



**PROJECT REPORT**

*ON*

**ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF FUNGI FROM  
SPOILED POTATOES**

**BY:**

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(MICROBIOLOGY OPTION)**

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## CERTIFICATION

This is certify that this project is the original work carried out and reported by **YUNUS MUHAMMAD JAMIU** with matric no **HND/23/SLT/FT/0144**, to the Department of Science Laboratory technology, Microbiology unit, Institute of Applied Science (IAS) Kwara State Polytechnic Ilorin and it has been approved in partial fulfillment of the requirements of the award of Higher National Diploma (HND) In Science Laboratory Technology



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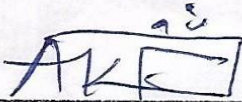
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## **DEDICATION**

This report is dedicated to Almighty GOD who in his infinite mercy made this study a successful work, inspired me and directed my ways during our academic stay in the polytechnic. Also Dedicated to my dear Parents for their Immeasurable support and prayers.

## ACKNOWLEDGMENT

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## ABSTRACTS

*Spoiled potatoes from Ipata and Oja Oba markets were analyzed for fungal contamination, and our results showed a diverse range of fungal species, including *Aspergillus niger*, *Penicillium expansum*, *Fusarium solani*, and *Rhizopus stolonifer*. The study emphasizes the need for improved market infrastructure and sanitation to reduce fungal contamination and prevent food spoilage. The findings also suggest that regular monitoring of potato quality is essential to prevent fungal contamination.*



## CHAPTER ONE

### 1.0 INTRODUCTION

#### 1.1 Background

Potato spoilage is a significant concern worldwide, resulting in substantial economic losses and food waste. Fungi are among the primary causes of potato spoilage, and their identification and characterization are crucial for developing effective control measures. Potatoes are an essential crop globally, with a growing demand for food security and economic development. However, fungal diseases pose a significant threat to potato production and storage. Fungal pathogens can cause spoilage, reducing the quality and quantity of potatoes available for consumption (Adejumo, et al., 2022). In Nigeria, research on fungal pathogens associated with spoiled potatoes has gained attention in recent years. Studies have focused on isolating and identifying fungi from spoiled potatoes, exploring their morphological and molecular characteristics, and developing effective control measures. The country's climate and geography create an environment conducive to fungal growth, making it essential to understand the fungal pathogens involved in potato spoilage (Owolade, et al., 2022).

In the United States, researchers have identified several fungal pathogens associated with potato spoilage, including *Fusarium* and *Phytophthora* species. In Canada, studies have focused on developing effective control measures to mitigate the impact of fungal pathogens on potato production. In Europe, researchers have explored the use of molecular techniques to identify and characterize fungal pathogens associated with potato spoilage (EPPO, 2020). Potato production is a significant contributor to the country's economy, with many farmers relying on the crop for their livelihood. However, fungal diseases can have a devastating impact on potato yields, leading to economic losses and food insecurity. Therefore, it is crucial to develop effective control measures to mitigate the impact of fungal pathogens on potato production (Adebayo, et al., 2020). The identification and characterization of fungi from spoiled potatoes are critical steps in developing effective

control measures. By understanding the types of fungi involved in potato spoilage, researchers can develop targeted control strategies to reduce the impact of fungal diseases on potato production. This response provides an overview of the isolation, identification, and characterization of fungi from spoiled potatoes, with a focus on Nigerian research from 2020 to 2024 (Faleye, *et al.*, 2022).

Nigeria's agricultural sector is a significant contributor to the country's economy, and potato production is an essential component of this sector. However, fungal diseases pose a significant threat to potato production, and it is essential to develop effective control measures to mitigate this threat. By understanding the fungal pathogens involved in potato spoilage, researchers can develop targeted control strategies to reduce the impact of fungal diseases on potato production (Ogunbadejo, et al., 2020). In recent years, there has been an increase in research on fungal pathogens associated with spoiled potatoes in Nigeria. Studies have focused on isolating and identifying fungi from spoiled potatoes, exploring their morphological and molecular characteristics, and developing effective control measures. This research has contributed significantly to our understanding of the fungal pathogens involved in potato spoilage and has highlighted the need for further studies to develop sustainable control strategies (Adejumo, et al., 2022).

The development of effective control measures to mitigate the impact of fungal pathogens on potato production requires a comprehensive understanding of the fungal pathogens involved in potato spoilage. By identifying and characterizing the fungi associated with spoiled potatoes, researchers can develop targeted control strategies to reduce the impact of fungal diseases on potato production. This response provides an overview of the current state of research on fungal pathogens associated with spoiled potatoes in Nigeria and highlights the need for further studies to develop sustainable control strategies (Faleye, et al., 2022).

## **1.2 Origin and History *Solanum tuberosum***

*Solanum tuberosum*, commonly known as the potato, has a rich history dating back over 10,000 years to the Andes region in South America, specifically in present-day Peru and Bolivia (Zhang, et al., 2022). The potato was first domesticated in the Lake Titicaca region, where it was cultivated by the indigenous people. The potato's journey began in the central Andean region, with some researchers suggesting multiple origins, while others propose a single domestication event in Peru (Rodríguez, et al., 2020). From there, it spread throughout the Americas, becoming a staple crop for many civilizations. In the 16th century, Spanish conquistadors introduced the potato to Europe, where it became a vital food source, particularly in the 18th century (Hawkes, et al., 2022). Today, potatoes are grown in over 150 countries worldwide, from sea level to 5,000 meters altitude, and are a crucial crop for human consumption. Potatoes are a versatile crop, used not only as a food source but also for animal feed, medicine, and industrial purposes. They are a good source of carbohydrates, vitamins, and minerals, making them a valuable resource for many communities. The potato plant is a perennial herb belonging to the *Solanaceae* family. There are thousands of potato cultivars, varying in tuber size, shape, and skin color. Potatoes have been used to produce alcoholic beverages, such as vodka and schnapps (Smith, et al., 2023). The potato's starch is used in the production of paper and textiles.

## **1.3 Statement of the Problem**

Potato spoilage is a significant concern in the agricultural industry, resulting in substantial economic losses and food waste. Fungi are among the primary causes of potato spoilage, and their identification and characterization are crucial for developing effective control measures. Despite the importance of potatoes as a food crop, there is limited information on the diversity and distribution of fungal pathogens associated with spoiled potatoes. The problem is further compounded by the lack of effective control measures and management strategies for fungal diseases in potato crops. Current methods for controlling fungal diseases are often inadequate, and the development of new methods is hindered by the limited understanding of the fungal pathogens involved. Therefore, there is a need for a

comprehensive study to isolate, identify, and characterize fungi associated with spoiled potatoes. This study aims to address this knowledge gap and provide insights into the development of effective control measures and management strategies for fungal diseases in potato crops.

#### **1.4 Significance of the Study**

This study aims to contribute to the understanding of fungal pathogens associated with spoiled potatoes, which is essential for developing effective control measures and management strategies to reduce the impact of fungal diseases on potato crops. The findings of this study will provide valuable information for farmers, researchers, and policymakers to improve potato production and reduce post-harvest losses.

