MICROBIAL QUALITY OF DIFFERENT TOMATO VARIETIES SOLD IN BENIN CITY, NIGERIA

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DEPARTMENT OF MICROBIOLOGY

UNIVERSITY OF BENIN

BENIN CITY

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN MICROBIOLOGY, UNIVERSITY OF BENIN, BENIN CITY.

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CERTIFICATION

This is to certify that this project work was carried out by Osamudiamen Eunice OSA-MORAL in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin city under the supervision of DR (MRS) C.G. DIMOWO.

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(HEAD OF DEPARTMENT)

DEDICATION

I wholeheartedly dedicate this research work to the Holy spirit, GOD Almighty and my creator for His unwavering guidance, grace, and strength throughout the course of this work. To Him be all the glory.

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ABSTRACT

Tomatoes (*Solanum lycopersicum*) are among the most consumed vegetables in Nigeria and constitute a vital component of human diets due to their high nutritional and economic value. This study investigated the microbial profiles of three tomato varieties: Beefsteak, Globe, and Roma sold in a selected market in Benin City, Edo State, Nigeria. Standard microbiological techniques were employed for the isolation, enumeration and identification of both bacterial and fungal contaminants using Nutrient Agar, MacKonkey Agar, Eosin Methylene Blue (EMB) Agar, and Potato Dextrose Agar (PDA). The total viable bacterial counts varied among varieties, with Beefsteak tomatoes exhibiting the highest total aerobic bacterial count ($5.0 \pm 0.5\times10^3$), Enteric bacterial count ($15.0 \pm 0.5\times10^3$) and Coliform bacterial count($7.6 \pm 0.5 \times10^3$) followed by Roma and Globe varieties ($4.2 \pm 0.5\times10^3$, $9.2 \pm 0.5\times10^3$, $6.4 \pm 0.5 \times10^3$) and ($4.2 \pm 0.5 \times10^3$, $3.2 \pm 0.5\times10^3$, $2.6 \pm 0.5 \times10^3$) respectively. Identified bacterial isolates included *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa,* while fungal isolates comprised *Aspergillus niger, Penicillium expansum, Rhizopus stolonifer,* and *Fusarium oxysporum*. The observed differences in microbial load were attributed to varietal characteristics such as texture, water content, and handling conditions. The results also revealed that poor post-harvest handling, unhygienic market environments, and the use of contaminated wash water contributed significantly to microbial contamination. It is recommended that vendors adopt improved hygiene, proper storage, and temperature control to reduce spoilage and ensure consumer safety.

CHAPTER ONE
INTRODUCTION
1.1 Background of the Study

Tomato (*Solanum lycopersicum*) is one of the most widely cultivated and consumed vegetables globally and is valued for its nutritional and economic importance. It serves as a rich source of essential vitamins, minerals, and antioxidants such as lycopene, which contribute to human health and disease prevention(Ghosh, 2009; Joosten *et al*., 2015). In Nigeria, tomatoes are consumed daily in both raw and processed forms and represent a major income source for farmers, traders, and vendors across various markets (Momoh *et al*., 2020).

Despite its significance, tomato is a highly perishable crop with a short shelf life due to its high moisture content and delicate epidermis. These properties make it susceptible to microbial contamination and subsequent spoilage (Khalid *et al*., 2024). During harvesting, handling, transportation, and marketing, tomatoes are exposed to a wide range of microorganisms, including bacteria and fungi, which contribute to loss of firmness, discoloration, off-odours, and decay (Saidu *et al.*, 2023).

Different tomato varieties, such as Beefsteak, Globe, and Roma differ in their physical characteristics, including firmness, moisture level, and surface smoothness. These factors may influence their susceptibility to microbial invasion and the rate of spoilage. In addition, environmental conditions such as temperature, humidity, handling practices, and sanitation levels in markets contribute significantly to the overall microbial load on the fruits (Onuorah and Orji, 2015).

In Benin City, where tomatoes are sold in open markets, poor post-harvest handling practices, exposure to dust and flies, and the use of contamina ted wash water often increase the risk of microbial contamination. Understanding the extent and nature of microbial contamination in different tomato varieties is therefore crucial to improving food quality, minimizing health risks, and reducing post-harvest losses. This study investigates the bacterial and fungal profiles of Beefsteak, Globe, and Roma tomatoes sold in Benin City to determine their microbial quality and safety for consumers (Saidu *et al*., 2023; Musa *et al*., 2022).

1.2 Statement of the Problem

Tomato spoilage and microbial contamination remain major challenges affecting food safety and economic stability in Nigeria. Contaminated tomatoes can serve as vehicles for the transmission of pathogenic microorganisms, posing serious public health risks (Khalid *et al*., 2024) While previous studies have examined microbial contamination of tomatoes in Nigerian markets, most have not compared the contamination levels of specific tomato varieties.

Moreover, limited research has been conducted in Benin City, a major trading centre for fresh produce, where environmental and handling conditions may differ from other regions. There is also a general lack of information on fungal involvement in tomato spoilage relative to bacterial contamination. As a result, data on microbial diversity, load, and varietal susceptibility are inadequate for designing effective control measures.

This research addresses these gaps by isolating, characterizing, and comparing bacterial and fungal contaminants associated with three distinct tomato varieties: Beefsteak, Globe, and Roma, sold in Benin City markets (Wogu and Ofuase, 2014; Momoh *et al*., 2020; Musa *et al*., 2022; Adedire *et al*., 2025). The findings are to contribute to the understanding of microbial ecology of tomato spoilage and to guide safety and quality improvement measures.

1.3 Aim/Objectives of the Study

The main aim of this study was to determine and compare the bacterial and fungal profiles associated with different tomato varieties (Beefsteak, Globe, and Roma) sold in selected markets in Benin City.

The specific objectives were to:

1. isolate and identify bacterial species present in different tomato varieties sold in Benin City markets.
2. isolate and identify fungal species present in these tomato varieties.
3. compare the microbial loads (bacterial and fungal) among the Beefsteak, Globe, and Roma varieties.
4. relate the types of microbial isolates to the observable physical characteristics of spoilage (e.g. mould growth, soft rot, discoloration).
5. compare the observed microbial counts with established microbial quality benchmarks for fresh produce to assess produce safety.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Fresh Tomatoes

Tomatoes (*Solanum lycopersicum*) are a staple food globally and are highly susceptible to spoilage due to their high moisture content (typically 90-95 %), relatively low acidity (pH 4.0-4.7), and rich nutrient composition, which provides an ideal substrate for microbial growth (Kumar *et al*., 2012; Saidu *et al*., 2023). Microbial spoilage is the primary cause of post-harvest losses, leading to significant economic repercussions and food safety concerns (Adedire *et al*., 2025; Musa *et al*., 2022). Spoilage manifests as visible signs of decay, including softening, discoloration, mold growth, off-odors, and eventually liquefaction of the fruit (Onuorah and Orji, 2015). These changes are a direct result of microbial enzymes breaking down complex polymers in the tomato cell walls and tissues.

2.2 Tomato varieties and their microbial susceptibility

Different tomato cultivated varieties (e.g., Beefsteak, Globe and Roma) vary in fruit morphology, skin thickness, surface topography and biochemical composition (sugars, organic acids). These intrinsic properties influence microbial attachment, survival and subsequent proliferation on fruit surfaces and in wounds (Ram *et al.,* 2017).

