MICROORGANISMS ASSOCIATED WITH VELVET TARMARIND (*Dialium guineense)* FRUIT

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BENIN CITY

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF CROP SCIENCE, FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE BACHELOR OF AGRICULTURE DEGREE (B.AGRIC.) IN CROP SCIENCE, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

NOVEMBER, 2025

CERTIFICATION

This is to certify that the work contained in this report titled "**Microorganisms associated with velvet tarmarind (*Dialium guineense)* fruit**" was carried out by **Shalom Oritsebemigho ESIJEMIDE-DORE** (**AGR2004340)** of the Department of Crop Science, Faculty of Agriculture, University of Benin, Benin City, Edo State, Nigeria.

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Head of Department

DEDICATION

This work is dedicated to God for His unending grace. To my family; my parents and my siblings, your love fuels me. And to myself, for perseverance.

ACKNOWLEDGEMENTS

This project's successful completion would not have been possible without the guidance, support and dedication of many individuals to whom I owe my sincere gratitude.

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ABSTRACT

Dialium guineense fruit is an important tropical fruit which faces substantial post-harvest losses and food safety concerns due to microbial contamination. This study investigated microorganisms associated with the shell, pulp and seed of *D. guineense* to determine infection rates, identify microbial species (fungal and bacterial) and assess their diversity. The research was conducted at the media preparation room of the Department of Crop Science, Faculty of Agriculture, University of Benin, Benin City, Edo State, Nigeria. Three (3) fruit parts were studied: Shell, pulp and seed on Potato Dextrose Agar (PDA) and Nutrient Agar (NA) laid out in a Completely Randomized Design (CRD) with six (6) replications. It involved evaluating infection percentages, sub-culturing and identification based on cultural, morphological and biochemical characteristics. Results showed a 100% infection rate across all fruit parts except one (1) replicate which had a 75% infection rate, indicating significant microbial pressure. A wide variety of fungal and bacterial species was observed, with specific microbial adaptations per fruit part. Pathogenic bacteria, including *Shigella* sp. and *Bacillus* cereus, were identified, posing potential health risks, alongside *Aspergillus* and *Fusarium* species. This study confirms the fruit's vulnerability to widespread microbial contamination. Findings underscore the critical necessity for improved safety measures against *Aspergillus* and *Fusarium* contamination, while identifying promising opportunities to utilize *Saccharomyces* sp. for industrial processing and seed extracts for natural preservation.

CHAPTER ONE

INTRODUCTION

Dialium guineense, commonly known as velvet tamarind, is a tall, perennial leguminous tree belonging to the family Fabaceae. It is indigenous to the tropical and subtropical regions of West and Central Africa, with its distribution stretching from Senegal to Sudan (Fern, 2024; Wikipedia, 2024*)*. In Nigeria, it is widely known by various local names, including 'awin' in Yoruba, 'icheku' in Igbo and 'tsamiyar kurmi' in Hausa (Wikipedia, 2024). The tree thrives in diverse environments, from lowland evergreen forests to savannah woodlands and is a characteristic feature of the local landscape, valued for both its ecological and economic contributions (Fern, 2024).

The fruit of *D. guineense* is a small, grape-sized drupe with a hard, brittle black shell that encloses a reddish-brown, edible pulp surrounding a single hard seed. This pulp is the most economically significant part of the plant, possessing a unique sweet and sour taste that makes it a popular snack, especially among children and rural dwellers. It is consumed raw, soaked in water to prepare a refreshing beverage or processed into jellies and preserves (Fern*,* 2024). Beyond its use as a snack, the fruit is a powerhouse of nutrients. It is exceptionally rich in vitamin C, a vital antioxidant and contains significant amounts of minerals such as potassium, magnesium, iron and sodium (Nwaukwu and Ikechi-Nwogu, 2012). The leaves and bark of the tree also have a long history of use in

traditional medicine for treating a variety of ailments, including coughs, stomatitis, and fever (Fern, 2024).

Despite its nutritional and economic importance, the full potential of *D. guineense* is severely hampered by its susceptibility to microbial contamination and spoilage. After harvesting, the fruits are subjected to handling, transportation and storage conditions that expose them to a wide range of microorganisms. These microbes, primarily fungi and bacteria, colonize the fruit, leading to rapid deterioration, loss of nutritional value and significant post-harvest losses (Ayessou *et al*., 2014; Besong *et al*., 2016; Ambrose and Ibiam, 2013).

1.1 Justification of the Study

The post-harvest loss of fruits and vegetables is a critical problem in developing countries like Nigeria, and *Dialium guineense* is no exception (Onwude *et al*., 2023; Yahaya and Mardiyya, 2019; Balana *et al*., 2022; Al-Amin *et al*., 2021). A significant portion of the harvest perishes before it can reach the consumer due to microbial activities (Saleh and Al-Thani, 2019; Udoh *et al*., 2015). This spoilage is not merely an economic issue for farmers and vendors; it has profound implications on food security and public health (Gbashi *et al*., 2020; Ajao *et al*., 2022).

Studies have shown that *D. guineense* fruits sold in markets are frequently contaminated with a diverse range of spoilage fungi, including species of *Aspergillus*, *Penicillium*,

Rhizopus and *Fusarium* (Ikechi-Nwogu and Nwaukwu, 2012; Ambrose and Ibiam, 2013). These fungi are responsible for causing various fruit rots, which lead to undesirable biochemical changes. Nwaukwu and Ikechi-Nwogu (2012) demonstrated that fungal infection drastically reduces the fruit's content of carbohydrates, lipids and essential vitamins, particularly its famously high vitamin C content, thereby diminishing the very nutritional benefits for which it is consumed.

More alarming is the threat of mycotoxin contamination. Several of the fungal species isolated from the fruit, such as *Aspergillus flavus* and *Aspergillus ochraceus*, are known producers of potent mycotoxins like aflatoxins and ochratoxins (Ambrose and Ibiam, 2013). These toxins are carcinogenic and can cause severe health problems, including liver damage and kidney disease (Pepple *et al*., 2016). A study by Pepple *et al.* (2016) confirmed the presence of aflatoxins in both visibly spoiled and healthy-looking fruits, highlighting a hidden danger for unsuspecting consumers.

Furthermore, the fruit is also susceptible to contamination by pathogenic bacteria, including enteric species like *Escherichia coli* and *Salmonella typhi*, which are indicators of poor hygiene and can cause serious foodborne illnesses (Ajiboye *et al.,* 2018). Given that the fruit is almost exclusively consumed raw without any heat treatment, the risk of ingesting these harmful microbes and their toxins is significantly elevated.