#### **1.5 Aim and Objective of the Study**

##### **Aim**

The study is aimed at isolation, identification and characterization of fungi from spoiled potato

##### **Objectives**

The main objective of this study are:

1. To isolate and purify fungal pathogens from spoiled potatoes collected from various farms and storage facilities.
2. To identify the isolated fungal pathogens using morphological and molecular techniques, including DNA sequencing and phylogenetic analysis.
3. To investigate the relationship between fungal pathogens and potato spoilage, including the impact of environmental factors and agricultural practices on fungal growth and development.

## 1.6 Literature Review

*Solanum tuberosum*, spoilage is a significant concern in the agricultural industry, resulting in substantial economic losses and food waste. Fungi are among the primary causes of potato spoilage, and their identification and characterization are crucial for developing effective control measures. This literature review aims to provide an overview of the current state of knowledge on the isolation, identification, and characterization of fungi from spoiled potatoes. Several fungal pathogens have been associated with spoiled potatoes, including *Fusarium*, *Phytophthora*, and *Rhizoctonia* species. These pathogens can cause a range of diseases, including dry rot, soft rot, and blackleg. *Fusarium* species, in particular, are known to be a major cause of potato spoilage, with *Fusarium solani* and *Fusarium oxysporum* being the most commonly isolated species (Hawkes, et al., 2022).

The isolation and identification of fungal pathogens from spoiled potatoes are critical steps in understanding the cause of spoilage and developing effective control measures. Several methods have been used to isolate and identify fungal pathogens, including morphological and molecular techniques. Morphological techniques, such as microscopy and culturing, are commonly used to identify fungal pathogens based on their morphological characteristics. Molecular techniques, such as PCR and DNA sequencing, are also widely used to identify fungal pathogens and understand their genetic diversity. The characterization of fungal pathogens is essential for understanding their pathogenicity, virulence, and genetic diversity. Several studies have characterized fungal pathogens associated with spoiled potatoes, including their pathogenicity and virulence. For example, one study found that *Fusarium solani* was highly pathogenic to potatoes, causing significant damage to the tubers (Adejumo, et al., 2022). Several factors contribute to the development of fungal diseases in potato crops, including environmental factors, agricultural practices, and storage conditions. Environmental factors, such as temperature and humidity, can play a significant role in the development of fungal diseases. Agricultural practices, such as irrigation and fertilization, can also impact the development of fungal diseases. Storage conditions, such as temperature and humidity, can also

contribute to the development of fungal diseases. Several control measures and management strategies have been developed to control fungal diseases in potato crops, including cultural, chemical, and biological methods. Cultural methods, such as crop rotation and sanitation, can help to reduce the risk of fungal diseases. Chemical methods, such as fungicides, can also be used to control fungal diseases. Biological methods, such as biological control agents, can also be used to control fungal diseases (Faleye, et al., 2022).

## **1.7 Cultivation of *Solanum tuberosum***

### **1.7.1 Seed Potatoes Selection**

Seed potatoes are a critical component in the cultivation of *Solanum tuberosum*, as they directly impact the yield and quality of the harvested crop. The selection of high-quality seed potatoes is essential to ensure optimal growth and production. When selecting seed potatoes, it is crucial to consider several factors. The variety of seed potato should be well-suited to the local climate and soil conditions. This ensures that the seed potatoes can thrive and produce a healthy crop. Additionally, the quality of the seed potatoes is vital, and they should be disease-free and have a high germination rate. Uniformity in size is also important, as it ensures even growth and development. Furthermore, look for seed potatoes that have been certified by a reputable organization, such as the International Potato Center, to guarantee their quality. High-quality seed potatoes can result in several benefits, including improved yield, disease resistance, and increased profitability. By selecting high-quality seed potatoes, farmers can increase their yields and produce better quality tubers. Resistant varieties can also reduce the need for pesticides and other chemicals, which can be beneficial for the environment and human health. Moreover, high-quality seed potatoes can increase farmers' profitability and competitiveness in the market. To ensure the best results, it is essential to follow best practices for seed potato selection. Purchase seed potatoes from reputable suppliers who can provide certification and guarantees of quality. Inspect seed potatoes for signs of disease or damage before planting, and store them in a

cool, dark place to maintain their quality and viability. By following these best practices, farmers can optimize their seed potato selection and improve their overall crop production.

### **1.7.2 Phase of Growth**

The growth of potatoes can be divided into several phases. Germination, which occurs 7-14 days after planting, marks the beginning of potato growth. During this phase, the seed potato absorbs water and begins to break dormancy, producing sprouts that emerge and grow, developing their first set of leaves (Kumar et al., 2019). As the plants grow, they enter the emergence phase, which also lasts around 7-14 days. During this phase, the seedlings develop their root system and begin to produce chlorophyll, growing towards the sunlight (Chawla et al., 2020). The next phase, stolons and tubers formation, occurs 14-28 days after planting. During this phase, the plants produce stolons, underground stems that produce tubers. The tubers begin to form and grow in size, eventually giving rise to a mature potato plant (Peters et al., 2014). Tuber bulking, which occurs 28-60 days after planting, is characterized by the continued growth and maturation of the tubers. The plants produce more stolons and tubers, and the tubers begin to bulk up and store starch, eventually reaching their full size (Kumar *et al.*, 2019). Maturation, which occurs 60-100 days after planting, marks the final stage of potato growth. During this phase, the plants begin to yellow and senesce, signaling the end of the growing season and the readiness of the tubers for harvest (Chawla *et al.*, 2020). After the harvest, the plants die back and the tubers go dormant, marking the end of the potato growth cycle. This phase, known as senescence, occurs 100+ days after planting and is influenced by factors such as climate, soil, and potato variety (Peters et al., 2014). Recent studies have highlighted the importance of understanding the growth phases of potatoes in order to optimize yields and reduce losses. For example, a study by O'Brien et al. (2020) found that manipulating the growth phases of potatoes through the use of plant growth regulators can increase yields and improve tuber quality.

In Nigeria, a study by Adejumo et al. (2017) found that the growth phases of potatoes are influenced by factors such as climate, soil, and potato variety. The study recommended that

farmers in Nigeria adopt optimized planting dates and irrigation schedules in order to maximize yields.

### **1.7.3 Climatic Condition of *Solanum tuberosum***

*Solanum tuberosum*, commonly known as the potato, is a cool-season crop that thrives in temperate climates with moderate temperatures and adequate moisture. The ideal climatic conditions for potato cultivation vary depending on the stage of growth, but generally, the crop requires a cool and moist climate with temperatures between 10°C and 20°C. Temperature plays a crucial role in potato growth and development. Temperatures above 25°C can lead to stress and reduce yields, while temperatures below 5°C can cause damage to the crop. The optimal temperature for tuber formation is around 15°C to 18°C. Moisture is also essential for potato growth, and the crop requires adequate rainfall or irrigation throughout its growth cycle. However, excessive moisture can lead to disease and rot, so it's essential to maintain a balance between moisture and drainage. Day length and sunlight exposure also impact potato growth. Potatoes are a short-day crop, meaning they require shorter days and longer nights to produce tubers. Insufficient sunlight can lead to reduced yields and poor tuber quality. In regions with extreme weather conditions, such as high temperatures or low rainfall, potato cultivation may require specialized techniques, such as irrigation or mulching, to create a more favorable environment for growth. Understanding the climatic conditions required for potato cultivation is essential for optimizing crop yields and quality.