2.2.1 Beefsteak Tomato Variety

Beefsteak tomatoes are large, meaty tomatoes, known for their large size and savoury flavour and they can be susceptible to common tomato diseases like Fusarium wilt, blight, and bacterial wilt, which can be managed with resistant varieties, sanitation, and cultural practices. Like all tomato varieties, they can be affected by bacterial and fungal pathogens, with the severity depending on environmental factors and specific strains. They are named for their “meaty” interior. Beefsteak tomatoes are the largest tomato variety, with fruits that can weigh a pound or more. Examples include "Big Beef" and "Burpee's Early Pick," with different cultivars offering variations in disease resistance and flavour. Flavour profiles vary, with some being more sweet than acidic. Beefsteak tomatoes can be susceptible to diseases such as Fusarium wilt which causes yellowing and wilting, Blight which can be particularly devastating to some varieties and Powdery mildew which inhibits photosynthesis. They can be affected by bacterial wilt, which thrives in warm, moist soil. Spoiled beefsteak tomatoes can host a variety of bacteria and fungi, including *Escherichia col*i, *Staphylococcus aureus, Fusarium oxysporum*, and *Rhizopus stolonifer*. Microbial susceptibility is influenced by environmental conditions such as soil temperature and moisture.

2.2.2 Globe Tomato Variety

Globe tomatoes are a classic, round-shaped slicing tomato variety known for their thick skin and mild flavour. They are susceptible to a wide range of microbes, including various bacteria and fungi that can cause spoilage. This susceptibility depends on a combination of genetic resistance and post-harvest handling. The severity of microbial contamination is heavily influenced by factors like handling, storage, and environmental conditions, rather than the variety alone. They have a classic, rounded "globe" shape, are medium-sized (averaging around six ounces), and are known for being juicy. They have a mild flavour and a thick skin, making them ideal for slicing and eating fresh. They are excellent for fresh consumption, slicing, and canning. Examples include the heirloom "Livingston's Globe" and the hybrid "Marglobe". Poor handling, such as display in unsanitary conditions, can introduce a variety of bacteria and fungi. While all tomatoes are susceptible to some degree, a specific variety's genetic makeup can influence its resistance to certain pathogens. The environment in which tomatoes are grown, harvested, and stored also plays a significant role in their microbial contamination. Common spoilage bacteria include *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*. Pathogenic bacteria can also be a risk, especially with improper preparation. Fungal spoilage is common, with pathogens like *Aspergillus niger*, *Penicillium notatum*, and *Mucor mucido* frequently isolated from spoiled tomatoes. The thick skin of globe tomatoes provides some protection, but they are still vulnerable to contamination, especially through the stem end or any cracks.

2.2.3 Roma Tomato Variety

Roma tomatoes are a determinate, plum-shaped variety known for their thick, meaty flesh and low water content, making them ideal for canning and sauces. They are resistant to common diseases like verticillium wilt (V) and Fusarium wilt (F), which is a key feature of the "VF" variety designation, though they are susceptible to other microorganisms and pests like other tomato varieties. Roma tomatoes are a determinate, open-pollinated variety with a plum shape and bright red colour. They are also known as "Roma VF" to denote their resistance to Verticillium and Fusarium wilt. They are meaty and thick with low water content, making them excellent for cooking down into sauces and pastes. They are high-yielding and prolific, with each plant producing many fruits. Each Roma fruit typically weighs around 60 grams and is about 3 inches long. They are primarily used for canning, making sauces, and pastes due to their low moisture content and thick walls. They grow well in a variety of soils but prefer deep, loamy, well-drained soil with a slightly acidic pH between 6.2 and 6.8. Roma tomatoes are bred for resistance to specific wilts, such as verticillium and fusarium wilt. Like all tomato varieties, they remain susceptible to a wide range of other microorganisms, including bacteria and fungi that cause spoilage. Fungi like *Aspergillus spp*., *Fusarium spp.*, and *Penicillium spp*., as well as bacteria like *Klebsiella spp*.

And *Enterobacter spp.*, can still grow on Roma tomatoes, especially as they age. They can also be susceptible to various pests, such as the cotton aphid, which can transmit harmful viral and fungal diseases.

2.3 Microflora of Tomato Varieties

A diverse community of microorganisms collectively referred to as "Tomato microflora" are commonly found in tomato. This microflora consists of bacteria, fungi, yeasts, and actinomycetes that inhabit both the surface (epiphytic) and internal tissues (endophytic) of the fruit. The microbial composition of tomato fruits varies widely among varieties, depending on factors such as genetic traits, fruit morphology, moisture content, pH, and environmental exposure (Ibrahim *et al*., 2023). Understanding the diversity and behavior of these microorganisms is critical for assessing spoilage patterns, ensuring food safety, and designing varietal-specific control strategies.

2.3.1 Composition of Tomato Microflora

Tomato fruits serve as substrates for a complex microbial ecosystem. Bacteria commonly isolated from tomato fuits include *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus* and *Escherichia coli* (Suleiman *et al*., 2021). Fungal isolates often include *Aspergillus niger*, *Penicillium expansum*, *Rhizopus stolonifer*, *Alternaria alternata* and *Fusarium oxysporum* (Olaniran *et al*., 2020). Yeasts, such as *Candida tropicalis* and *Rhodotorula mucilaginosa*, are also found, particularly in overripe or bruised fruits where they contribute to fermentation and softening. The abundance of each group depends on storage duration, environmental humidity, and the tomato's physicochemical attributes.

2.3.2 Factors Influencing Microbial Diversity Among Tomato Varieties

Fruit Surface Morphology

Microbial attachment and colonization depend largely on the tomato's epidermal structure. Beefsteak varieties have thin and delicate skin which permits easier microbial penetration and harbour higher microbial loads. Globe tomatoes exhibit moderate resistance due to their balanced moisture and acidity. Roma tomatoes, with thick pericarps and lower water activity, often contain fewer surface microorganisms (Nwaogu *et al*., 2022).

Nutritional Composition

Tomatoes provide abundant carbohydrates, organic acids, and amino acids that support microbial growth. Varieties with higher soluble solids and moisture, like Beefsteak, encourage faster microbial proliferation, while Roma varieties, with firmer texture and lower sugar concentration, show slow microbial metabolism (Adeyemi *et al*., 2023).

Handling and Environmental Exposure

Environmental factors during harvest, transportation, and storage greatly influence the tomato microflora. Tomatoes displayed in open markets are constantly exposed to dust, insects, and contaminated water. Studies in south western Nigeria reported higher bacterial counts on tomatoes sold at open stalls compared to those stored under shaded or refrigerated conditions (Uche *et al*., 2021).

pH and Acidity

Tomato pH ranges between 4.0 and 4.6, favouring acid-tolerant microorganisms. High-acid varieties, like Globe tomatoes, suppress certain bacteria but allow the growth of moulds and yeasts. Roma varieties are slightly less acidic and favour facultative anaerobes such as *Lactobacillus plantarum* and *Bacillus cereus* (Eze *et al*., 2023).

2.3.3 Bacterial Flora of Tomato Varieties

2.3.3.1 Gram-Negative Bacteria

Gram-negative bacteria predominate on tomato surfaces, with *Pseudomonas aeruginosa, Klebsiella pneumoniae*, *Enterobacter aerogenes*, and *Escherichia coli* being most common. These species thrive in moist environments and are responsible for enzymatic degradation of tomato tissues, leading to soft rot (Oluwaseun *et al*., 2021). Some strains of *Salmonella enterica* have also been isolated from tomatoes irrigated with contaminated water or handled under poor hygiene.

2.3.3.2 Gram-Positive Bacteria

Species such as *Bacillus subtilis*, *Micrococcus luteus* and *Staphylococcus aureus* are frequent contaminants. While some *Bacillus* spp. are harmless and spore-forming, *Staphylococcus aureus* and *Enterococcus faecalis* can pose serious health risks if consumed (Adebayo *et al*., 2020).

2.3.3.3 Varietal Patterns

Beefsteak tomatoes often harbour high populations of *Pseudomonas* and *Erwinia*, leading to rapid tissue degradation. Globe tomatoes typically support moderate populations of *Micrococcus* and *Bacillus* species, resulting in slower spoilage. Roma tomatoes show reduced bacterial counts, dominated by spore-formers capable of surviving dry conditions (Ashaolu *et al*., 2022).

