistency and thickening properties of cocoyam. In addition, fungal growth on cocoyam tubers development has serious implications for food safety. On the other hand, the fungal contamination of food commodities is well known as a possible public health risk. Finaly, the results of this study adds to the existing body of knowledge on the mycology of cocoyam spoilage in Nigeria by reporting the occurrence of *Scopulariopsis sp., Rhopalomyces sp. and Basidiobotrys sp*. in decayed cocoyam obtained from Abakpa Market. It emphasizes the significance of proper harvesting, handling, sanitation and storage practices in minimizing post-harvest fungal losses and maintaining the quality, safety and economic value of cocoyam.

5.3 Recommendations

Improved harvesting and field handling: Farmers should use less aggressive harvesting tools and handling practices to reduce cuts, bruises and skinning damage of cocoyam tubers. Protection of tubers from mechanical injury is very important because physical wounds are natural entry points for invasion of pathogens which greatly increases the rate of tuber deterioration and spoilage (Azubuike *et al.,* 2023; Raju, 2023).

Optimised storage environments: Market vendors must stop storing cocoyam in damp sacks, on bare ground or in poorly ventilated rooms. Instead, tubers should be cleaned of excess soil, cured after harvesting and stored in dry and well-aerated conditions to control humidity, increase shelf life and prevent fast deterioration (Raju, 2023).

Public health education and food safety messaging: Mould growth is closely associated with mycotoxin contamination, therefore, regulatory bodies and extension workers should work to educate the consumers on the dangers of eating visibly rotten tubers. Most mycotoxins are chemically stable and easily survive common food processing procedures.

Thus, public awareness should be clearly highlighted regarding the risks of mould-related spoilage and the potential of harmful toxin exposure (World Health Organization, 2023).

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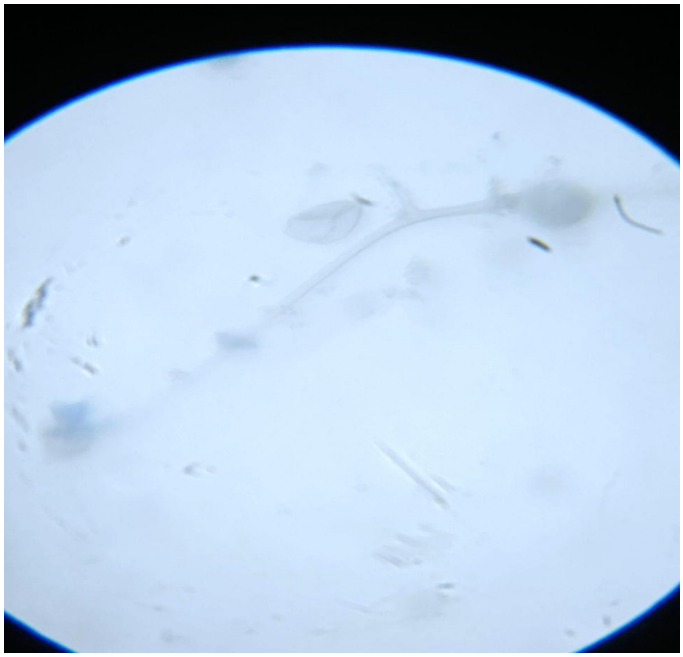
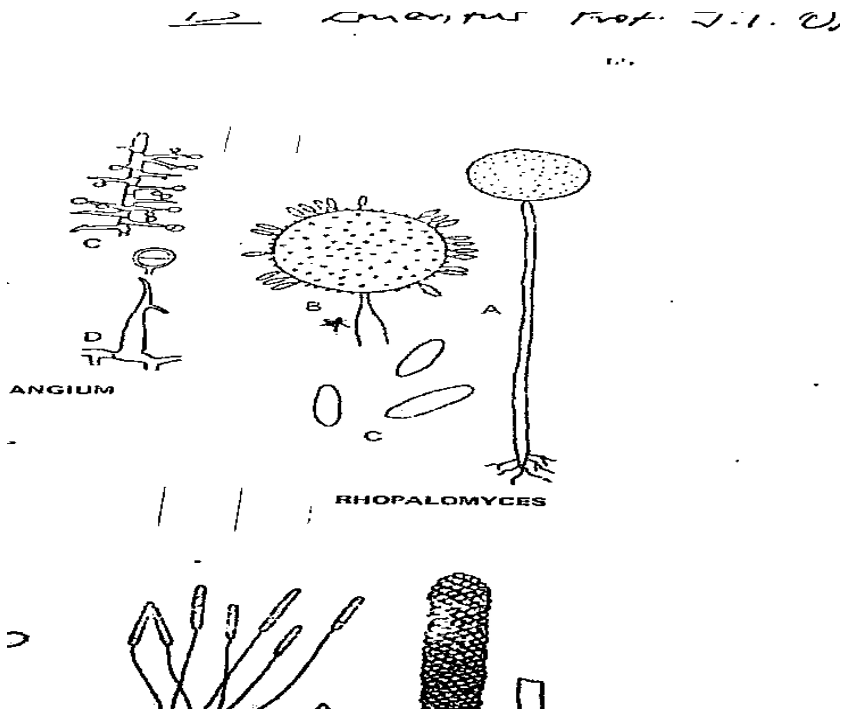
APPENDICES