Therefore, a thorough investigation into the microorganisms associated with *Dialium guineense* is essential. Understanding the specific microbial species involved, the nature of the spoilage they cause and the potential food safety hazards they pose is the first step toward developing effective strategies to mitigate post-harvest losses and protect consumer health.

1.2 Specific Objectives of the Study

The specific objectives of the study were to;

i. ascertain the percentage infection of microorganisms on the shell, pulp and seed of *Dialium guineense* fruits.
ii. isolate and identify the microorganisms (fungal and bacterial) species associated with the shell, pulp and seed of *Dialium guineense* fruits.
iii. determine the microbial diversity and similarities.

CHAPTER TWO

LITERATURE REVIEW

2.1 Microbial Diversity Associated with *Dialium guineense* Fruit

The *Dialium guineense* fruit is home to a diverse array of microorganisms, including various fungi and bacteria. These microorganisms play multiple roles, from contributing to post-harvest spoilage to offering potential for biotechnological applications.

2.1.1 Fungi Associated with *Dialium guineense*

Fungi are significant microbial inhabitants of *Dialium guineense*, influencing the fruit at different stages, including pre-harvest contamination, post-harvest spoilage and involvement in fermentation processes.

2.1.1.1 Field Fungi: Pre-harvest Contamination

Fungal pathogens have been identified in association with *Dialium guineense* fruits in the field. For instance, a study in the Port Harcourt Metropolis specifically investigated fungal pathogens affecting the forest fruit *Dialium guineense* (Ikechi-Nwogu and Nwaukwu, 2012). These pre-harvest fungal infections can negatively impact the fruit's quality and biochemical composition. Research indicates that such infections can lead to the degradation of essential food nutrients (Ambrose and Ibiam, 2013) and a significant decrease in the fruit's mineral and vitamin content (Nwaukwu and Ikechi-Nwogu, 2012). However, some findings suggest that pathogenic fungi may not be isolated from freshly

harvested fruits, implying that contamination patterns or detection rates can vary (Ambrose and Ibiam, 2013).

2.1.1.2 Storage Fungi and Spoilage

After harvest, *Dialium guineense* fruits are highly susceptible to spoilage by a variety of fungi. Common fungal genera isolated from stored *Dialium guineense* fruits include *Aspergillus niger, Aspergillus flavus, Aspergillus japonicum, Penicillium chrisogenum, Fusarium moniliforme, Fusarium oxysporum, Penicillium italicum, Penicillium notatum, Aspergillus fumigatus* and *Aspergillus ochraceus* (Ambrose and Ibiam, 2013). Other fungi, such as *Rhizopus microcrosporus, Penicillium purpureum*, and *Penicillium minioluteum*, have also been detected in shelved *D. guineense* fruits (Pepple *et al*., 2016).

The activities of these storage fungi induce considerable biochemical changes within the fruit. These alterations typically involve a reduction in crucial nutritional components, such as crude protein, fiber, carbohydrates, fats and vitamins (A and C) (Ambrose and Ibiam, 2013). However, there may be an increase in moisture content and ash content in the infected fruits (Ambrose and Ibiam, 2013). The presence of these pathogenic fungi also leads to a significant decrease in mineral content and certain vitamins, while possibly increasing antioxidant content as a plant response to the stress (Nwaukwu and Ikechi-Nwogu, 2012).

2.1.1.3 Yeast and Other Fungi in Fermentation Processes

Beyond their role in spoilage, certain fungal communities, particularly yeasts, found in *Dialium guineense* can be utilized for beneficial fermentation. *Saccharomyces cerevisiae var. ellipsoids* have been identified in the context of *D. guineense* fruit fermentation for producing products like wine (Ojukwu and Ozugha, 2019). Furthermore, naturally occurring fungi isolated from the fruit pulp of *Dialium guineense* have been explored for their biotechnological potential in industrial applications, such as the production of citric acid (Ajiboye and Sani, 2015). This demonstrates a positive role for specific fungal communities in valorizing the fruit.

2.1.2 Bacteria Associated with *Dialium guineense*

Bacteria also constitute a part of the microbial ecosystem of *Dialium guineense*, particularly through internal colonization as endophytes.

2.1.2.1 Endophytic Bacteria: Internal Colonization

Endophytic bacteria have been successfully isolated and identified from *Dialium guineense*. Specifically, research has focused on the stem bark of *Dialium guineense*, revealing the presence of several bacterial genera (Omokpo and Adetunji, 2023). Identified genera include *Pseudomonas, Halopseudomonas, Burkholderia, Streptococcus* and *Bacillus* (Omokpo and Adetunji, 2023). These endophytic bacteria are not merely present but can also exhibit significant biological activities. Extracts from these

endophytic bacteria, especially from the *Halopseudomonas* genera, have demonstrated antibacterial activity (Omokpo and Adetunji, 2023). This suggests that endophytic bacteria in *Dialium guineense* could play a role in the plant's natural defenses or provide new leads for developing powerful antimicrobial drugs. Their presence as endophytes also points to their potential involvement in plant growth promotion and other beneficial host interactions, which are commonly observed roles for endophytes in various plant species (Omokpo and Adetunji, 2023).

2.2 Post-Harvest Microbial Deterioration, Mycotoxin Contamination and Food Safety

Post-harvest handling and storage are critical periods for *Dialium guineense* fruit, as they are highly susceptible to microbial deterioration, which can lead to significant quality loss, mycotoxin contamination and pose food safety risks.

2.2.1 Microbial Activity on Different Fruit Parts: Shell, Pulp and Seed

Microorganisms interact differently with various parts of the *Dialium guineense* fruit, affecting its integrity and safety.

2.2.1.1 Shell

The fruit shell often serves as a primary protective barrier against microbial invasion. While some studies indicate that no pathogenic fungi were isolated from freshly

harvested *Dialium guineense* fruits, suggesting a potential protective role, microbial colonization of the shell can still occur under certain conditions (Ambrose and Ibiam, 2013). The integrity of the shell therefore influences the overall contamination patterns of the fruit.

2.2.1.2 Pulp

The fruit pulp, being rich in nutrients, is particularly vulnerable to fungal spoilage. This microbial activity in the pulp can lead to considerable nutritional degradation (Ambrose and Ibiam, 2013).

The work of Nwaukwu and Ikechi-Nwogu (2012*)* provides a detailed account of the biochemical devastation wrought by pathogenic fungi on the pulp. Their inoculation studies demonstrated how fungi like *Aspergillus niger, Botryodiplodia theobromae* and *Rhizopus stolonifer* actively metabolize the pulp's components. They documented a significant decrease in carbohydrates, lipids and crude fiber, as the fungi consumed these for energy and growth. Simultaneously, the fungi caused a drastic reduction in essential vitamins, particularly vitamin C and minerals like calcium, magnesium and potassium. This process not only results in visible rot (e.g., the black mould of *A. niger* or the soft rot of *R. stolonifer*) but also constitutes a severe degradation of the fruit's nutritional value.