2.3.3.4 Fungal Flora of Tomato Varieties

Fungi are the principal agents of visible spoilage in tomatoes, especially under warm, humid conditions. *Aspergillus niger, A. Flavus, Penicillium expansum, Fusarium oxysporum,* and *Rhizopus stolonifer* are frequently associated with mouldy or decayed fruits (Tunde *et al*., 2020).

Alternaria alternata and *Cladosporium herbarum* are common post-harvest invaders causing black spots and internal decay. Yeasts like *Candida utilis* and *Rhodotorula glutinis* initiate fermentation, producing ethanol and CO_2 that cause swelling and sour odours (Nwankwo *et al*., 2023).

2.3.4.1 Variety-Specific Fungal Profiles

Beefsteak tomatoes are prone to *Rhizopus* and *Aspergillus* infections due to high moisture. Globe tomatoes are frequently colonized by *Penicillium* species through cracked surfaces. Roma tomatoes are mainly invaded by *Fusarium* species under long-term storage or humid conditions.

2.3.5 Microbial Interactions and Spoilage Progression

Spoilage often involves sequential microbial colonization. Bacteria like *Pseudomonas* degrade cell walls leading to the release of nutrients that promote fungal invasion. Conversely, fungal metabolites such as organic acids alter the pH, favouring bacterial proliferation. These synergistic interactions explain why mixed bacterial-fungal infections are common in stored tomatoes (Ojo *et al*., 2022). Varietal texture influences these interactions. Firm Roma tomatoes delay bacterial entry, whereas soft Beefsteak types rapidly succumb to mixed spoilage.

2.3.5 Implications for Microbial Safety and Quality

The diversity of tomato microflora has direct implications for food safety, shelf life, and market quality. High bacterial counts, particularly of *E. Coli* or *Salmonella*, signal faecal contamination, whereas dominance of *Aspergillus* or *Fusarium* indicates poor storage or excessive humidity. The microbial spectrum thus serves as an indicator of both varietal vulnerability and environmental hygiene (Ibrahim *et al*., 2023). Regular monitoring and microbial profiling of tomato varieties can help identify specific hazards and guide safety interventions.

2.4 Microorganisms Implicated in Tomato Spoilage

A diverse array of microorganisms, primarily bacteria and fungi, contribute to the spoilage of fresh tomatoes. The specific dominant species can vary depending on factors such as geographical location, handling practices, environmental conditions during storage and transport and the intrinsic properties of the tomato variety itself (Khalid *et al*., 2024; Momoh *et al*., 2020).

2.4.1 Bacterial Spoilage Microflora

Bacterial spoilage of tomatoes often presents as soft rot, characterized by a rapid breakdown of tissue. Several bacterial genera have been consistently isolated from spoiled tomatoes, with recent studies in Benin City confirming their prevalence. For instance, a study in Benin City identified *Bacillus subtilis* as the most prevalent bacterial isolate, followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which were associated with significant rot(Wogu and Ofuase, 2024). Other studies corroborate the prevalence of these organisms, often linking their pressence to poor hygiene and potential faecal contamination, especially in the case of *E. Coli* and *Klebsiella* species (Ogofure and Igbinosa, 2023; Onuorah *et al*., 2025; Wogu *et al*., 2024). The high resistance of some isolates like *Pseudomonas aeruginosa* and *Salmonella typhi* to antibiotics also raises significant public health concerns (Wogu and Ofuase, 2024).

***Bacillus* species**: These are common soil bacteria that can readily contaminate tomatoes during pre-harvest and post-harvest handling. Species like *Bacillus subtilis* and *Bacillus* *cereus* have been frequently isolated from spoiled tomatoes in Nigerian markets (Abdullahi *et al.,* 2022; Saidu *et al*., 2023). They are known for their ability to produce heat-resistant spores, which allows them to survive harsh conditions, including some processing treatments, making them difficult to eliminate. These bacteria are potent producers of pectinolytic enzymes, which directly contribute to the soft rot observed in infected tomatoes. The presence of *Bacillus cereus* also raises food safety concerns due to its potential to produce toxins that can cause foodborne illness. (Saidu *et al*., 2023).

***Pseudomonas* species**: *Pseudomonas species*, particularly *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*, are frequently isolated from spoiled tomatoes (Momoh *et al*., 2020; Saidu *et al.*, 2023). They are psychrotrophic, meaning they can grow at refrigeration temperatures, which can be problematic for cold storage conditions that are not consistently maintained. They produce a variety of extracellular enzymes, including pectinases, proteases, and cellulases, which efficiently degrade tomato tissues, leading to a characteristic slimy soft rot. *Pseudomonas* species can also produce pigments and off-flavours, further diminishing the quality and marketability of the fruit. Their adaptability to various environmental conditions makes them persistent contaminants (Abdullahi *et al*., 2022).

Escherichia coli: *E. Coli* is often an indicator of fecal contamination, and thus poor hygiene, it has also been identified in spoiled tomato samples, particularly in markets with poor sanitary conditions (Momoh *et al*., 2020; Saidu *et al*., 2023). The presence of *E. Coli* in fresh produce raises significant public health concerns due to its potential pathogenicity, as certain strains can cause severe gastrointestinal illnesses. Its detection underscores the importance of stringent hygiene practices throughout the tomato supply chain, from farm to fork, to prevent cross-contamination.

***Klebsiella* species**: *Klebsiella aerogenes* is another Gram-negative bacterium commonly associated with spoiled tomatoes in Nigeria (Abdullahi *et al*., 2022; Momoh *et al*., 2020). Like *E. Coli*, its presence often suggests contamination from environmental sources or poor hygiene.

Klebsiella species can contribute to soft rot and may also pose health risks, particularly for immunocompromised individuals.

***Staphylococcus* species**: *Staphylococcus aureus* is a significant human pathogen that has been found on spoiled tomatoes, likely due to human handling (Saidu *et al.*, 2023; Momoh *et al.,* 2020). The ability of *S. Aureus* to produce various toxins (e.g., enterotoxins) is a serious food safety concern, as these toxins can cause food poisoning even if the bacteria themselves are no longer viable. This highlights the critical need for proper hand hygiene among all individuals involved in the tomato value chain.

***Proteus* species**: *Proteus mirabilis* and other *Proteus* species have also been isolated from spoiled tomatoes (Momoh *et al*., 2020). These bacteria are known for their distinctive swarming motility on agar plates, and their rapid growth can contribute significantly to the quick progression of spoilage. They can produce enzymes that degrade plant tissues and contribute to off-odours.

***Salmonella* species and *Shigella* species**: While less frequently cited as primary spoilage organisms, the presence of these highly pathogenic bacteria in spoiled tomatoes from Nigerian markets has been reported. Their detection is a critical public health warning, as both *Salmonella* and *Shigella* can cause severe gastrointestinal diseases, including typhoid fever and bacillary dysentery, respectively. Their presence often indicates significant faecal contamination and necessitates immediate intervention to improve food safety practices.(Saidu *et al.*, 2023).

The total bacterial count in spoiled tomatoes in Nigeria has been reported to range from 10^3 to 10^8 CFU/g, indicating heavy microbial loads (Abdullahi *et al*., 2022; Saidu *et al*., 2023).

2.4.2 Fungal Spoilage Microflora

Fungi are arguably the most significant group of spoilage microorganisms for fresh tomatoes, often causing more extensive visible damage and producing mycotoxins. Their filamentous growth habit allows them to penetrate deeply into the fruit tissue, leading to widespread decay (Ghosh, 2009; Khalid *et al.*, 2024).