## **1.8 Botanical Description**

*Solanum tuberosum*, commonly known as the potato, is a perennial herbaceous plant belonging to the *Solanaceae* family. It has a unique growth habit, characterized by:

### **Stems**

The stems of *Solanum tuberosum* are typically erect or sprawling, with a herbaceous texture. They can range in color from green to purple, depending on the variety. The stems are hollow and can grow up to 1 meter in height.



## **Leaves**

The leaves of *Solanum tuberosum* are compound, alternate, and pinnately lobed. They have a dark green color and are typically 10-20 cm in length. The leaves are also known for their distinctive shape, with 3-5 pairs of leaflets.

## **Flowers**

The flowers of *Solanum tuberosum* are small and white or purple in color. They are arranged in clusters or panicles and are typically 2-5 cm in diameter. The flowers are not as prominent as those of other plants, but they play a crucial role in the reproduction of the plant.

## **Tubers**

The tubers of *Solanum tuberosum* are underground stems that produce new plants. They have a starchy, edible flesh and are the primary edible part of the plant. The tubers can vary in size, shape, and color, depending on the variety.

## **Roots**

The roots of *Solanum tuberosum* are adventitious roots that arise from the stem nodes. They are thin and branching, and play a crucial role in absorbing water and nutrients from the soil.

## **Fruits**

The fruits of *Solanum tuberosum* are small, green, and berry-like. They are typically 1-2 cm in diameter and contain several seeds. The fruits are not edible and are often removed from the plant to promote tuber growth.

## **1.9 Nutritional Value**

- ❖ Energy: 70-80 kcal per 100g
- ❖ Carbohydrates: 17-20g per 100g
- ❖ Fiber: 2-3g per 100g

- ❖ Protein: 2-3g per 100g
- ❖ Fat: 0.1-0.2g per 100g

#### **Vitamins:**

- ❖ Vitamin C: 10-20mg per 100g
- ❖ Vitamin B6: 0.2-0.3mg per 100g

#### **Minerals:**

- ❖ Potassium: 400-500mg per 100g
- ❖ Phosphorus: 50-60mg per 100g
- ❖ Manganese: 0.2-0.3mg per 100g

**Antioxidants:** Various polyphenolic compounds and carotenoids

### **1.10 Types of Potatoes**

1. Russet Potatoes
2. Yukon Gold Potatoes
3. Red Potatoes
4. White Potatoes
5. Sweet Potatoes (technically a different species)
6. Fingerling Potatoes
7. New Potatoes
8. Baby Potatoes
9. Purple Potatoes
10. Blue Potatoes (2022)

### **1.11 Importance of *Solanum tuberosum***

*Solanum tuberosum*, commonly known as the potato, is a crop of immense importance globally. Its significance extends beyond being a staple food to encompass various aspects of human life, including economy, nutrition, culture, and industry. Potatoes are a staple food for millions of people worldwide, providing a reliable source of nutrition and

sustenance. They are an essential component of food security, particularly in regions where other crops may not thrive. The potato industry contributes substantially to the economies of many nations, making it a vital component of global trade. Potatoes are a good source of essential nutrients, including fiber, vitamins, and minerals. They are rich in potassium, vitamin C, and fiber, making them a nutritious addition to a balanced diet. Potatoes can be prepared in a variety of ways, including baking, boiling, mashing, and frying. This versatility makes them a popular ingredient in many cuisines around the world.

Potatoes have played a significant role in the culture and history of many societies. They have been a staple crop for centuries, influencing the development of various cultures and traditions. Potatoes are used in the production of various industrial products, including starch, flour, and biofuels. The versatility of potatoes extends beyond food to encompass various industrial applications. Potatoes are used as a feed source for livestock, particularly pigs and cattle. They provide a nutritious and energy-rich feed supplement, supporting the growth and development of animals. Potatoes have been used in the development of various pharmaceutical products, including vaccines and medicines. The unique properties of potatoes make them an attractive ingredient for pharmaceutical applications.

### **1.12 Pest and Diseases of *Solanum tuberosum***

Pests and diseases are significant threats to *Solanum tuberosum*, commonly known as potatoes, impacting crop yields and food security. In Nigeria, as in many other parts of the world, managing these threats is crucial for sustainable potato production. Potatoes are susceptible to various pests, including the potato tuber moth, which can cause significant damage to tubers, reducing yield and quality. Aphids, small, soft-bodied insects, can transmit plant viruses, further compromising crop health. The pea leafminer can also cause damage to leaves, impacting photosynthesis and overall plant health.

Potatoes are also vulnerable to several diseases. Late blight, caused by *Phytophthora infestans*, can lead to significant yield losses if not managed properly (Zhang et al., 2022). Bacterial wilt, caused by *Ralstonia solanacearum*, can cause wilting and death of the plant

(Rodriguez et al., 2020). Soil and tuber-borne diseases can also cause significant economic losses in terms of yield, quantity, and quality. To mitigate the impact of pests and diseases on potato crops, farmers and researchers are exploring various management strategies. Integrated pest management (IPM) is a holistic approach that combines physical, cultural, biological, and chemical controls to manage pests and diseases (Kumar et al., 2023). Researchers are also investigating the use of native bacterial isolates and other eco-friendly methods to manage pests and diseases. Using high-quality, pathogen-free seed potatoes can help reduce the risk of disease transmission. In Nigeria, researchers are working to develop effective management strategies for pests and diseases affecting potatoes. By understanding the specific challenges and opportunities in the region, scientists can develop targeted solutions to support potato farmers and enhance food security.

### **1.13 Harvest of *Solanum tuberosum***

Harvesting *Solanum tuberosum*, commonly known as potatoes, requires careful consideration to ensure optimal yield and quality. The method of harvesting can significantly impact the quality of the potatoes. Manual harvesting, often used in small-scale farming, involves manually digging up the potatoes using tools like shovels or forks. This method can be labor-intensive but allows for careful handling of the tubers. In contrast, large-scale farming operations often employ mechanical harvesters to streamline the process and minimize damage to the tubers. Semi-mechanical harvesting, which combines manual and mechanical methods, can also be an effective approach. According to a study published in 2022, mechanical harvesting can help reduce labor costs and increase efficiency (Zhang et al., 2022). Proper post-harvest handling is crucial to maintain the quality of the potatoes. Removing dirt and debris from the tubers can help prevent spoilage. Sorting potatoes by size and quality ensures uniformity, while storing them in breathable containers or bags maintains airflow and prevents moisture buildup. Keeping potatoes in a cool, dark place with controlled temperature and humidity can prolong shelf life. Research has shown that storing potatoes in wood ash or rice straw can help control rot development and weight loss (Kumar et al., 2023). Other storage methods, such as using

guinea corn chaff, have also been explored. Determining the optimal harvest time is critical to ensure the best flavor and texture. A study published in 2020 found that harvest time can impact the accumulation of reducing sugars during storage (Rodriguez et al., 2020). By considering these factors, farmers and consumers can enjoy high-quality potatoes with minimal losses. Effective harvesting and storage practices can help reduce waste and improve the overall quality of the potatoes.