Moreover, the pulp is where mycotoxin production becomes a critical issue. Fungi like *Aspergillus flavus* and *Fusarium* spp., upon colonizing the pulp, can produce aflatoxins

and fumonisins, respectively (Ambrose and Ibiam, 2013). The study by Pepple *et al.* (2016) confirmed the presence of aflatoxins in both infected and seemingly healthy fruits, suggesting that fungal growth and toxin production can occur within the pulp even before visible signs of spoilage are apparent.

However, the pulp also holds biotechnological potential, as naturally occurring fungi from this part has been explored for beneficial processes, such as fermentation for citric acid production (Ajiboye and Sani, 2015).

2.2.1.3 Seed

The seeds of *Dialium guineense* are also susceptible to microbial contamination. Fungal presence, including mycotoxigenic fungi, has been observed on or within *D. guineense* seeds (Pepple *et al*., 2016; Nwaukwu and Ikechi-Nwogu, 2012).

The most significant microbial-related activity concerning the seed is not its spoilage, but its potent antimicrobial properties. As demonstrated by Ajiboye *et al.* (2018), extracts from the seed are highly effective at inhibiting key enteric pathogens. This suggests that the seed's primary role in the microbial context is defensive. It concentrates on a specific set of non-polar bioactive compounds (alkaloids, saponins, etc.) that protect it from degradation. This is an evolutionarily sound strategy, as protecting the seed is essential for the plant's reproduction.

2.2.2 Mycotoxin Contamination: A Major Public Health Concern

Mycotoxins, toxic secondary metabolites produced by certain fungi, represent a significant public health concern associated with *Dialium guineense* fruit.

Mycotoxigenic Fungi Isolated from *D. guineense*: Several mycotoxigenic fungi have been isolated from *Dialium guineense*, indicating a risk of mycotoxin contamination (Pepple *et al*., 2016). These fungi can colonize the fruit, particularly during post-harvest storage.

Mycotoxins of Concern: Aflatoxins have been a specific focus, with studies detecting aflatoxin contamination in *D. guineense* fruits, both infected and healthy-looking (Pepple *et al*., 2016). The presence of aflatoxins highlights a significant food safety issue.

Factors Influencing Mycotoxin Production: Mycotoxin production is influenced by a complex interaction of environmental and other intrinsic factors. These include moisture content, relative humidity, temperature, substrate composition and the presence of competing micro-organisms (Pepple *et al*., 2016). Warm and humid conditions during harvesting, handling, storage and processing are particularly conducive to mycotoxin development (Bouelet Ntsama *et al*., 2023). Other factors contributing to high levels of mycotoxin contamination in African foods include climatic and environmental variables, farming systems, processing and storage techniques and socio-political factors (Nji *et al*., 2022).

Health Risks Associated with Mycotoxin Consumption: The consumption of mycotoxin-contaminated *Dialium guineense* poses several health risks. These include the potential for food poisoning (Nwaukwu and Ikechi-Nwogu, 2012). Reports of children experiencing stomach aches after consuming the fruit pulp suggest adverse effects from ingesting degraded or contaminated fruits (Ambrose and Ibiam, 2013).

2.3 Antimicrobial Properties of *Dialium guineense* Extracts and its Microbial Context

Dialium guineense has garnered attention for its inherent antimicrobial properties, which are attributed to various parts of the plant and even its associated microorganisms. These properties suggest its potential in traditional medicine and for developing new antimicrobial agents.

2.3.1 Antimicrobial Activity of *Dialium guineense* Fruit's Components

The antimicrobial efficacy of *Dialium guineense* has been investigated across different parts of the fruit, revealing a broad spectrum of activity against various microbial strains.

2.3.1.1 Antimicrobial Activity of the Fruit Pulp

The pulp of the *D. guineense* fruit, being the primary edible portion, has been a major focus of antimicrobial research. Its rich phytochemical composition is believed to be the source of its inhibitory effects against pathogenic and spoilage microorganisms.

Ajiboye *et al.* (2018) conducted a comprehensive study on the antimicrobial activity of *D. guineense* fruit pulp extracts using different solvents (n-hexane, ethanol and aqueous) against a panel of clinically important bacteria and a yeast. Their findings demonstrated that the ethanol and aqueous extracts were particularly effective. The ethanol extract showed a broad spectrum of activity, inhibiting the growth of *Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis* and the yeast *Candida albicans*. The aqueous extract was similarly effective against all tested organisms. In contrast, the n-hexane extract showed no activity, indicating that the bioactive compounds responsible for the antimicrobial effects are polar in nature and are not effectively extracted by non-polar solvents. This is a crucial finding, as it guides future efforts to isolate and identify these specific compounds. The study also determined the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of the extracts. The ethanol extract, for instance, had an MIC of 25 mg/ml against *K. pneumoniae* and *P. mirabilis*, and 50 mg/ml against the other bacteria and *C. albicans*. The fact that the MBC/MFC values were often identical or close to the MIC values suggests that the pulp extracts are largely deadly (killing the microbes) rather than just static (inhibiting their growth), which is a desirable characteristic for a therapeutic agent.

Further supporting these findings, Airaodion *et al.* (2021) investigated the bactericidal potential of aqueous extracts of *D. guineense* pulp against both Gram-positive (*Bacillus*

cereus, Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus viridans) and Gram-negative *(Salmonella typhi, Escherichia coli)* bacteria. Their results confirmed the work of Ajiboye *et al.* (2018), showing that the aqueous extract was potent against all tested bacteria. The study highlighted that the extract's effectiveness was concentration-dependent, with higher concentrations of the extract resulting in larger zones of inhibition. This dose-response relationship is a classic indicator of antimicrobial activity. Airaodion *et al.* (2021) attributed these properties to the presence of various phytochemicals like tannins, saponins, flavonoids, alkaloids and phenols, which are well-known for their antimicrobial actions. For example, tannins can inactivate microbial enzymes and proteins, while flavonoids can disrupt microbial cell membranes.

2.3.1.2 Antimicrobial Activity of the Seed

Ajiboye *et al.* (2018) specifically focused on the antibacterial activity of *D. guineense* seed extracts against selected enteric bacteria, which are a major cause of foodborne illnesses and are relevant contaminants of the fruit itself. They tested aqueous, methanolic and ethanolic extracts against *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae* and *Salmonella typhi*. Their results were striking: the methanolic and ethanolic extracts demonstrated significant antibacterial activity against all the tested pathogens, whereas the aqueous extract showed no activity. This contrasts with the findings for the pulp, where aqueous extracts were effective. This difference suggests that the chemical nature of the bioactive compounds in the seed differs from that in the pulp.