***Aspergillus* species**: Species such as *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus ochraceus* are among the most prevalent fungal isolates from spoiled tomatoes in Nigeria (Adedire *et al*., 2025; Khalid *et al*., 2024). *Aspergillus* species are known for causing black mould rot and are a major concern due to their ability to produce mycotoxins (e.g., aflatoxins from *A. Flavus*), which are highly carcinogenic and pose severe health risks (Saidu *et al*., 2023; Khalid *et al.*, 2024).

Aspergillus niger is commonly associated with black mould rot, characterized by dark, velvety patches on the fruit surface. A major concern, however, is *Aspergillus flavus*, known for its ability to produce aflatoxins, which are highly carcinogenic mycotoxins posing severe health risks to consumers.

Aspergillus ochraceus can produce ochratoxin A, another potent mycotoxin. The presence of these toxigenic *Aspergillus* species emphasizes the severe public health implications of fungal spoilage in tomatoes.

***Rhizopus* species**: *Rhizopus stolonifer* (bread mold) is a very common and aggressive spoilage fungus on tomatoes, causing a rapid watery soft rot, where the fruit quickly turns soft and watery due to extensive tissue degradation (Saidu *et al*., 2023). Its rapid growth and widespread presence in the environment make it a frequent contaminant of harvested tomatoes, especially through wounds(Onuorah and Orji, 2015).

***Penicillium* species**: Various *Penicillium* species including *Penicillium digitatum* and *Penicillium chrysogenum* are also significant spoilage agents, often causing blue or green mould rots (Momoh *et al*., 2020; Saidu *et al*., 2023). *Penicillium species*, such as *Penicillium expansum* can also produce mycotoxins, such as patulin, which is a concern for human health.

***Fusarium* species**: *Fusarium oxysporum* and other *Fusarium* species are frequently isolated from spoiled tomatoes (Adedire *et al*., 2025; Momoh *et al*., 2020). They can cause various forms of rot, including soft rot and internal discoloration, and some species produce mycotoxins like fumonisins and trichothecenes which are harmful to human and animal health.

***Alternaria* species**: *Alternaria alternata* is a common post-harvest pathogen causing black mould spots or blotches on tomatoes, especially in areas where there has been some physical damage, providing an entry point for the fungus (Momoh *et al*., 2020). While typically causing superficial damage, severe infections can lead to significant aesthetic and quality losses.

***Saccharomyces* species (Yeasts)**: While less commonly the sole cause of spoilage, yeasts like *Saccharomyces cerevisiae* have been isolated from spoiled tomatoes, especially when the fruit has been damaged, contributing to fermentation and off-flavors(e.g., alcoholic, vinegary) and off-odours. In some cases, gas production from fermentation can lead to bloating of packaged fruits (Khalid *et al.*, 2024; Saidu *et al.*, 2023).

***Mucor* species**: *Mucor mucido* and other *Mucor* species have also been reported to contribute to tomato spoilage, often causing a soft, watery rot similar to that caused by Rhizopus. They are fast growing fungi that can rapidly colonize damaged fruit.(Abdullahi *et al*., 2022).

Fungal counts in spoiled tomatoes have been reported to range from 10^3 to 10^7 CFU/g (Colony Forming Units per gram), indicating substantial fungal contamination and potential for widespread spoilage (Saidu *et al*., 2023; Onuorah and Orji, 2015).

2.5 Mechanisms of Microbial Spoilage

The spoilage of tomatoes by microorganisms is a complex process involving several mechanisms, primarily enzymatic degradation and metabolic activity.

Enzymatic Degradation: Both bacteria and fungi produce a wide array of extracellular enzymes that break down the structural components of the tomato fruit into simpler molecules that can be absorbed and utilized by the microbes.

Pectinases: These are crucial enzymes that degrade pectin, a major component of the middle lamella and plant cell walls. Pectinases (e.g., polygalacturonases, pectin lyases) produced by bacteria like *Pseudomonas* and *Bacillus*, and fungi like *Rhizopus* and *Aspergillus*, lead to the softening and liquefaction characteristic of soft rot. This enzymatic action effectively dissolves the cellular cement, causing the fruit to lose it's firmness and become mushy (Khalid *et al.*, 2024).

Cellulases: These enzymes break down cellulose, another important structural polysaccharide found in plant cell walls. While pectinases are often the primary cause of initial softening, cellulases further contribute to the disintegration of tissue, leading to a more complete breakdown of the fruit's structure.

Proteases and Lipases: These enzymes degrade proteins and lipids, respectively, which are also essential components of tomato cells. The breakdown of the macromolecules alter the texture of the fruit and releases smaller compounds that can serve as substrates for other microbial metabolic activities leading to the formation of off-flavours and odours. For instance, the degradation of proteins can lead to the production of putrefactive compounds.

Acid Production: Many spoilage microorganisms particularly certain bacteria and yeasts, ferment the sugars abundant in the tomato fruit. This fermentation process often results in the production of various organic acids such as lactic acid, acetic acid and succinic acid. While tomatoes are already acidic (pH 4.0-4.7), significant acid production by spoilage microbes further alter the pH, creating conditions favourable for the growth of other acid-tolerant spoilage organisms or contributing to a noticeable souring of the fruit. This alteration in pH also affects the activity of various enzymes within the fruit, accelerating decay.

Gas Production: Anaerobic or facultative anaerobic bacteria and yeasts can produce gases, primarily carbon dioxide, as by-products of their fermentation activities. In packaged tomatoes, this gas accumulation can lead to visible bloating of the packaging or internal pressure within the fruit, which can cause bursting or splitting. The presence of gas indicates active microbial metabolism and spoilage.

Pigment Degradation: Certain microorganisms possess enzymes or metabolic pathways that can degrade natural pigments present in tomatoes, such as lycopene (responsible for the red color) and chlorophyll (green color in unripe fruit). This degradation leads to undesirable discoloration, manifesting as browning, blackening, or a general loss of vibrant color, which significantly impacts the visual appeal and marketability of the fruit.

Toxin Production: A critical aspect of fungal spoilage is the production of mycotoxins (e.g., aflatoxins by *Aspergillus flavus*, patulin by *Penicillium* species). These toxins are harmful to human health and can persist even after the visible signs of spoilage are removed, posing a hidden danger. This makes vigilance against fungal contamination paramount for food safety (Khalid *et al*., 2024; Saidu *et al*., 2023).

2.6 Factors Influencing Microbial Spoilage of Fresh Tomatoes

Several intrinsic and extrinsic factors interact to influence the type and rate of microbial spoilage in fresh tomatoes.

2.6.1 Intrinsic Factors (Tomato Characteristics)

Physiological Maturity and Ripeness: The physiological stage of the tomato at harvest profoundly influences its susceptibility to spoilage. Over-ripe tomatoes are more susceptible to spoilage. This is because, as tomatoes ripen, their cell walls naturally undergo softening processes due to the activity of endogenous enzymes(e.g., Pectinases). This pre-existing softening makes it easier for spoilage microorganisms to penetrate the fruit's tissues (Khalid *et al*., 2024). The natural protective barriers, such as the cuticle and epidermal layers, weaken with increased ripening, offering less resistance to microbial entry. Green, unripe tomatoes are generally more resistant due to their firmer texture and higher acidity.

pH: The relatively low pH (4.0-4.7) of tomatoes generally favours fungal growth over many common spoilage bacteria, as most bacteria prefer a more neutral pH. However ,acid-tolerant bacteria can still thrive and contribute to spoilage in this pH range. The specific pH can also influence the types of microbial enzymes that are most active.