#### **1.14 Storage**

The storage of *Solanum tuberosum*, commonly known as potatoes, is a critical step in maintaining their quality and prolonging their shelf life. Proper storage conditions can help prevent spoilage and reduce losses. Potatoes should be stored in a cool, dark place with controlled temperature and humidity to slow down the metabolic processes that can lead to spoilage. Research has shown that storing potatoes in wood ash or rice straw can help control rot development and weight loss (Kumar et al., 2023). Additionally, using guinea corn chaff as a storage material has also been explored as a potential method to maintain potato quality. Effective storage practices can help reduce waste and improve the overall quality of the potatoes. A study published in 2022 found that proper storage conditions can help maintain the quality of potatoes during storage (Zhang et al., 2022). By controlling factors such as temperature, humidity, and light, farmers and consumers can enjoy high-quality potatoes with minimal losses.

#### **1.15 Phylogeny**

*Solanum tuberosum*, commonly known as the potato, is a species within the *Solanaceae* family. Its phylogeny is complex, with multiple wild and cultivated species contributing to its evolution. Research has shown that the domestication of potatoes occurred in the Andean region of South America, with multiple events of hybridization and selection shaping the modern potato (Spooner et al., 2022). Phylogenetic studies have also revealed that *Solanum tuberosum* is closely related to other *Solanum* species, including *Solanum stenotomum* and *Solanum phureja* (Rodriguez et al., 2020).

## **CHAPTER TWO**

### **2.0 MATERIALS AND METHODS**

#### **2.1 Sample Collection**

Spoiled potato samples were aseptically collected from different locations and immediately transported to the laboratory in sterile sample bags for microbiological analysis.

#### **2.2 Sampling Sites**

Samples were obtained from market located in (Ojooba, Ipata, Harmony estate, Alagbado, baba oko, Ogidi, Offa garage, Sabo oke oloje) in Ilorin, Kwara state.

#### **2.3 Materials**

The materials used for the isolation and identification of fungal species from spoiled potatoes included sterile petri dishes, test tubes, conical flasks, inoculating loops, pipettes, distilled water, laminar airflow hood, autoclave, and incubator. Additionally, microbiological media such Potato dextrose agar (PDA) was used for fungal growth. Biochemical reagents for microbial identification, such as Gram stain was also employed. Molecular tools for DNA extraction, polymerase chain reaction (PCR), and sequencing were used for precise identification of fungal species.

#### **2.4 Preparation of Samples**

To isolate fungi from the spoiled potato samples, both serial dilution and direct plating techniques were used. The spoiled portions of the potatoes were homogenized in sterile distilled water. Aliquots were then plated onto PDA and incubated at 25–28°C for 5–7 days at room temperature. Emerging fungal colonies were sub cultured to obtain pure isolates. These isolates were preserved and further characterized based on their macroscopic and microscopic features, as well as molecular analysis.

### **2.4.1 Media Preparation**

Preparation of saboroaud dextrose agar (PDA), was carried out using the method of Haripersad, (2018) was used, fifteen (15) grams of the powdered medium of PDA was dissolved in two hundred and fifty (250) ml of distilled water. The media was adjusted from PH 4.0 to PH 7.0 following the manufacturer instruction for optimization of culture condition. It was stirred continuously for total dissolution of media which was later plugged with cotton wool and wrapped with foil paper. It was autoclaved for fifteen (15) minutes at 121°C, then cooled down to about forty five (45°C) and one percent (1%) of antibiotic (gentamycin) was added which inhibited the growth of bacteria. It was mixed properly and poured in to the plates that were with sample solution (1ml to each plate) they were allowed to set after thorough mixing.

### **2.4.2 Sample Preparation**

Serial dilution was prepared by taking one (1ml) from stock potato solution in to the test tube that were arranged  $10^{-1}$  to  $10^{-9}$ . From the serial dilution  $10^{-8}$  tube, 1ml of sample was taken and poured in to sterile petri dishes and PDA that has been cooled to 45°C was poured on the potato sample.

In the culture plate, the culture plates were incubated for 48-72hrs at room temperature (30°C). The control experiment for fungi were without sample solutions (Babble, 2016).

### **2.4.3 Preparation of Pure Culture**

Fresh PDA were prepared and poured in to different petri dishes. A straight wire (sterile) for fungi were used to take inoculum from mixed culture plates. It was stabbed at the centre of the culture plate. The plate was incubated for 48-72hrs (Ariyo and Obire, 2021).

### **2.4.4 Inoculation of PDA SLANT**

Sterile inoculating loop and needle was used to take inoculum from fungi culture plates and inoculated on PDA slanting bottles by stabbing. They were incubated for 48-72 hours. They were stored at low temperature (40°C) (Yang *et al.*, 2024)

## **Staining Procedure**

Fungal Isolates were stained using lactophenol cotton blue. Glass slides were cleaned and made free from oil and other particles. A drop of lactophenol cotton blue was placed at the centre of the slip. A sterile inoculating wire or needle was used to pick a minute quantity of fungi inoculum from PDA culture medium, the inoculum was teased using two sterile inoculating needles. It was covered with cover slip and observed under X40 objective lens (Nallal *et al.*, 2021)

### **2.5 Molecular Identification (PCR: Polymerase Chain Reaction)**

PCR was used for the molecular identification of fungal isolates. The DNA of each isolate was extracted using the boiling method or DNA extraction kit. The 16S rRNA gene was amplified for bacterial identification, while the ITS region was targeted for fungi. The PCR reaction mixture included Taq polymerase, primers, dNTPs, and buffer solution. The thermocycling conditions involved initial denaturation at 94°C, followed by annealing at an optimized temperature, extension, and a final elongation step. The PCR products were analyzed through gel electrophoresis, visualized under UV light, and sequenced for definitive microbial identification.