2.3.1.3 Antimicrobial Activity of the Shell

The shell of *Dialium guineense* also possesses noteworthy antimicrobial properties. Studies have shown that the dichloromethane fraction obtained from the fruit coat exhibits wound-healing capabilities alongside its antimicrobial effects (Okeke *et al*., 2016). This suggests that the outer layers of the fruit contain active compounds that can combat microbial growth, potentially contributing to the fruit's natural defense mechanisms.

2.3.1.4 Antimicrobial Activity of Endophytes

The microorganisms residing within the tissues of *Dialium guineense* also contribute to its antimicrobial profile. Endophytic bacteria isolated from the stem bark of *Dialium guineense* have been found to exhibit antibacterial activity (Omokpo and Adetunji, 2023). Specifically, isolates from the *Halopseudomonas* genera showed notable antibacterial effects, suggesting that the host plant's internal microbial communities can produce bioactive compounds with antimicrobial potential (Omokpo and Adetunji, 2023).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Location

This study was carried out at the media preparation room of the Department of Crop Science, Faculty of Agriculture, University of Benin, Benin City, Edo State, Nigeria. The laboratory is geologically located at coordinates 6°24'03.1"N 5°37'28.4"E (Google Maps, 2025).

The microorganism's identification was carried out at the Department of Microbiology laboratory, Faculty of Life Science, University of Benin, Benin City, Edo State, Nigeria.

3.2 Source of Fruits

Velvet tamarind fruits were purchased from June 12 mini market, University of Benin, Ugbowo, Benin City.

3.3 Materials and Equipment

The materials used for the study: Potato Dextrose Agar (PDA), Nutrient Agar (NA), sterile distilled water, petri dish, filter paper, aluminum foil, cotton wool, ethanol, sodium hypochlorite (bleach), masking tape, forceps, stirring rod, syringe and bunsen burner.

Equipment used: Inoculation chamber (laminar flow hood), autoclave, weighing scale, microscope, incubator and conical flask.

3.4 Sterilization

The inoculation chamber (laminar flow hood) was swabbed with cotton wool and 98% ethanol. Sterilization of the petri dishes, other glass wares and filter paper were carried out by wrapping them in aluminum foils and placed in a hot air oven at 160°C for 2 hours.

3.5 Surface Sterilization of Fruits

The fruit samples were randomly selected and surfaced sterilized in 1% sodium hypochlorite for 60 seconds and was then rinsed in three changes of sterile distilled water. The fruit samples were then placed on a sterile filter paper for absorption of moisture.

3.6 Media Preparation and Plate Pouring

Media was prepared according to the manufacturer's instruction by weighing 39g of Potato Dextrose Agar (PDA) and 28g Nutrient Agar (NA) into separate conical flasks and dissolving in 1,000mls of distilled water. These were thoroughly stirred using the stirring rod to allow them to dissolve. The flasks were then corked with cotton wool and aluminum foil. The media was then autoclaved at 121°c for 15minutes. The media (NA and PDA) were transferred to the inoculation chamber and allowed to cool for about 45°c (cheek hot). 20ml of each medium was poured into individual petri dishes and allowed to gel.

3.7 Plating of Fruit Materials, Isolation and Purification of Culture

Plating was done by picking various parts of velvet tamarind fruit samples (shell, pulp and seeds) from sterile filter paper using forceps sterilized by flaming to red hot and then

placed directly to the petri dishes already containing media (NA and PDA) respectively. Four (4) of each fruit sample were placed per petri dish. Each fruit sample had six (6) replications per media. A total of 36 petri dishes were used. The plates were then sealed with masking tape to prevent contamination.

Observation for bacterial growth was done 24 hours after inoculation on plates, while observation was made for fungal growth after 48 hours of plating.

3.8 Evaluation of Percentage Infection

All the plates containing the inoculated samples were observed after 24 hours of inoculation.

Percentage infection was evaluated thus:

$\frac{1}{4}$ infected inoculations = 25%, $\frac{2}{4}$ infected inoculations = 50%,

$\frac{3}{4}$ infected inoculations = 75% and $\frac{4}{4}$ infected inoculations = 100%.

3.9 Sub-culturing of Microorganisms

Freshly prepared NA and PDA were poured into already sterilized petri dishes, and they were allowed to solidify. From the old plates containing microorganisms, the streaking method was used to isolate the individual bacterial culture into a fresh plate for further studies. The fungal culture was sub-cultured by carefully taking a fresh growing portion with a sterilized needle into a fresh PDA plate. Sub-culturing was done to obtain a pure

culture.

3.10 Evaluation of Microbial Diversity

All the plates containing the inoculated samples were observed after 48 hours of inoculation. Similarities and differences were noted.

3.11 Identification of Isolates

3.11.1 Fungal Identification

The fungal isolate was identified based on their cultural characteristics such as the colony colours, margins, elevation and colony reverse colours. Microscopic features were examined under a microscope fitted with a camera (Motic B1 digital camera) using the cover-slip method in which a little quantity of each culture was transferred to the base cover slips buried in PDA. The fungal isolates were identified using the method described by Barnett and Hunter (1998) and Nyongesa *et al* (2015).

3.11.2 Bacteria Identification

3.11.2.1 Gram Staining Techniques

The clean microscope slide was greased for preparation of the smear. A smear was made on the slide and heat was fixed. The slide was then placed on the staining tray. It was then stained with a crystal violet and allowed to stay for 1 minute. The slide was tilted and gently rinsed with distilled water. It was then stained with gram's iodine and left to stand for a minute, then rinsed with distilled water. De-colonization using 95% acetone was

done and was immediately rinsed with water. Drops of Safranin were added to the counter stain and left for 45 seconds. It was then slightly rinsed with distilled water and blot dried. The slide was viewed using a light microscope under oil immersion. Blue or purple stained cell walls indicate the isolate is gram positive bacteria while pink or red walls indicate gram negative bacteria.

3.11.2.2 Catalase Test

This test was used to identify microorganisms that would produce enzyme catalase. The procedure includes sterilization of the working area with cotton wool and 98% ethanol. The spirit lamp was put on; a smear of bacteria's isolate was made on a clean glass slide. 1-2 drops of hydrogen peroxide were added. The presence of bubbles indicating the production of oxygen shows a catalase positive result; absence of the bubbles shows a catalase negative result.