Nutrient Content: The rich nutritional composition of tomatoes, including an abundance of sugars(glucose, fructose), vitamins(e.g., Vitamin C), minerals and organic acids provides an ideal and readily available substrate for microbial growth and metabolic activities, enabling rapid multiplication and enzyme production, thus accelerating the spoilage process (Khalid *et al*., 2024). It is estimated that ripe tomato fruits contain approximately 94 % water, 4.3 % carbohydrates, 1 % protein, 0.1 % fat, 0.6 % fibre and vitamins. The nutrients support the growth of microorganisms such as fungi and bacteria, which produce enzymes that degrade the nutrients. Tomato fruits contain a lot of water which makes them more susceptible to spoilage by microorganisms.

Physical Damage: This is arguably one of the most significant intrinsic factors contributing to spoilage. Any form of physical damage, such as bruises, cuts, cracks, and insect punctures provide direct entry points for spoilage microorganisms, bypassing the natural protective skin of the fruit (Khalid *et al*., 2024). Once this barrier is breached, microbes can easily colonize the nutrient- rich internal tissues, bypassing the plant's natural defenses. This is a major factor in spoilage during harvesting, handling, and transportation in Nigeria (Adedire *et al*., 2025).

2.6.2 Extrinsic Factors (Environmental and Handling)

Temperature: Temperature is arguably the most critical extrinsic factor. Higher ambient temperatures, common in tropical climates like Nigeria, significantly accelerate microbial growth and enzymatic activity, leading to rapid spoilage (Khalid *et al*., 2024). Optimal storage temperatures for tomatoes (around 13°C to 18°C depending on ripeness) are often not maintained in open markets or during transit (Adedire *et al*., 2025).

Relative Humidity (RH): High relative humidity (above 90 %) promotes the growth of many spoilage fungi and bacteria (Khalid *et al*., 2024). It also reduces water loss from the fruit, maintaining its turgor and fresh appearance for a short period , but this also supports microbial activity. Conversely, very low humidity can lead to excessive water loss and shriveling, making the fruit more susceptible to certain forms of decay.

2.7 Pre-Harvest Handling Practices

Contamination from pre-harvest practices arises from soil, water, animal waste, insects, and farm workers. Preventing contamination at this stage is more effective and economical than attempting to remove pathogens later. These practices include:

2.7.1 Use of clean irrigation water

A major determinant of microbial safety is water quality. Irrigation water contaminated with faecal matter or untreated wastewater introduces *E. Coli, Salmonella, Shigella,* and other pathogens to the tomato surface (Alegbeleye *et al*., 2018). Farmers should use borehole or treated water, regularly test water sources, and apply irrigation methods (e.g., drip irrigation) that minimize direct contact between water and fruit surfaces.

2.7.2 Soil management and manure treatment

The use of fresh or inadequately composted animal manure contributes enteric bacteria and fungi to the field ecosystem. Proper composting, which involves maintaining temperatures above 55 °C for at least 3 days and periodic turning, which destroys most pathogens (Balali *et al*., 2020). Synthetic or well-treated organic fertilizers are preferred to minimize microbial load.

2.7.3 Field and crop sanitation

Removing decaying plant debris, maintaining weed-free fields, and disinfecting harvesting tools are essential pre-harvest practices. Crop rotation and soil solarization reduce the persistence of soil-borne pathogens such as *Fusarium* spp. and *Rhizoctonia* spp. (Taddesse *et al*., 2019).

2.7.4 Worker hygiene and protective practices

Washing of hands by farm workers before harvesting and avoiding contact between tomatoes and the soil is very necessary. Using gloves or clean containers helps prevent transfer of skin or faecal bacteria to fruits (Cho, 2023).

2.7.5 Choice of tomato variety and resistant cultivars

Varieties with thicker cuticles and firm skins (e.g., some Roma cultivars) are less prone to bruising and microbial entry. Breeding programs now focus on developing cultivars resistant to post-harvest pathogens such as *Alternaria solani* and *Fusarium oxysporum* (Lawal *et al*., 2025).

2.7.6 Pest and vector control

Insects such as fruit flies and thrips can serve as mechanical carriers of microbial pathogens. Regular pest control using safe, approved pesticides helps reduce this route of transmission.

2.8 Post-Harvest Handling Practices

The way tomatoes are handled after harvest plays a crucial role in determining their shelf life.

2.8.1 Harvesting

Rough harvesting methods, such as throwing or dropping tomatoes into containers, can cause mechanical damage (bruises or cracks), creating entry points for microbes and initiate physiological deterioration (Obeng *et al*., 2018).

2.8.2 Sorting and Grading

Inadequate sorting allows spoiled or damaged fruits to contaminate healthy ones through contact. This contact facilitates the rapid spread of spoilage microorganisms from infected to healthy fruits, a phenomenon known as " Nesting spoilage".

2.8.3 Packaging

Traditional packaging methods in Nigeria, such as baskets, sacks or simply piling tomatoes, offer little protection against physical damage during transport and provide poor ventilation, leading to localized high humidity and heat accumulation within the bulk, creating environments favourable for microbial growth (Yahaya and Mardiyya, 2019).

2.8.4 Transportation

Long transportation distances over poor roads, often with tomatoes piled high in open trucks, lead to extensive bruising and crushing. This mechanical stress significantly accelerates the initiation and progression of spoilage (Naglaa *et al*., 2023).

2.8.5 Storage Conditions

Lack of proper cool storage facilities and storage in open markets exposed to high temperatures, direct sunlight, and dust significantly contribute to microbial proliferation and dehydration (Momoh *et al*., 2020).

2.8.6 Sanitation

Poor hygiene throughout the value chain, from farm to market, are significant sources of microbial contamination and spread. This includes contaminated harvesting tools, unsanitary washing water(if used), unwashed hands of handlers, and unclean storage surfaces and containers. These unhygienic practices can introduce and spread spoilage microorganisms. Contaminated soil adhering to the fruit is also a major source of inoculum, introducing a wide array of soil-borne spoilage microorganisms to the tomato surface (FAER, 2022).

2.9 Ecological Role of Tomato Microflora

The microflora associated with tomatoes play two ecological roles: beneficial and detrimental roles depending on the species, environmental context, and interactions with the host fruit.

2.9.1 Beneficial Roles

Some microorganisms contribute positively to tomato health and post-harvest quality. Non-pathogenic bacteria such as *Bacillus subtilis* and *Pseudomonas fluorescens* decompose organic matter, releasing nutrients that enrich the soil around tomato roots. Certain endophytic bacteria and fungi antagonize spoilage or pathogenic microbes by producing antibiotics, lytic enzymes, or volatile organic compounds. For example, *Trichoderma harzianum* and *Bacillus amyloliquefaciens* have demonstrated inhibitory effects against *Fusarium oxysporum* and *Alternaria alternata* (Haruna *et al*., 2022). Some epiphytic microflora form biofilms that outcompete pathogens for space and nutrients, thus reducing infection rates on tomato surfaces.

2.9.2 Detrimental Roles

Many microorganisms on the other hand can cause spoilage and decay through enzymatic degradation of fruit tissues. Bacteria such as *Erwinia carotovora* produce pectinolytic enzymes that break down cell walls, leading to soft rot. Fungi like *Aspergillus flavus* and *Fusarium verticillioides* generate mycotoxins (aflatoxins and fumonisins) hazardous to humans. Yeasts accelerate post-harvest fermentation, reducing fruit shelf life and altering flavour.

2.10 Ecological Interactions

Microbial succession occurs naturally on tomato surfaces. Early colonizers (e.g., *Bacillus* spp.) may suppress pathogenic species, while opportunistic fungi proliferate later as the fruit's defense systems weaken. This dynamic interplay defines the tomato's microbial ecology and determines whether the fruit decomposes rapidly or remains stable during storage (Sharma *et al*., 2021).

2.11 Human and Environmental Implications

Beneficial microflora support sustainable agriculture by enhancing nutrient cycling and biocontrol, while harmful microflora pose health risks and economic losses. Balancing these microbial populations through proper sanitation, biocontrol, and handling practices is key to maintaining tomato quality and ecological harmony in post-harvest systems.