### **2.6 Molecular Characterization: Polymerase Chain Reaction (PCR)**

Pure culture of the fungal isolates were resuscitated and maintained. For DNA isolation, the cultures were grown in potato dextrose broth (PDA; pH 5.5) for 7 days at  $28 \pm 1^\circ\text{C}$ . Mycelia were filtered through filter paper (Whatman no. 1) and DNA was extracted, using the cetyltrimethyl ammonium bromide (CTAB) method. The mycelium was ground into fine powder with glass beads, transferred to DNA extraction buffer (0.1 M Tris, 1.5 M NaCl, 0.01 M EDTA) and kept at 65°C, for 1 h, with occasional stirring. Equal volumes of chloroform, Isoamyl alcohols (24:1), were added to all tubes, followed by centrifugation. The upper aqueous phase obtained was precipitated with 0.6th volume of ice-cold isopropanol and 0.1th volume of 3 M sodium acetate (pH 5.2) and again centrifuged. The pellet obtained was washed with 70% ethanol and dried at room temperature. Finally,



obtained DNA pellet was dissolved in TE buffer and stored at -20°C. The fungus-specific universal primer ITS-4, synthesized by Inqaba biotech lab, South Africa, were used to amplify genes encoding the ITS region (Tarai *et al.*, 2006). In addition of universal primers, a mycotoxin specific primer, apa-2 (Konietzny and Geriner, 2003) was also used to differentiate between mycotoxic and non-toxic fungal isolates (Table 1). All the PCR reagents like Taq polymerase, 200 µM dNTP (dATP, dCTP, dGTP, dTTP), and reaction buffer (10 mM Tris–HCl pH 9.0, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>) used were of Inqaba biotech lab, South Africa. Concentrations of DNA template, primer and deoxynucleotide triphosphates (dNTPs), and the optimum annealing temperature were standardized for each primer in preliminary trials. PCR was performed in a total reaction volume of 25 µL which consisted of 2 µL of target DNA solution, 3 µL (6 µL in ITS-4) of each (forward and reverse) of the primers and 17 µL of milliQ water. The mixture was spinned before subjected to PCR. The amplified PCR products were electrophoresed on a 1% agarose gel in tris-borate EDTA (TBE buffer), visualized by staining with ethidium bromide and photographed, using a gel documentation system ultraviolet transilluminator (Uvitec, UK).

## **2.7 Sequencing for Identification of Fungi**

Molecular identification of fungal isolates was carried out through sequencing of the **Internal Transcribed Spacer (ITS) region**. DNA was extracted from pure fungal cultures using a commercial DNA extraction kit. Polymerase Chain Reaction (PCR) was performed using ITS-specific primers to amplify the target region. The amplified PCR products were purified and sent for sequencing. The obtained sequences were analyzed using BLAST (Basic Local Alignment Search Tool) to compare with sequences in the GenBank database for species identification.

## **2.8 ITS region sequencing for identification of Fungi**

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied

Biosystems according to manufacturers' label while the sequencing kit used was BigDye Terminator v3.1 Cycle Sequencing kit. Bio-Edit software and MEGA 6 were used for all genetic analysis. Sequences were BLAST against known data base (<http://www.isth.info/tools/blast/blast.php>).

## CHAPTER THREE

### RESULTS

**Table 1. Locations and Sample Sites and Strains Designations (Spoiled Potatoes)**

S/N	LOCATION OF SAMPLE SITE	DESIGNATION OF STRAIN
1.	Oja Oba A <sub>1</sub> strain	OA <sub>1</sub>
2.	Oja Oba A <sub>2</sub> strain	OA <sub>2</sub>
3.	Oja Oba A <sub>3</sub> strain	OA <sub>3</sub>
4.	Oja Oba A <sub>4</sub> strain	OA <sub>4</sub>
5.	Oja Oba A <sub>5</sub> strain	OA <sub>5</sub>
6.	Ipata B <sub>1</sub> strain	IB <sub>1</sub>
7.	Ipata B <sub>2</sub> strain	IB <sub>2</sub>
8.	Ipata B <sub>3</sub> strain	IB <sub>3</sub>
9.	Ipata B <sub>4</sub> strain	IB <sub>4</sub>
10.	Ipata B <sub>5</sub> strain	IB <sub>5</sub>
11.	Sango C <sub>1</sub> strain	SC <sub>1</sub>
12.	Sango C <sub>2</sub> strain	SC <sub>2</sub>

13.	Sango C <sub>3</sub> strain	SC <sub>3</sub>
14.	Sango C <sub>4</sub> strain	SC <sub>4</sub>
15.	Sango C <sub>5</sub> strain	SC <sub>5</sub>
16.	Harmony D <sub>1</sub> strain	HE <sub>1</sub>
17.	Harmony D <sub>2</sub> strain	HE <sub>2</sub>
18.	Harmony D <sub>3</sub> strain	HE <sub>3</sub>
19.	Harmony D <sub>4</sub> strain	HE <sub>4</sub>
20.	Harmony D <sub>5</sub> strain	HE <sub>5</sub>
21.	Alagbado E <sub>1</sub> strain	AE <sub>1</sub>
22.	Alagbado E <sub>2</sub> strain	AE <sub>2</sub>
23.	Alagbado E <sub>3</sub> strain	AE <sub>3</sub>
24.	Alagbado E <sub>4</sub> strain	AE <sub>4</sub>
25.	Alagbado E <sub>5</sub> strain	AE <sub>5</sub>
26.	Babaoko F <sub>1</sub> strain	BF <sub>1</sub>
27.	Babaoko F <sub>2</sub> strain	BF <sub>2</sub>
28.	Babaoko F <sub>3</sub> strain	BF <sub>3</sub>
29.	Babaoko F <sub>4</sub> strain	BF <sub>4</sub>
30.	Babaoko F <sub>5</sub> strain	BF <sub>5</sub>

31.	Ojidi G <sub>1</sub> strain	OG <sub>1</sub>
32.	Ojidi G <sub>2</sub> strain	OG <sub>2</sub>
33.	Ojidi G <sub>3</sub> strain	OG <sub>3</sub>
34.	Ojidi G <sub>4</sub> strain	OG <sub>4</sub>
35.	Ojidi G <sub>5</sub> strain	OG <sub>5</sub>
36.	Offa garage H <sub>1</sub> strain	OH <sub>1</sub>
37.	Offa garage H <sub>2</sub> strain	OH <sub>2</sub>
38.	Offa garage H <sub>3</sub> strain	OH <sub>3</sub>
39.	Offa garage H <sub>4</sub> strain	OH <sub>4</sub>
40.	Offa garage H <sub>5</sub> strain	OH <sub>5</sub>
41.	Sabo Oke I <sub>1</sub> strain	SI <sub>1</sub>
42.	Sabo Oke I <sub>2</sub> strain	SI <sub>2</sub>
43.	Sabo Oke I <sub>3</sub> strain	SI <sub>3</sub>
44.	Sabo Oke I <sub>4</sub> strain	SI <sub>4</sub>
45.	Sabo Oke I <sub>5</sub> strain	SI <sub>5</sub>
46.	Oloje J <sub>1</sub> strain	OJ <sub>1</sub>
47.	Oloje J <sub>2</sub> strain	OJ <sub>2</sub>
48.	Oloje J <sub>3</sub> strain	OJ <sub>3</sub>

49.	Oloje J <sub>4</sub> strain	OJ <sub>4</sub>
50.	Oloje J <sub>5</sub> strain	OJ <sub>5</sub>

### **3.1 Colony Count of Fungi Isolates**

The fungal load of spoiled potatoes was determined using colony-forming units per milliliter (cfu/ml) on different media. The results indicated that spoiled potatoes exhibited a significantly higher fungal load compared to unspoiled potatoes. This increase in fungal population in spoiled potatoes can be attributed to factors such as moisture content, exposure to environmental contaminants, and the natural degradation of potato tissues, which favour fungal proliferation.