FUNGAL IDENTIFICATION REFERENCE KEYS (MONOGRAPHS) 1

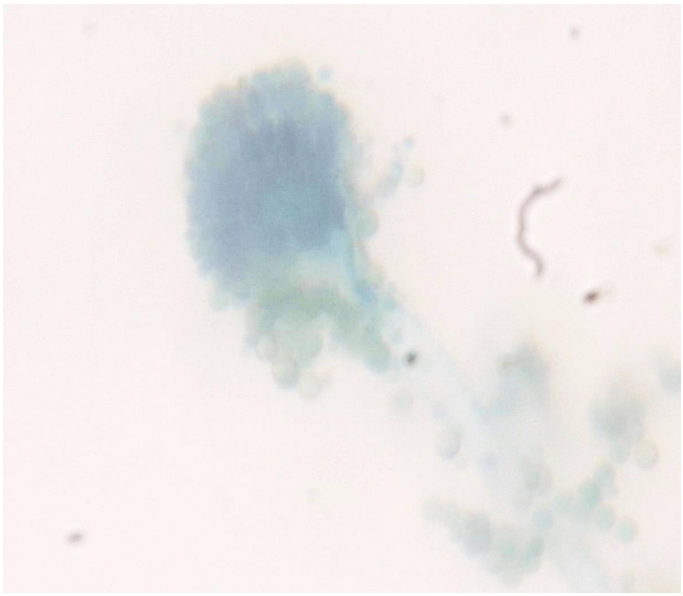


Appendix 1: Morphological features of *Rhopalomyces sp*. (Isolate F1)