2.12 Antimicrobial Resistance in Tomato Microflora

The emergence and spread of antimicrobial-resistant microorganisms in food systems have become a major global public health concern. Antimicrobial resistance (AMR) occurs when microorganisms such as bacteria and fungi develop the ability to survive exposure to drugs that were previously effective in inhibiting or killing them. In the context of fresh produce like tomatoes, antimicrobial-resistant microbes can be transferred from the environment to humans through the food chain, contributing to the global AMR crisis.

2.12.1 Sources and Routes of Resistance in Tomatoes

Tomatoes can acquire antimicrobial-resistant microorganisms at various stages of production and distribution. The primary sources include the use of untreated animal manure, contaminated irrigation water, and contact with resistant bacteria from soil or human handlers. Studies have shown that *Escherichia coli, Klebsiella pneumoniae, Salmonella enterica,* and *Staphylococcus aureus* isolated from fresh produce often exhibit multidrug resistance patterns (Balali *et al*., 2020; Adegoke *et al*., 2019).

Environmental contamination plays a key role, as antibiotic residues from livestock and aquaculture waste can enter agricultural soils, promoting the selection and persistence of resistant genes. In addition, poor market hygiene and repeated handling of tomatoes with unwashed hands or dirty containers facilitate cross-contamination between resistant strains.

Fungal isolates from tomatoes, such as *Aspergillus, Penicillium*, and *Fusarium species*, have also shown decreased susceptibility to conventional fungicides due to excessive use of agricultural chemicals. This contributes not only to spoilage persistence but also to potential mycotoxin accumulation.

2.12.2 Common Resistant Bacteria and Fungi Isolated from Tomatoes

Numerous studies across Africa and Asia have reported the presence of antibiotic-resistant bacteria on fresh tomatoes and other vegetables. Common resistant bacterial species include *E. Coli, Salmonella* spp., *Enterobacter* spp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

These isolates often show resistance to ampicillin, tetracycline, chloramphenicol, ciprofloxacin, and cefotaxime, which are commonly used antibiotics in both clinical and agricultural settings (Alonso *et al*., 2021).

In Nigeria, multidrug-resistant *E. Coli* and *Klebsiella* strains have been recovered from tomatoes sold in open markets, suggesting environmental exposure to resistant organisms originating from animal waste or contaminated irrigation sources. Similarly, *Pseudomonas* and *Bacillus species*, though often regarded as spoilage organisms, have demonstrated tolerance to antibiotics such as erythromycin and streptomycin (Akinyemi *et al*., 2022).

Fungal isolates such as *Aspergillus niger*, *Penicillium expansum* and *Fusarium oxysporum* have also exhibited tolerance to fungicides like benomyl, carbendazim, and azole-based compounds, complicating post-harvest control efforts (Naglaa *et al*., 2023).

2.12.3 Mechanisms of Antimicrobial Resistance

The mechanisms by which tomato-associated microorganisms develop resistance are multifaceted. Bacteria may acquire resistance through: Mutation of target sites, reducing antibiotic binding efficiency. Enzymatic degradation of antimicrobial compounds (e.g., β-lactamases that inactivate penicillins). Efflux pump systems, which actively expel antibiotics from the cell.Horizontal gene transfer, where resistance genes are exchanged via plasmids, transposons, or bacteriophages.

Environmental exposure to sub-lethal levels of antimicrobials in fertilizers or water sources encourages these adaptive responses. Once acquired, these genes can persist in microbial populations and even spread to human pathogens through the food chain.

For fungi, resistance mechanisms often involve mutations in target enzymes, overexpression of efflux transporters, or cell wall modifications that reduce fungicide penetration. These adaptations enable spoilage fungi to survive repeated fungicide applications, leading to persistent infection in stored tomatoes.

2.12.4 Public Health and Food Safety Implications

The presence of antibiotic and fungicide-resistant microorganisms on tomatoes represents a serious public health concern. Consumers may be exposed to resistant bacteria during handling or ingestion of raw tomatoes. Such exposure increases the likelihood of horizontal gene transfer to commensal gut bacteria, facilitating the emergence of antibiotic-resistant infections in humans.

Resistant fungal contaminants also pose a dual risk: they prolong spoilage and can produce mycotoxins such as aflatoxins and fumonisins, which are carcinogenic and hepatotoxic. Moreover, the continued presence of resistant microorganisms reduces the effectiveness of commonly used disinfectants and storage treatments, undermining food safety systems.

The persistence of AMR in the tomato supply chain underscores the need for integrated control strategies, including prudent use of agricultural antibiotics, improved water quality management, enforcement of hygiene standards, and continuous monitoring of resistance patterns in produce microflora.

2.13 Microbial Quality Standards and Risk Benchmarks

Microbiological quality standards establish the acceptable limits of microorganisms in foods and serve as important indicators of hygiene, safety, and shelf life. These standards help interpret laboratory results by defining when a food is considered satisfactory, marginal, or unacceptable for human consumption. The International Commission on Microbiological Specifications for Foods (ICMSF) and the Codex Alimentarius Commission (CAC) of FAO/WHO are the two major bodies that provide globally recognized guidance on microbial limits and risk assessment frameworks for foods (FAO 2016; ICMSF, 2018).

2.13.1 Concept and Application of Microbiological Criteria

A microbiological criterion is defined as a statement that describes the acceptability of a food, batch, or process based on the presence, absence, or number of microorganisms and/or the quantity of their toxins or metabolites per unit of mass or volume (ICMSF, 2018). Such criteria are essential for ensuring food safety, validating manufacturing processes, and monitoring sanitary conditions during production and distribution (Jaffrezic, 2020).

Microbiological criteria typically include three parameters:

1. Sample plan: This is the number of samples and analytical units to be tested;

2. Analytical methods: These are the standardized detection and enumeration techniques (e.g., ISO 4833 for total plate count, ISO 21528-2 for Enterobacteriaceae).

3. Acceptance limits (m, M): The threshold counts determining acceptability.

Foods exceeding the maximum limit (M) are considered unsafe or of unsatisfactory hygienic quality.

2.13.2 Global and Regional Standards for Fresh Produce

For fresh fruits and vegetables such as tomatoes, international standards focus primarily on indicator organisms rather than strict pathogen limits, since raw produce is not sterile.

The ICMSF (2018) recommends that the total aerobic plate counts for fresh produce should generally be below 10^5 CFU/g and Coliform counts below 10^3 CFU/g for acceptable hygiene.

The FAO (2000) recommends that the total bacterial count for fresh vegetables generally should be $\leq 10^5$ CFU/g) and microorganisms constituting a direct hazard to health, including pathogenic microorganisms and agents causing parasitic diseases should have a count of zero.

The Codex Alimentarius (FAO/WHO, 2016) provides a framework for assessing microbial safety of ready-to-eat foods, emphasizing hazard identification, exposure assessment, and risk characterization rather than fixed numeric limits.

The Food Standards Australia New Zealand (FSANZ, 2022) compendium suggests that fresh ready-to-eat produce should have aerobic counts $< 10^6$ CFU/g, and enteric pathogens such as *Salmonella* or *Listeria monocytogenes* must be absent in 25 g of product.

In Nigeria, while there are no formal national microbial standards for fresh tomatoes, the Standards Organisation of Nigeria (SON) aligns many food quality requirements with Codex and WHO guidelines, emphasizing Good Agricultural Practices (GAPs) and Good Hygiene Practices (GHPs) to minimize contamination (SON, 2020).

2.13.3 Risk Assessment Frameworks

Modern microbial quality assurance relies on risk assessment rather than fixed limits alone. The Codex framework identifies four steps: Hazard identification (e.g., presence of *E. Coli* O157:H7, *Salmonella* spp.), Hazard characterization (severity of illness, infectious dose), Exposure assessment (probability of consumption of contaminated produce) and Risk characterization (overall likelihood of adverse health effects).