3.11.2.3 Indole Test

This test is used to identify bacteria isolates which have the ability to decompose amino acid tryptophane to indole. The procedures include the sterilization of the working area. Several drops of indole spot reagent were placed on filter paper. Using an inoculation loop, apportion of the bacteria isolate was picked and smear was made on the saturated area of the filter paper. It was observed immediately. The presence of pink reddish colour shows a positive indole test while absence of this colour indicates a negative indole test.

3.11.2.4 Oxidase Test

This test is used to identify bacteria isolates capable of synthesizing the enzyme's cytochrome oxides. The procedure includes sterilization of the working area. A piece of filter paper was placed in a clean petri dish and 2-3 drops of fresh or nascent oxidase reagent was added. A colony of test microorganisms was collected using a glass rod and smeared on the filter paper and observed. Blue-purple color within a few seconds showed a positive test.

3.11.2.5 Citrate Test

This test is used to identify bacteria isolates which have the ability to utilize citrate as a source of energy. The procedures involved sterilization of the working area, a streak of bacteria isolate was made in the slant for the prepared citrate agent. It was incubated aerobically at 35°C. Development of colour change from green to blue indicates a positive citrate test while otherwise indicates a negative test.

3.11.2.6 Urease Test

This test is used to identify isolates which have the ability to hydrolyze urea with enzyme urease. The procedure includes sterilization of the working area. The test organism was heavily inoculated onto Christensen's urea broth in a bijou bottle using a sterile wire loop and incubated at 35°C- 37°C for 18-24 hours and examined. Thereafter a pink colour in the medium showed a positive test.

3.11.2.7 Hydrogen Sulfide (H_2S) Formation Test

The SIM medium was inoculated and incubated at 37°C for 24-48 hours. Blackening of the medium indicated H_2S production.

3.11.2.8 Glucose Fermentation Test

Glucose broth with phenol red and a Durham tube was inoculated and incubated at 37°C for 24-48 hours. Yellow colour indicated acid; gas bubbles indicated gas production.

3.11.2.9 Lactose Fermentation Test

Lactose broth containing phenol red and a Durham tube was inoculated and incubated at 37°C for 24-48 hours. Acid production turned the broth yellow; gas formation produced bubbles.

3.11.2.10 Mannitol Fermentation Test

Mannitol broth with phenol red and a Durham tube was inoculated and incubated at 37°C for 24-48 hours. Yellow colour showed acid production; gas bubbles showed gas formation.

3.12 Experimental Design

The experiment was laid out using Completely Randomized Design (CRD) with six (6) replications.

CHAPTER FOUR

RESULTS

4.1 Percentage Infection

The evaluated percentage infection of the inoculated fruit shell, pulp and seed samples are shown in Figure 1, 2 and 3. This was evaluated 24 hours after inoculation. Each plate had a 100% infection rate except NA shell plate five (5) which had a 75% infection rate.

4.2 Identified Isolates

4.2.1 Fungal Isolates

A total of eleven (11) fungi isolates were identified.

The fungi isolates were identified as *Aspergillus niger, Saccharomyces* sp., *Fusarium* sp., *Rhizopus* sp., *Mucor, Aspergillus fumigatus, Alternaria* sp., *Aspergillus vadensis, Cladosporium* sp., *Botrytis cinerea* and *Mucor mucedo*.

Table 1 shows the cultural and microscopic characteristics of identified fungi microorganisms.

4.2.2 Bacteria Isolates

A total of five (5) bacteria isolates were identified.

The bacteria isolates were identified as *Bacillus* sp., *Micrococcus* sp., *Proteus* sp., *Shigella* sp. and *Bacillus cereus.*

Table 2 shows the cultural, morphological and biochemical characteristics of identified bacteria microorganisms.

4.3 Microbial Diversity

The microbial diversity of *Dialium guineense* fruit from the seed, pulp and shell is shown in Table 3. The result shows how diverse microorganisms in *Dialium guineense* fruit are as all the different fruit parts had different microorganisms except for *Mucor* which was present in the pulp and seed.

4.4 Figures and Tables

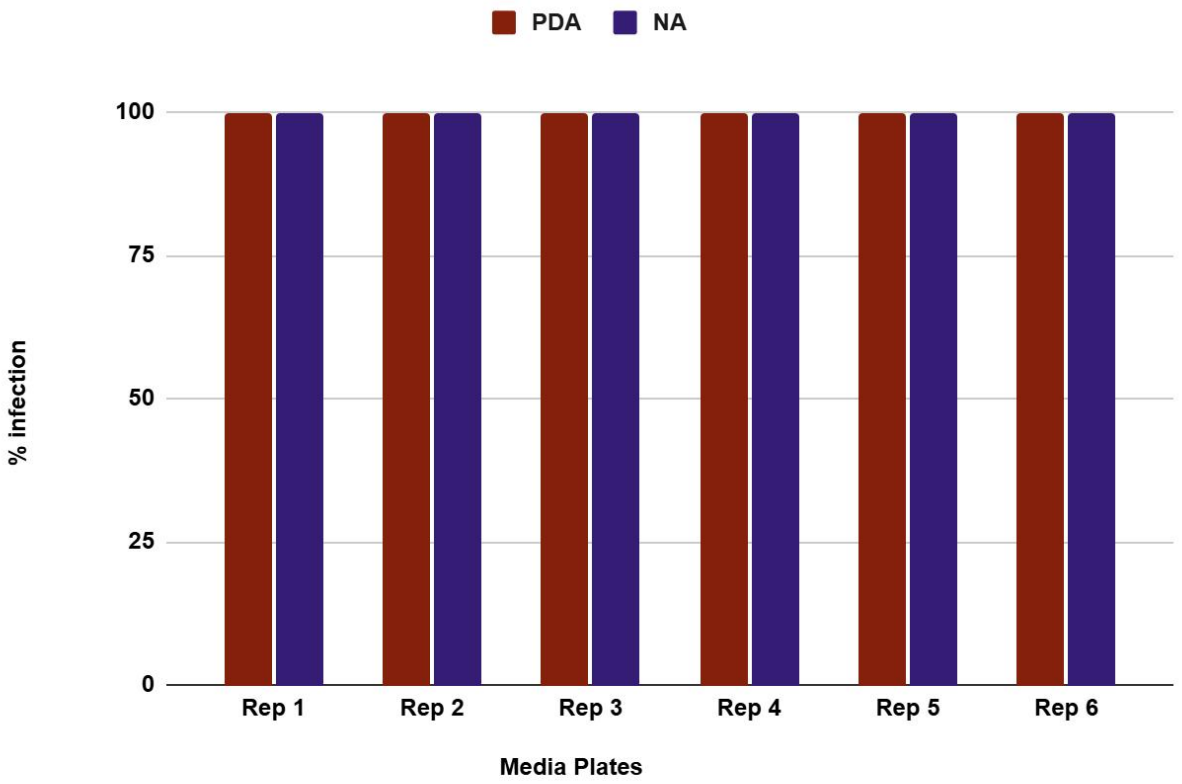

Figure 1: Percentage Infection of Velvet Tamarind Seed in Potato Dextrose Agar (PDA) and Nutrient Agar (NA).