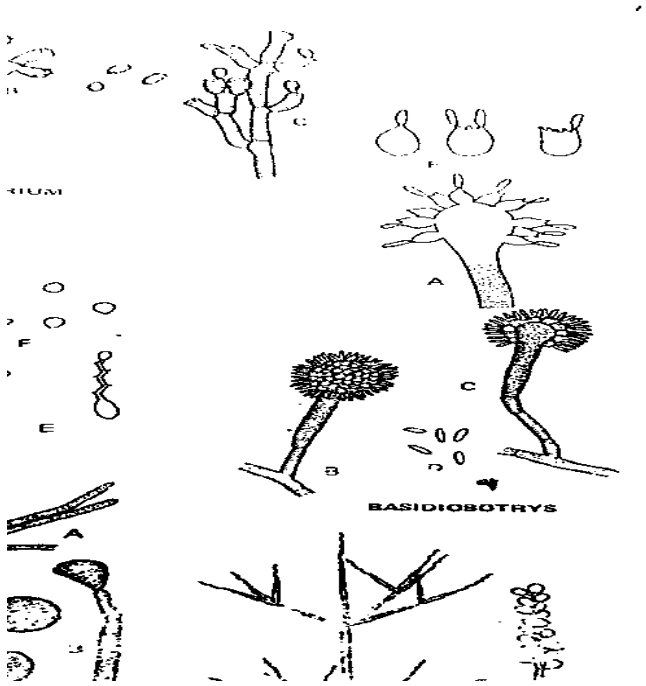


Appendix 2: Textbook reference -Morphological features of *Rhopalomyces sp.*

FUNGAL IDENTIFICATION REFERENCE KEYS (MONOGRAPHS) 2

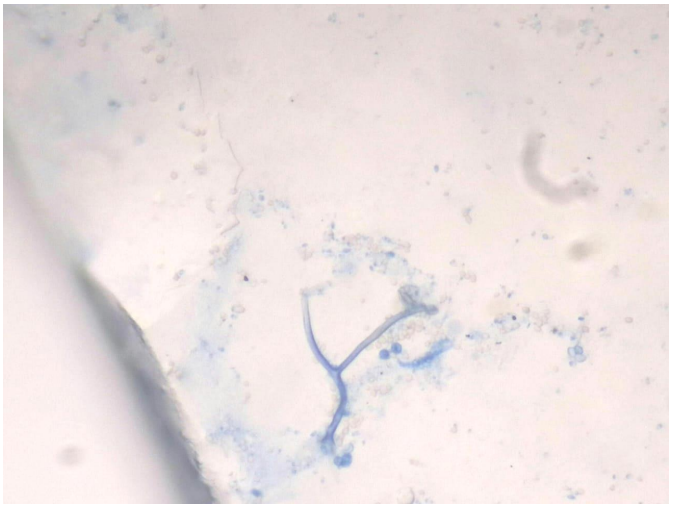


Appendix 3: Morphological features of *Basidiobotrys* sp. (Isolate F2)

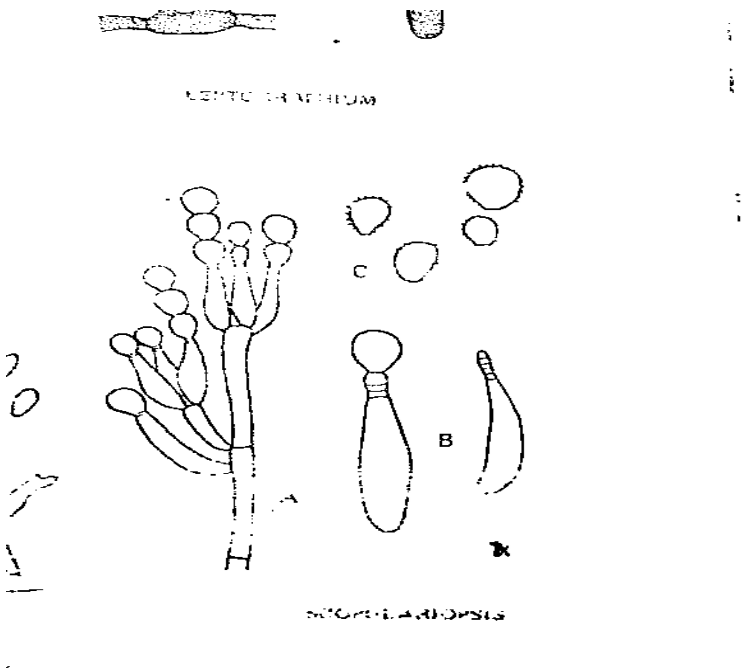


Appendix 4: Textbook reference - orphological features of *Basidiobotrys* sp. (Isolate F2)

FUNGAL IDENTIFICATION REFERENCE KEYS (MONOGRAPHS) 3



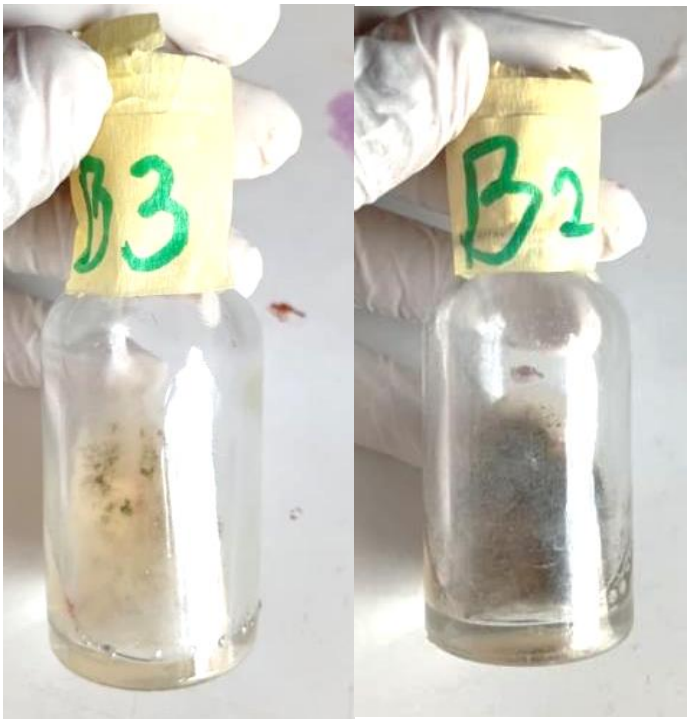
Appendix 5: Morphological features of *Scopulariopsis sp.* (Isolate F3)



Appendix 6: Textbook reference -Morphological features of *Scopulariopsis sp.* (Isolate F3)

LABORATORY PROCEDURAL PICTURES

Appendix 7: Image of initial culture in Culture media



Appendix 8: Image of Pure culture in Bijou bottles