2.14 Control and Prevention of Tomato Spoilage in Different Varieties

Complex interactions between microbial activity, varietal characteristics, and post-harvest handling conditions results in tomato spoilage. The high moisture content, thin epidermis, and nutrient-rich composition of tomatoes make them particularly vulnerable to both bacterial and fungal invasion. Since each variety possesses unique physicochemical traits, such as fruit firmness, acidity, and skin thickness, their susceptibility to spoilage differs significantly. Effective control and prevention measures must therefore combine pre-harvest, harvest, and post-harvest interventions tailored to the specific tomato variety, environmental condition, and market handling practices (Cho, 2023; Lawal *et al*., 2025).

2.14.1 Overview of Spoilage Mechanisms in Tomato Varieties

Tomato spoilage in different varieties commonly involves microbial species such as *Pseudomonas fluorescens*, *Bacillus subtilis, Erwinia carotovora, Aspergillus niger,* *Fusarium oxysporum* and *Penicillium expansum*. These organisms break down cell walls and utilize sugars and organic acids, resulting in soft rot, discoloration, and off-odours. Varietal differences affect the rate and pattern of deterioration.

Beefsteak tomatoes, with high water activity and thin pericarp, are highly prone to bacterial soft rot. Globe tomatoes exhibit moderate firmness but tend to crack under fluctuating humidity, creating entry points for fungi. Roma tomatoes, being denser and less juicy, possess better natural resistance to microbial invasion and hence longer shelf life (Balali *et al*., 2020; Yahaya and Mardiyya, 2019).

2.14.2 Pre-Harvest Control Measures

Variety Selection and Breeding

Selection of disease-resistant and firm-fleshed varieties is the foundation of spoilage prevention. Modern breeding programs have introduced cultivars with thicker skins, elevated phenolic content, and resistance to Fusarium and Alternaria infections (Lawal *et al*., 2025). Roma cultivars demonstrate superior field resistance, whereas Beefsteak varieties require greater attention to field sanitation.

Soil and Irrigation Management

Contaminated irrigation water and manure are major contamination routes for tomatoes. The use of treated irrigation water and properly composted manure significantly reduces E. Coli and Salmonella contamination (Alegbeleye *et al*., 2018). Drip irrigation systems are recommended to minimize direct fruit contact with contaminated water or soil.

Field Sanitation and Crop Rotation

Frequent removal of diseased fruits, disinfection of tools, and rotation with non-host crops interrupt microbial persistence. Crop rotation with legumes or cereals reduces soil-borne *Fusarium* and *Rhizoctonia* species (Taddesse *et al*., 2019).

Pest and Vector Management

Insects such as fruit flies and thrips mechanically transmit bacteria and fungi. Integrated Pest Management (IPM) using pheromone traps and biological control agents limits these vectors and consequently reduces microbial spoilage incidence (FAO, 2016).

Worker Hygiene

Adherence to Good Agricultural Practices (GAPs) like hand washing, clean gloves and sanitized containers reduces human-mediated contamination in the field. Training and awareness programs among farmers have been shown to lower microbial contamination levels (Cho, 2023).

2.14.3 Harvest and Post-Harvest Handling

1. Harvest Timing and Technique
2. Harvesting at the appropriate maturity stage during cool hours prevents mechanical injury and heat accumulation. Overripe Beefsteak fruits should be harvested early and handled gently, while Roma tomatoes may be picked at a later stage without compromising firmness (FAER, 2022).
3. Cleaning and Disinfection

   Post-harvest washing with potable water is essential for removing soil and debris. Chlorinated water (50–100 ppm), citric or lactic acid rinses, and ozonated water are proven to lower microbial loads by 2–3 log units (Kumar *et al*., 2021). However, water reuse must be avoided to prevent cross-contamination.

4. Sorting and Grading

   Physically damaged or decayed tomatoes act as focal points for microbial spread. Sorting and removing such fruits before packaging minimizes cross-infection. This is especially crucial for Beefsteak tomatoes, which bruise easily under pressure (Obeng *et al*., 2018).

5. Packaging and Container Hygiene

   Ventilated plastic crates or food-grade boxes are preferable to jute sacks and wooden baskets, which harbor spores and retain moisture (Yahaya & Mardiyya, 2019). Clean, dry containers reduce microbial persistence and maintain fruit integrity.

6. Temperature Management

   Temperature control is vital to extending shelf life. Storing tomatoes at 12–15°C with 85–90% relative humidity slows microbial metabolism and prevents condensation. Rapid pre-cooling of Beefsteak tomatoes can extend freshness by up to five days (FAER, 2022).

2.14.4 Storage and Transportation Controls

Controlled Atmosphere Storage

Reducing oxygen and increasing carbon dioxide concentrations during storage limits respiration and microbial growth. Controlled atmosphere storage has been shown to preserve Roma and Globe tomatoes for longer durations without affecting taste (Yahaya and Mardiyya, 2019).

Use of Natural Preservatives and Biocontrol Agents

Edible coatings such as chitosan, aloe vera gel, or plant-based essential oils (neem, thyme, cinnamon) reduce microbial attachment and moisture loss. Biocontrol agents like *Bacillus subtilis* and *Pseudomonas fluorescens* inhibit fungal pathogens by producing antifungal compounds (Naglaa *et al*., 2023).

Fungicide and Antimicrobial Treatments

Synthetic fungicides remain effective but should be applied sparingly to prevent resistance and residue accumulation. Combining reduced fungicide doses with biological control and natural coatings offers sustainable control strategies (Lawal *et al*., 2025).

Transport and Market Hygiene

Transportation vehicles and market stalls should be regularly sanitized. Over-stacking must be avoided to reduce bruising. Vendors should display tomatoes on raised, shaded platforms and avoid sprinkling contaminated water to enhance freshness (Obeng *et al*., 2018).

2.14.5 Variety-Specific Control Strategies

Beefsteak Tomatoes require early harvesting and rapid pre-cooling. It benefit from single-layer storage and low mechanical stress. Regular surface disinfection is essential due to thin skin and high moisture. Globe Tomatoes need careful irrigation management to prevent cracking. Chitosan or citric acid coatings enhance surface protection. Its moderate firmness allows short-term storage under cool, ventilated conditions. Roma Tomatoes exhibit higher resistance to microbial invasion due to low water activity. They can tolerate stacking but still require good ventilation to prevent mould. They are ideal candidates for long-distance transport and processing applications.

2.14.6 Emerging Technologies for Spoilage Prevention

Recent innovations offer eco-friendly alternatives for tomato preservation:

Ultraviolet-C (UV-C) irradiation effectively reduces surface microbes without chemical residues.

Cold plasma treatment inactivates pathogens while preserving sensory quality.

Nanocomposite edible coatings incorporating silver or zinc oxide nanoparticles inhibit bacterial and fungal growth (Lawal *et al*., 2025).

Smart packaging technologies embedded with freshness indicators or antimicrobial agents monitor spoilage in real time (Cho, 2023).

2.14.7 Integrated Control Approach

A combination of Good Agricultural Practices (GAPs), Good Handling Practices (GHPs), and Hazard Analysis and Critical Control Points (HACCP) ensures the best outcomes for spoilage prevention. Continuous monitoring at each stage, from production to retail, maintains product safety and extends shelf life.

2.15 Gaps in Literature

Despite the wealth of studies on the microbial contamination of fruits and vegetables in Nigeria and other developing countries, several critical gaps still exist in the current body of knowledge regarding tomato microbiology, particularly within Benin City and its surrounding markets.