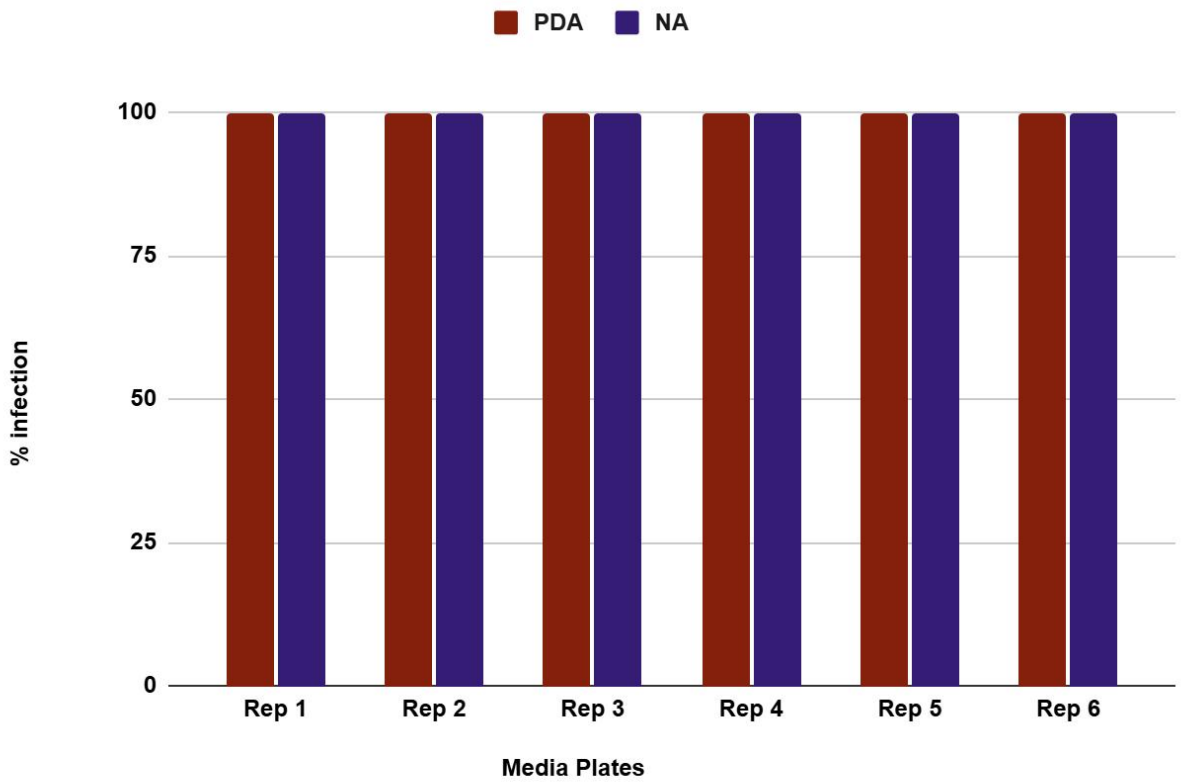

Figure 2: Percentage Infection of Velvet Tamarind Pulp in Potato Dextrose Agar (PDA) and Nutrient Agar (NA).

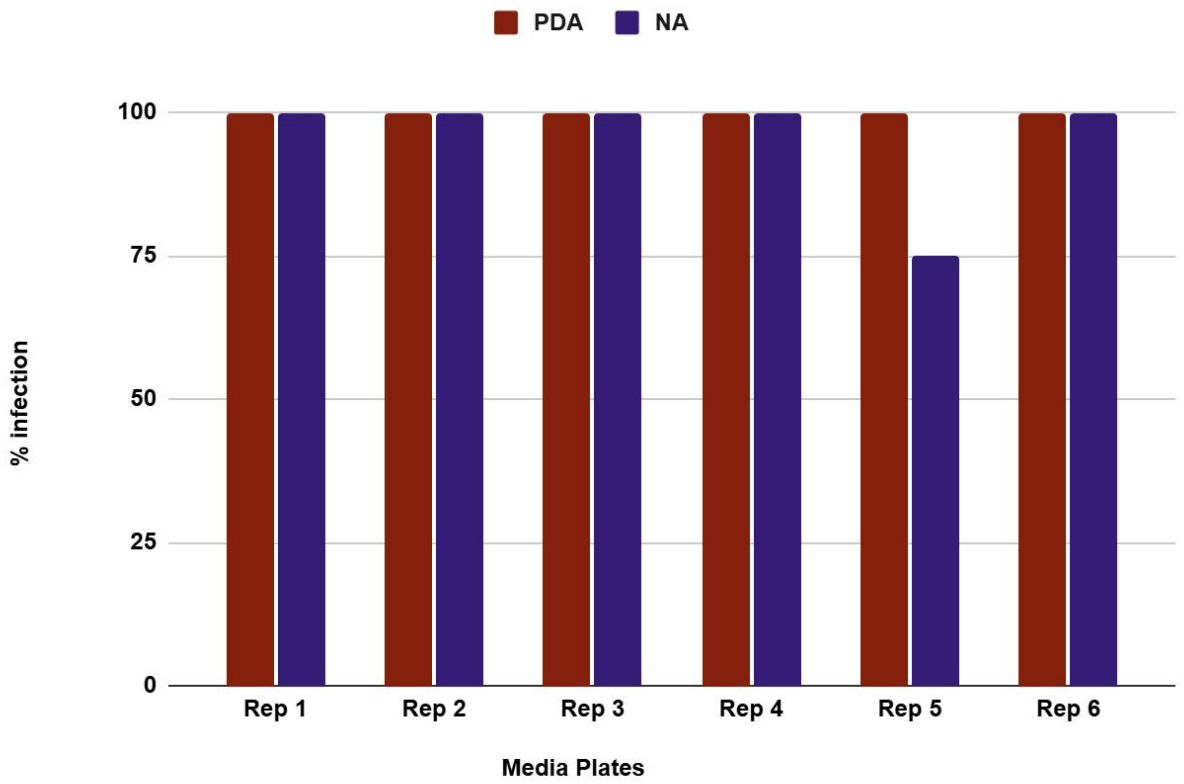

Figure 3: Percentage Infection of Velvet Tamarind Shell in Potato Dextrose Agar (PDA) and Nutrient Agar (NA).