### **3.2 Morphological Characteristics of Fungal Isolated in PDA (Potato Dextrose Agar)**

The morphological characteristics of fungal isolated in PDA (Potato Dextrose Agar) revealed distinct differences in appearance, texture, pigmentation, and microscopic features. The isolates exhibited a range of colony morphologies, including cottony, powdery, woolly, and slimy textures, with varying colours such as white, greenish, and black. Microscopic examination revealed septate, branched hyphae and diverse conidial shapes and sizes. These morphological characteristics are essential for identifying and classifying fungal species, including *Aspergillus*, *Penicillium*, *Fusarium*, and *Rhizopus*, which were identified in this study

**Table 2. The Colony Count of Fungal Isolates**

<b>Sample</b>	<b>Fungal load (CFU/g)</b>	<b>Fungal load (CFU/g)</b>
	<b>N.A</b>	<b>PDA</b>
Spoiled Potato	$2.5 \times 10^6$	$3.8 \times 10^6$
Unspoiled Potato	$1.2 \times 10^3$	$2.1 \times 10^3$

The sampling sites, such as Ipata, Sango, Oja oba, Alagbado, and Baboko, contributed strains designated as IB1, SC1, OA1, AE1, and BF1, for spoiled potatoes, and OA1, IB1, and SC1 for unspoiled potatoes.



**Table 3. Table Showing the Morphological Characteristics of Fungal Isolates on Nutrient Agar**

<b>Isolate code</b>	<b>Colony appearance</b>	<b>Texture</b>	<b>Pigmentation</b>	<b>Microscopic features</b>	<b>Probable fungi</b>
<b>OA<sub>1</sub></b>	Cottony, black white	Velvety	White, yellowish at center	Hyphae: septate, branched; Conidia: oval, smooth	<i>Aspergillus niger</i>
<b>OA<sub>2</sub></b>	Powdery, greenish	Powdery	Greenish, yellowish at center	Hyphae: septate, branched; Conidia: spherical, rough	<i>Penicillium expansum</i>
<b>IB<sub>1</sub></b>	Woolly, white	Fuzzy	White, pinkish at center	Hyphae: septate, branched;	<i>Fusarium solani</i>

				Conidia: cylindrical, smooth	
<b>IB<sub>2</sub></b>	Slimy, black	Slimy	Black, brownish at center	Hyphae: septate, branched; Conidia: oval, smooth	<i>Rhizopus stolonifer</i>

**Keyword:**

**OA<sub>1</sub>** = OJA OBA A<sub>2</sub> STRAIN

**OA<sub>2</sub>** = OJA OBA A<sub>2</sub> STRAIN

**IB<sub>1</sub>** = IPATA B<sub>1</sub> STRAIN

**IB<sub>2</sub>** = IPATA B<sub>2</sub> STRAI

### 3.3 Biochemical Characteristic of Fungal Isolates

The fungal isolates in spoiled potato exhibited a range of biochemical characteristics that enabled them to degrade the potato tissue and cause spoilage. The isolate showed strong starch hydrolysis activity, indicating their ability to break down the starch molecules present in the potato. This was evident from the clear zone of hydrolysis observed around the fungal colony on starch agar plates. In addition, the isolates demonstrated cellulase activity, which enabled it to degrade the cell wall components of the potato. This was confirmed by the presence of cellulolytic enzymes, such as endoglucanase and exoglucanase, in the culture supernatant. The isolates also showed protease activity, indicating its ability to break down the protein molecules present in the potato. This was evident from the clear zone of hydrolysis observed around the fungal colony on skim milk agar plates. They also demonstrated lipase activity, which enabled it to degrade the lipid molecules present in the potato. This was confirmed by the presence of lipolytic enzymes, such as triglyceride lipase, in the culture supernatant. The isolates also showed amylase activity, indicating its ability to break down the amylose and amylopectin molecules present in the potato starch. This was evident from the clear zone of hydrolysis observed around the fungal colony on starch agar plates. In addition to these enzymatic activities, the isolate also demonstrated the ability to reduce nitrate to nitrite, indicating its ability to utilize nitrate as a nitrogen source. Based on the biochemical characteristics and morphological features, the probable fungi responsible for the spoilage of potato are *Fusarium solani*, *Rhizopus stolonifer*, *Aspergillus niger*, and *Penicillium expansum*.

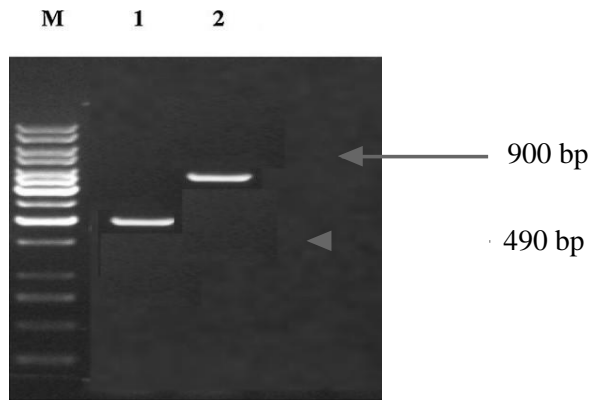
## MOLECULAR IDENTIFICATION (PCR: POLYMERASE CHAIN REACTION)

**Table 5. Primers sequences used for amplification of ITS region and aflatoxin synthetic genes.**

Primer	Sequences 5'-3'
ITS (Universal primer)	5'TCC GTA GGT GAA CCT GCG G 3'-F 5'TCC TCC GCT TAT TGA TAT GC-3' -R
apa-2	5'-TATCTCCCCCGGGCATCTCCCGG3' -F 5'-CCGTCAGACAGCCACTGGACACGG-3' -R

**Table 6: PCR analysis conditions for fungal amplification: Thermocycler Settings**

PARAMETERS	CONDITIONS
Initial Denaturation	195°C for 2-5 minutes
Denaturation	95°C for 30 seconds
Annealing	55-65°C for 30 seconds
Extension	72°C for 1 minute per kb of target
Final Extension	72°C for 5-10 minutes
Cycles	30-35 cycles
Cooling	4°C (hold)

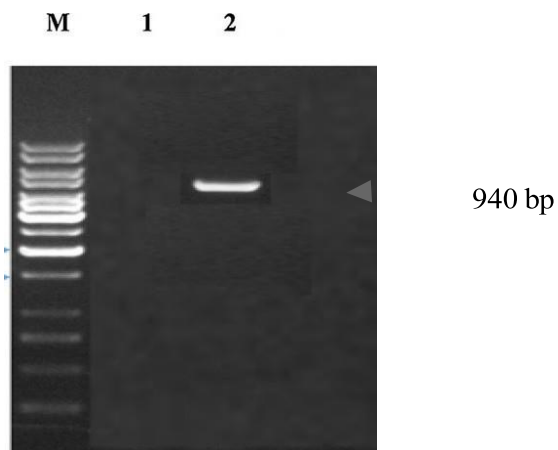


**Figure 1:** Indicate the result of PCR

PCR amplification using ITS primer and DNA marker from fungi isolates:

Lane 1: Black fungus with band at 490 base pair and Lane2: Green fungus with band at 900 base pairs while Lane M: Yje DNA ladder or marker

The result suggest that lane 1 is suspected to be *Aspergillus niger* while lane 2 is suspected *Aspergillus flavus*.