Table 1: Cultural and Microscopic Characteristics of Fungi Isolates

PARAMETERS	ISOLATE 1	ISOLATE 2	ISOLATE 3	ISOLATE 4	ISOLATE 5	ISOLATE 6
CULTURAL CHARACTERISTICS						
Colour of mycelium on agar plate	Dark coloured growth	Cream front colour	Brown cotton with white margins and reddish-brown reverse colour	Black wooly with black reverse colour	Cotton candy texture, white with pale yellowish brown reverse colour	Grey hipped cottony colour with black reverse colour
Colour of plate culture reverse	Dark	Dark cream	Dark	Yellowish	Brown	Green
MICROSCOPIC CHARACTERISTICS						
Nature of hyphae	Septate	Septate	Septate	Septate	Septate	Septate
Type of Spore	Conidiospore	Conidiospore	Sporangiospore	Sporangiospore	Conidiospore	Conidiospore
Conidia	Present	Present	Present		Present	Present
Rhizoids	Absent	Absent	Absent	Absent	Absent	Absent
Spore colour	Absent	Absent	Absent	Green	Absent	Absent
Appearance of special structure	Dark	Fruiting heads	Dark	Black	White	Dark
Class of fungi	Ascomycetes	Ascomycetes	Ascomycetes	Ascomycetes	Ascomycetes	Ascomycetes
Possible Identity	***Aspergillus niger***	***Saccharomyces* sp.**	***Fusarium* sp.**	***Rhizopus* sp.**	***Mucor***	***Aspergillus fumigatus***

Table 1 Continued…

PARAMETERS	ISOLATE 7	ISOLATE 8	ISOLATE 9	ISOLATE 10	ISOLATE 11
CULTURAL CHARACTERISTICS					
Colour of mycelium on agar plate	Brown mass of mycelium	Dark wooly coloured growth	Black colour with white margin	Grey colour	White wooly with cream colour reverse
Colour of plate culture reverse	Black	Black	Black	Brown	Cream
MICROSCOPIC CHARACTERISTICS					
Nature of hyphae	Non-Septate	Septate	Septate	Septate	Septate
Type of Spore	Conidiospore	Conidiospore	Conidiospore	Sporangiospore	Conidiospore
Conidia	Present	Present	Present	Present	Present
Rhizoids	Absent	Absent	Absent	Absent	Absent
Spore colour	Absent	Absent	Absent	Absent	Absent
Appearance of special structure	Lack Pseudohyphae	Dark	Fruiting heads	Dark	White
Class of fungi	Ascomycetes	Ascomycetes	Ascomycetes	Ascomycetes	Ascomycetes
Possible Identity	***Alternaria* sp.**	***Aspergillus vadensis***	***Cladosporium* sp.**	***Botrytis cinerea***	***Mucor mucedo***

Table 2: The Cultural, Morphological and Biochemical Characteristics of Identified Bacteria Microorganisms.

PARAMETERS	ISOLATE 1	ISOLATE 2	ISOLATE 3	ISOLATE 4	ISOLATE 5
CULTURAL CHARACTERISTICS					
Colour	Cream	Cream	Cream	Grey	Grey
Shape	Circular	Circular	Irregular	Circular	Circular
Elevation	Convex	Convex	Effuse	Convex	Convex
Margin	Entire	Entire	Entire	Entire	Entire
Size	Small	Large	Small	Small	Small
MORPHOLOGICAL CHARACTERISTICS					
Gram stain	+	+	-	-	+
Cell morphology	Rod	Cocci	Rod	Rod	Rod
Cell arrangement	Single	Single	Single	Single	Clusters

Table 2 continued…

PARAMETERS	ISOLATE 1	ISOLATE 2	ISOLATE 3	ISOLATE 4	ISOLATE 5
BIOCHEMICAL CHARACTERISTICS					
Catalase	+	+	+	+	+
Indole	+	+	-	-	-
Oxidase	-	-	-	-	-
Citrate	-	-	+	+	+
Urease	-	-	+	+	-
H2S Formation	-	-	-	+	-
Glucose	+	+	+	+	+
Lactose	+	+	+	-	+
Mannitol	+	+	+	-	+
Gr. Diff.	Straw (BCA)	Black spot (NA)	Dark cream (NA)	Straw (SSA)	Blue (BCA)
Identity	***Bacillus* sp.**	***Micrococcus* sp.**	***Proteus* sp.**	***Shigella* sp.**	***Bacillus cereus***

KEY: + = Positive, - = Negative

Table 3: Microbial Diversity of Isolates

Organisms	Seed	Pulp	Shell
Fungal Isolates			
Aspergillus niger	+	-	-
Saccharomyces sp.	+	-	-
Fusarium sp.	+	-	-
Rhizopus sp.	-	+	-
Mucor	+	+	-
Aspergillus fumigatus	-	-	+
Alternaria sp.	-	-	+
Aspergillus vadensis	+	-	-
Cladosporium sp.	-	-	+
Botrytis cinerea	-	+	-
Mucor mucedo	+	-	-
Bacteria Isolates			
Bacillus sp.	+	-	-
Micrococcus sp.	+	-	-
Proteus sp.	+	-	-
Shigella sp.	-	+	-
Bacillus cereus	+	-	-

KEY: + = Present, - = Absent

CHAPTER FIVE

DISCUSSION

This research was designed to ascertain the percentage infection of microorganisms on the shell, pulp and seed of *Dialium guineense* fruits, isolate and identify the microorganisms (fungal and bacterial) species associated with the shell, pulp and seed of *Dialium guineense* fruits and determine the microbial diversity and similarities. The findings provide a clear confirmation of the fruit's vulnerability to microbial contamination, aligning with and expanding upon existing research. The insights gained from this study are crucial, not only for the post-harvest management of this culturally significant fruit but also for understanding its broader ecological and health implications.

To ascertain the percentage infection was undoubtedly met. The finding of a 100% infection rate except one (1) replicate which had a 75% infection rate across all fruit parts on both Potato Dextrose Agar (PDA) and Nutrient Agar (NA) highlights a critical post-harvest challenge. This high susceptibility suggests that from the moment of harvest, the fruit is under immense microbial pressure, which validates the high post-harvest losses reported by Ambrose and Ibiam (2013).

This observation aligns with challenges faced globally in preserving tropical fruits, where warm climates and high humidity often create perfect breeding grounds for microbes, leading to rapid spoilage and significant post-harvest losses (Dieme *et al*., 2022).