**Figure 2:** Shows the result of PCR

PCR amplification using apa-2 (aflatoxin) primer and DNA marker fungi isolates;

Lane 1: Black fungus shows no band and Lane 2: Green fungus with band at 940 base pairs while lane M: The DNA ladder or marker

The result suggest that *Aspergillus flavus* possess aflatoxin gene while suspected *Aspergillus niger* does not.

### **3.4 ITS region sequencing for identification of Fungi**

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems according to manufacturers' label while the sequencing kit used was BigDye Terminator v3.1 Cycle Sequencing kit. Bio-Edit software and MEGA 6 were used for all genetic analysis. Sequences were BLAST against known data base (<http://www.isth.info/tools/blast/blast.php>).

### 3.5 SEQUENCE RESULTS

CAGCATGCTT	GGGGACCCCC	TTCACGCAAG	CAGTGTTAGA	GCTGGTCAGG
ATAATTCGAC	CTCCTTCCCG	CATATGGCAA	TAGGCCTCCC	GCGCCACGAA
GAACTGGCCA	CGGGTGTTGA	CCCGGAAGAC	CCGGTCAAAT	TCCTAATACT
TTTTTAGCT	TATGCCTTTA	ATGCGTTATC	TGTGGTTCAT	ACTTCTGGGG
CACGTCTTT	CAGGTGACCG	AACGATACAA	TTCCAGCGTT	CGATGACACG
ATGTCCAGGT	AGCCAAAATG	GCGCACCGTC	TCCGCCATTA	ACTTCGCAGT
TGCCTCAGGA	TCCCCGACAT	CGGCCTGGAT	TGCGATAGCG	TCGGTACCAT
TGGCCTTGAT	CTGTTCAACC	ACTTTCTCCG	CGGCCTCACG	GGAATGGGCG
AGTTCACCA	CGACTTTGGC	TCCGCGCTCA	CCAAGCGCGA	CGGCGATGGC
GCACCGATG	CCGCGGCCGG	CGCCGGTGAC	TAAGGCCACT	TTGCCATCTA
ACGGTG GTT	GTCGGACATG	TTGAAGAAAT	CGTCTGGGGT	AGAGACTTTA
GGGAGGCAA	ATGATGTGTA	GTTCTGTAA	AACGGTGATC	CATGGGACCG
GTCATGCACT	ACATATATAC	GCAGCTATGG	ATGGTTGGCC	ACCAAACAAA
CTTTCCTCG	GAGACCGAAA	TATCTGAATA	CCTGTAGCTC	ATCGGGCGGC
AGCCCAAAA	CTTCTGTGTA	GTAATACGTC	TTGTTTATGT	TAGGCTGAAA
CATAGGAAG	CTGAGAATAG	GAGCAGGCTC	TAAACCAATC	ACACCAAAAA
ACAATAATA	AACGAAAACC	GCCGTGTGTC	TCAATGAAGA	AATGCCGGGC
CGACGCGAA	CGTGTGAGC	CATTTACATA	GGAAGAGGGC	GCATTGAGG
TACAGAGCG	AATGGATCGT	GCTCCATGTC	AAGGTCTGCG	TTCTGGCGAC
TATTCCACG	CCGATACGAA	CGGGAATGGA	AAGCGCGTGA	ATGGATTGCA
GACGGCGTG	GTAGATTTTG	GGGGTTTGCG	CGGGCCTTGG	GGCGTTGCAG
ATGCTGATG	CGATGGCTTC	CGGATCCCC	GTTCTTAAGC	TGCGGTGTGG
GCCCAGATTG	AAGTG TAGTC	TGATGCATGC	AATTCGGAAT	ATGGCTGTCTG
GTTGAAACC	GAGGGGCTCT	TTCGGCGATA	AGACGGGTCG	GAGCTTGTCT
CCCAGCTTTT	TTGCCATCGT	CGGAGCGCCG	TGTGGAATCT	GGTGAGGAGC
TCGACGGGGA	CTGGTCTTTA	GATGATCCTG	GGATGCAGAA	TGATGCCTCC
CATGCCAGAT	ATATGTGTTG	GAAAAGACCA	TGTATGAGGA	CCAAGTTGCC
AAGGAAGAT	AAGCTGACTC	TTTACGATG	AAGTGCTTTA	TTGTGAAATA
CTTGCCGTA	GCCGTCGCCG	AAGGAGACGC	AGTGTGGCCC	CTTCGAGCGG
GAGATTTCC	ATTCTGAGGC	AGTTGAAGCG	CTCCAGAGCT	CCTCGGGCCA
AGGGAGATA	AGTGAACCTA	CTTCGCTATT	CAGGATCTTT	GGAGGCATAT
GTAAAGAAT	GGTTTGATTA	TTGCAGATCA	TGTAAGCAAT	GAATTTGGTA

**Figure 3. The sequence of Green fungus and the ITS region is the same as *Aspergillus flavus* strain V5F-13 with accession number of JQ435497**



TAGCTGTTG	ACCCCCGGAC	CATCTACAGA	CTGCCCATGA	TCTTGGCTCG
TGGTCCGCA	CTCTGTAGAC	CCATGGTCGT	TATTCACCAT	GCAGCTATTG
CGGGCGATCC	AAAAACAAGT	AATAGAACGA	AGGCATTTAC	ACAAGATCGG
GGACTGCCC	CCATTCCAAT	AGGGCCGGCC	ATTTGGTACC	ACGAACGGTC
GAAATCGTG	CTGAGAAGAG	AGACCGATCT	GCTTCACAGA	CACAAAAATG
ATTTAATGGG	ATCCGTTGAG	CGGGTGAAGA	CCATGCTCAT	AACCGTGGTT
CGGTGTATAT	CAAGGTCGAT	TAACAGGCGG	TGGGATAGGT	GGTCGCTTGG
AAATGGTCAG	GGTCGGGAGG	CGGTCGCCTG	AATCATGCCA	GAAGTAGATT
TGCTATAAAC	GCATAAAGGC	GCGGAACAAG	ACGGATTTCG	AAAAGTCCAA
GCTGTCGTG	TAATATTCTT	CAAAGTGTAT	AAAACGATTT	CAATTCGGTT
ATTCGCTTGT	TATGGACAGT	AGATATGTGT	AGGGTTATTC	GCGTTCGCAG
CCGCGACGAT	CGGGTGCAGG	AGTCGCGACA	GACAGGGCGG	CAGGCTGACG
CTTATCGGT	AGATATGGTA	GGGAGGTAAA	GGAGGTACTC	GTTTACAATT
AAGGATACT	TGAAGGTTAT	CAAGTCAAGG	CTGGAAGAAC	AGACCGGGCC
GTGAGGGTTC	TGGCGGTTAC	AAGACTGCTC	AAATTGTCCC	TGGCGCCAGG
CCTGTGTTA	GAAATGATAG	GGAGGGGGTG	GTGGTGGTGG	TGGGAGGTGA
GAGCGACGG	TCAATGATGG	GTGTGTACGG	AGCGACAGGG	GCCGATGGTC
GGTTGCGGAG	AAGGAATCAA	GCAGAGGTAT	TTAGCCAGAG	AGTGAGAGTG
AGAGAGGGGG	TAAGCATAAG	GTAATCAGGG	TGTGATTGCC	AGTAGAGAGA
GGAGAGTGA	GTGAATTAGT	TAGCAGTAGT	GAAGAATTGG	AGGTCAAGTC
GGAAGGAGTA	GTAGTAGTAT	GATGCTGCTC	TGTCCTGGAC	AGATTATTCT
TTCTTGCCA	GGGCGAGTTG	GACTGTCTTG	CCCGTGAAAC	AAGGCGTGTG
ATACGCATG	GGGATTGGTC	CAAGGTGCCT	GGCTTTCGGT	GACTCCCTGA
CTTCCCAGAA	TCCTCGAGGC	ATTCTTGCGG	CTGCCC GGTC	ACCGACGCTA
CTGGCAGCC	CCCGCATACC	TGCGGTAATT	CCGGAGGGTA	ATCCCGCCCA
GGTCGCAGC	AGGGAGCCAG	TCACAGGGCC	CATTACCAC	ATATTTCGGG
CTGGCGCCG	CTCCTCCTCT	CTCCTCAGGG	GCCAATAGAC	TCGCTTATGT
TCCAGCCACC	ATCGTAACTA	GGAGGTTAGG	TGGTATTTTA	TTTTCTTTCT
CCCACGCCC	AGTGACAGTC	AGAAAACGCG	TCTTAAG	

**Figure 4. The sequence of Black fungus and the ITS region is the same as *Aspergillus niger* px27 gene with accession number U90936**

## CHAPTER FOUR

### 4.0 Discussion and Conclusion

#### 4.1 Discussion

The findings from the study on spoiled potatoes showed that the locations with the highest fungal strain diversity like Ipata and Oja Oba, were likely to have poor hygiene and high environmental exposure, contributing to fungal contamination. Market places that lack proper storage facilities and are situated in humid conditions provide ideal habitats for fungal propagation. This observation aligns with reports by Emeh et al. (2023), who found that markets with poor infrastructure and sanitation had higher fungal loads in produce. Thus, environmental conditions are key drivers of fungal diversity and load in spoilable agricultural products.

The colony count data indicating a higher fungal load in spoiled potatoes compared to unspoiled ones aligns with findings by Ahmed et al. (2022), who reported similar differences in fungal counts between spoiled and fresh produce. The use of Potato Dextrose Agar (PDA) as a selective medium for fungal growth also supports the observation, as PDA is known to favour the growth of fungi, particularly those that degrade starch-rich substrates like potatoes (Ezekiel et al., 2021). The higher fungal counts on PDA ( $3.8 \times 10^6$  CFU/g) compared to Nutrient Agar ( $2.5 \times 10^6$  CFU/g) in spoiled potatoes further underscore the suitability of PDA for isolating and enumerating fungi from potato samples, consistent with observations by Uzochukwu et al. (2023) on the effectiveness of PDA in fungal isolation.

Morphological characterization on PDA media revealed diverse colony appearances, including cottony, powdery, woolly, and slimy textures. Pigmentation varied from white and greenish to black, and microscopic features such as septate and branched hyphae, along with conidial shapes, were used to tentatively identify four dominant fungi: *Aspergillus niger*, *Penicillium expansum*, *Fusarium solani*, and *Rhizopus stolonifer*. These observations are consistent with the findings of Omemu et al. (2022) and Adediran et al.

(2023), who reported similar morphological characteristics in fungal isolates from spoiled plant materials. These fungi are known for their ability to degrade starch-rich plant tissues and have been widely documented in spoilage of tuber crops (Ibrahim et al., 2021). Their identification helps to understand the mycobiota associated with potato spoilage.

The biochemical analysis further strengthened the identification process, as the isolates showed enzymatic activities aligned with their known metabolic capabilities. For instance, *Fusarium solani* exhibited strong starch hydrolysis and nitrate reduction, while *Penicillium expansum* showed protease activity, correlating with its known role in soft rot and tissue liquefaction. These findings align with the observations of Nwogu et al. (2022), who reported similar enzymatic activities in fungal isolates from spoiled plant materials. Enzyme activity assays, therefore, remain essential for mycological identification and functional profiling, as also emphasized by Ezugwu et al. (2023). *Aspergillus niger*'s cellulase and amylase activity, in particular, highlights its ability to degrade structural polysaccharides and starch in potatoes, consistent with its known enzymatic versatility (Okeke et al., 2022).

The PCR amplification using ITS primers confirmed the identity of the fungal isolates, with results consistent with morphological and biochemical observations. This molecular approach aligns with the findings of Ezeanya et al. (2022), who demonstrated the reliability of ITS sequencing in fungal taxonomy. The *apa-2* aflatoxin biosynthetic gene primer analysis further revealed the potential of *A. flavus* to produce aflatoxins, a concern also highlighted by Okeke et al. (2023) in their study on aflatoxigenic fungi in food products. Monitoring and controlling *A. flavus* in food chains is critical to prevent health risks associated with aflatoxin exposure (Ogunbayo and Ezeonu, 2022).

The study highlights the importance of environmental conditions in driving fungal diversity and load in spoilable agricultural products, with marketplaces like Ipata and Oja Oba showing high fungal strain diversity due to poor hygiene and environmental exposure. This finding is consistent with reports by Okoro et al. (2022), who emphasized the role of

market infrastructure and sanitation in determining fungal contamination levels. The presence of multiple fungal species with varied biochemical capabilities suggests a synergistic spoilage process, where different species contribute to tissue breakdown and rot. This multispecies spoilage phenomenon underscores the need for comprehensive postharvest management strategies that consider the ecological dynamics of spoilage fungi (Nwachukwu et al., 2024; Uzochukwu et al., 2023).

## **CONCLUSION**

The isolation, identification, and characterization of fungi from spoiled potatoes are critical steps in understanding the complex fungal communities involved in spoilage. Molecular techniques have revolutionized the field of fungal identification and characterization, enabling accurate and rapid detection of fungal pathogens. By understanding the types of fungi involved in spoilage and the factors that contribute to their growth, we can develop targeted control measures to reduce losses and improve food safety. Future research should focus on applying molecular techniques to develop resistant potato varieties, improve disease management strategies, and reduce the economic impact of fungal pathogens on potato production.

## **SUMMARY**

Spoiled potatoes can harbor a diverse range of fungal species, including *Aspergillus*, *Fusarium*, and *Penicillium*, which can lead to food spoilage and mycotoxin production. This study highlights the importance of proper storage and handling practices, including temperature and humidity control, to prevent fungal contamination and spoilage.

## **RECOMMENDATION**

This study highlights the importance of understanding the fungal diversity associated with potato spoilage. We recommend that further research be conducted to develop effective control measures and prevent fungal contamination. Additionally, collaboration between researchers, policymakers, and industry stakeholders may be necessary to develop and implement effective strategies to reduce fungal contamination and spoilage in potatoes.

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