A total of eleven (11) fungal (*Aspergillus niger, Saccharomyces* sp*., Fusarium* sp*., Rhizopus* sp*., Mucor, Aspergillus fumigatus, Alternaria* sp*., Aspergillus vadensis,*

Cladosporium sp*., Botrytis cinerea* and *Mucor mucedo)* and five (5) bacterial species (*Bacillus* sp., *Micrococcus* sp., *Proteus* sp., *Shigella* sp. and *Bacillus cereus)* were isolated and identified.

The fungal isolates, particularly *Aspergillus niger*, *Fusarium* sp. and *Rhizopus* sp., are well-documented as the principal agents of spoilage in tropical fruits (Ambrose and Ibiam, 2013). Their presence is a direct threat to the fruit's shelf life, causing the very rots that lead to nutrient degradation as described by Nwaukwu and Ikechi-Nwogu (2012). Furthermore, the isolation of *Aspergillus* and *Fusarium* lends significant weight to the public health concerns regarding mycotoxin contamination, a risk previously highlighted by Pepple *et al*. (2016) and Egbuta (2012). The identification of *Saccharomyces* sp. (a type of yeast) is also noteworthy; while it is known for its role in beverage fermentation (Ojukwu and Ozugha, 2019), its presence on the fruit indicates a natural source for both potential value-added processing and unwanted fermentative spoilage.

The bacterial isolates, however, represent the most acute food safety finding of this study. The identification of *Bacillus cereus* and, most alarmingly, *Shigella* sp. shifts the conversation from mere spoilage to a significant public health hazard. *Shigella* is a major cause of dysentery and is transmitted due to poor sanitation and hygiene practices. Its presence in the pulp strongly implies contamination from poor handling, washing with contaminated water, or contact with contaminated surfaces, which are widespread challenges in informal food supply chains (Granum, 2006; Allamin *et al*., 2015; Sané *et al*., 2024). Similarly, *Bacillus cereus* is frequently linked to foodborne illnesses due to its

ability to produce toxins (Jeßberger *et al*., 2020; Jovanović *et al*., 2022; Turnbull, 1981). These toxins can cause two types of food poisoning: a vomiting-related form, often linked to a preformed toxin called cereulide, and a diarrhoea form caused by enterotoxins produced by viable bacteria in the intestine (Jovanović *et al*., 2022; Masquelier *et al*., 2023). While Omokpo and Adetunji (2023) focused on *Bacillus* as an endophyte, the presence of the pathogenic *B. cereus* and *Shigella* sp. indicates that external contamination is a more immediate threat to the consumer.

Determining microbial diversity, revealed a wide variety of microbial life (fungal and bacterial). The organisms found on the shell (e.g., *Alternaria* sp., *Cladosporium* sp.) are classic examples of field fungi (Patriarca *et al*., 2014; Martín *et al*., 2022), likely contaminating the fruit before harvest. In contrast, the pulp and seed isolates represent a mix of invasive spoilage fungi (*Rhizopus*, *Mucor*) and handling-associated bacteria (*Shigella*, *Bacillus*).

While *Mucor* showed its versatility by appearing in both the pulp and seed suggesting a broader ability to colonize different environments within the fruit, the overall microbial composition varied significantly. This suggests that certain microbes are specially adapted to the specific conditions of each fruit part, playing distinct roles in either spoilage or perhaps, contributing to the fruit's natural processes. Understanding this spatial distribution is like having a map; it guides us toward more targeted and effective strategies for managing these microbial populations.

5.1 Conclusion

This study successfully characterized the diverse microbial landscape of the *Dialium guineense* fruit. It confirmed that the fruit parts harbor a heavy load of microorganisms, with near-total infection rates.

The microbial diversity was found to be wide and specific to each fruit part, indicating that contamination might have originated from multiple sources, including the field (pre-harvest) and human handling (post-harvest).

This research can directly inform the development of strategies aimed at reducing post-harvest losses and, most importantly, safeguarding consumer health (Allamin *et al.*, 2015; Sané *et al*., 2024). Considering that similar challenges are faced by other fruits like *Detarium senegalense*, where drying has been found effective in reducing rot (Dieme *et al*., 2022), there might be transferable lessons for *Dialium guineense*.

5.2 Recommendations

Given the high infection rates, consumers should be advised to discard any fruits that show visible signs of mould, discolouration or an "off" smell, given the high risk of mycotoxin presence from *Aspergillus* and *Fusarium* species.

Given the presence of *Aspergillus* and *Fusarium* species, future research should focus on conducting detailed analyses to assess the potential for mycotoxin production on *Dialium guineense* fruit, particularly under different storage conditions. This is crucial in fully understanding the health risks associated with consuming infected fruits.

The isolation of *Saccharomyces* sp. reinforces the fruit's potential for value-added processing, such as wine or juice (Ojukwu and Ozugha, 2019). This could create a more stable product, reduce post-harvest losses and provide a safer alternative to consuming contaminated raw fruit.

The antimicrobial properties of the *D. guineense* seed, noted by Ajiboye *et al*. (2018), should be tested *in vitro* against the specific spoilage organisms isolated in this study like *Aspergillus niger* and *Rhizopus* sp. to explore its potential as a natural bio-preservative.

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Plate 1: Isolated *Aspergillus niger* (Fungal Isolate 1)

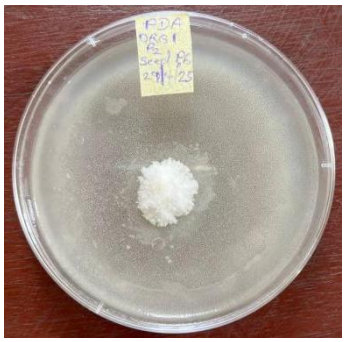

Plate 2: Isolated *Fusarium* sp. (Fungal Isolate 3)

Plate 3: Isolated *Rhizopus* sp. (Fungal Isolate 4)

Plate 4: Isolated *Mucor* (Fungal Isolate 5)

Plate 5: Isolated *Aspergillus fumigatus* (Fungal Isolate 6)

Plate 6: Isolated *Alternaria* sp. (Fungal Isolate 7)

Plate 7: Isolated *Aspergillus vadensis* (Fungal Isolate 8)

Plate 8: Isolated *Cladosporium* sp. (Fungal Isolate 9)

Plate 9: Isolated *Botrytis cinerea* (Fungal Isolate 10)

APPENDIX

Plate 10: Isolated *Bacillus* sp. (Bacteria Isolate 1)

Plate 11: Isolated *Micrococcus* sp. (Bacteria Isolate 2)

Plate 12: Isolated *Proteus* sp. (Bacteria Isolate 3)

Plate 13: Isolated *Shigella* sp. (Bacteria Isolate 4)

Plate 14: Isolated *Bacillus cereus* (Bacteria Isolate 5)