Limited comparative data on tomato varieties

Most existing studies on microbial contamination of tomatoes in Nigeria focus on general assessments of market samples without differentiating among specific tomato varieties. Yet, tomato cultivars such as Beefsteak, Globe, and Roma differ in morphology, moisture content, and surface texture. These are factors that significantly affect microbial colonization and spoilage potential. Comparative studies examining microbial load variations among these distinct varieties remain scarce or non-existent within the local context.

Insufficient integration of bacterial and fungal assessments

Previous research has often treated bacterial and fungal analyses separately, with few studies simultaneously quantifying and identifying both groups of microorganisms within the same set of tomato samples. This separation limits understanding of the microbial ecology of spoilage, where both bacterial and fungal communities interact to influence deterioration rate and shelf life.

Scarcity of research linking microbial counts to global safety benchmarks

Although several studies report total viable counts, there is limited effort to interpret these results in relation to international microbial standards such as those of ICMSF, Codex Alimentarius, or WHO/FAO guidelines. Consequently, it remains unclear whether tomato products sold in Nigerian markets meet the recommended safety limits for consumption or hygiene.

Lack of antibiotic resistance profiling among tomato isolates

With rising global concern over antimicrobial resistance (AMR), very few local studies have explored the resistance characteristics of bacteria isolated from fresh tomatoes. Understanding whether environmental and handling factors contribute to the spread of resistant bacteria via produce remains a major research gap in Nigeria.

Minimal focus on market-level hygiene practices in Benin City

Most published studies on produce contamination have been conducted in Lagos, Ibadan, or Northern Nigerian markets, while in Benin City, a major commercial hub for tomato distribution, has received limited scientific attention. There is therefore inadequate documentation of how vendor handling practices, water sources, and environmental sanitation contribute to microbial contamination within Benin's tomato markets.

Neglect of post-harvest fungal diversity and mycotoxin potential

While bacterial counts are commonly reported, the mycological aspect of tomato spoilage is underexplored. Limited information exists on the diversity of fungal isolates (e.g., *Aspergillus*, *Fusarium*, *Penicillium*) and their potential for mycotoxin production, especially in warm, humid environments typical of southern Nigeria.

Limited use of combined morphological and biochemical identification methods

Many market-based studies stop at colony counting or simple Gram reactions without performing detailed biochemical or molecular identification. This restricts accurate classification of microbial isolates and weakens the reliability of findings regarding potential pathogenicity.

Inadequate linkage between microbial load and storage/handling conditions

There is insufficient research connecting quantitative microbial results with the specific handling and storage conditions under which the tomatoes were sold. Studies rarely quantify how practices such as exposure to sunlight, stacking density, or water reuse correlate with bacterial or fungal proliferation**.**

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research design

This study employed an experimental and descriptive design to evaluate the microbial diversity across three tomato varieties: Beefsteak, Globe and Roma. The experimental phase involved culturing, isolating and identifying the microbial species using standard microbiological techniques. This design enabled the collection of both qualitative and quantitative data critical for understanding the dynamics of microbial analysis in different tomato varieties.

3.2 Study Area

Tomato varieties were purchased from a selected market in Benin city, Edo state, Nigeria (Oluku market).

3.3 Sample Collection

Three (03) Tomato varieties (Roma, Globe and Beefsteak) were selected randomly. Three to five of each variety were brought in sterile polythene bags. The samples were labelled and transported to the microbiology laboratory within one hour of collection. The samples were kept in a cool, dry place before analysis.

3.4 Sterilization of Materials

The materials used in this study were sterilized by appropriate methods to free them from microbial contamination. All glass wares were sterilized in hot air oven at 160°C for 2 hours. The inoculating loop and wire were sterilized by flaming in the Bunsen burner until red hot. The working surface was sterilized using 70% Ethanol.

3.5 Preparation of Media

All media were prepared according to manufacturer's instruction under aseptic conditions and sterilized properly at 121°C for 15 minutes.

3.6 Isolation of Microorganisms by Serial dilution

The tomatoes were rinsed with clean, sterile water. 25g of each sample was blended and aseptically diluted in 225ml of sterile distilled water to prepare a stock solution. 9ml of distilled water was measured into test tubes and sterilized. For appropriate dilutions, 1ml of stock solution was diluted into four test tubes labelled 10^1 to 10^4 (4-fold dilution) for each sample. These dilutions were used for the enumeration of bacteria and fungi using appropriate selective and differential media. 1 ml of 10^2 and 10^4 dilutions for each sample were plated onto Nutrient agar(for total bacterial count), MacKonkey agar(for coliforms), Eosine Methylene Blue Agar(for Gram- negative enteric bacteria and lactose and non lactose fermenters) and Potato Dextrose Agar (for Fungi). The plates were gently swirled to mix and ensure even distribution of the inoculum. The plates were inverted and incubated at 37°C for 24-48 hours(for bacteria) and at 28°C for 3-5 days(for fungi). The colonies observed after growth were counted using a colony counter and recorded as CFU/g (Jasuja *et al.,* 2013).

3.7 Enumeration of Isolates

After the period of incubation, the plates were observed for growth. Colonies present on the agar medium were counted using a colony counter. The total viable count for each microbial group was calculated by multiplying the number of colonies by the dilution factor and results were expressed in CFU/g of tomato (Willey, 2008). The number of colony-forming united per milligram (CFU/g) was calculated using the formula below:

$$\text{Colony forming unit per milligram } \frac{cfu}{g} = \frac{number\ of\ colonies \times 1}{volume\ plated \times dilution\ factor}$$

3.8 Purification of Isolates

Distinct colonies observed on the different agar plates were carefully sub-cultured onto freshly prepared agar plates. This was done using the streak plate method to obtain distinct colonies. The agar plates were inverted and incubated at 37°C for 24 hours. The pure colonies observed thereafter were further identified based on morphology or biochemical methods (Dimowo and Omoregie, 2023).

3.9 Characterization of Bacterial Isolates

The purified isolates were characterized both morphologically and biochemically.

3.9.1 Morphological Characterization

The bacterial colonies were examined for their colour, shape, margin, elevation, surface and appearance. Gram staining was carried out to determine Gram reaction and cell morphology.

3.9.2 Biochemical Characterization

The bacterial isolates were analyzed biochemically using a series of tests, which included Catalase test, Coagulase test, Oxidase test, Indole test, Urease test, Citrate utilization test and Triple Sugar Iron agar test. The results were compared with Bergey's Manual(19^{th} Edition, 1994).

3.10 Characterization and Identification of Fungal Isolates

Characterization and identification of fungal isolates were based on macroscopic and microscopic examination.

3.10.1 Macroscopic Examination

It was carried out by observing the colonial characteristics especially the colour formation on both the front and reverse sides of the plates.

3.10.2 Microscopic Examination

Lactophenol cotton blue stain was used. A drop of the solution was placed on a clean grease-free slide. A fragment of the fungal isolate was emulsified in the solution after which the slide was covered with a cover slip, avoiding bubbles. The slide was thereafter viewed under the microscope. Fungal growth on plate culture was observed : surface, spore, underside colour, structure of house and details of sporulating structure.

3.11 Data Analysis

Data obtained from microbial enumeration were recorded and statistically analyzed. The results were expressed as mean ± standard deviation.

CHAPTER FOUR

RESULTS

The results for the microbial analysis of different Tomato varieties sold in Benin city are presented below. The total aerobic bacterial counts of the different tomato varieties are shown in Table 4.1. The highest aerobic bacterial count was gotten from Beefsteak variety ($5.0 \pm 0.5 \times 10^3$ CFU/g), while the lowest was gotten from Globe variety (4.2 ± 10^3 CFU/g).

The total enteric bacterial counts presented in Table 4.2 show that the highest contamination was recorded from Beefsteak variety ($15.0 \pm 0.5 \times 10^3$ CFU/g), followed by Roma variety ($9.2 \pm 0.5 \times 10^3$ CFU/g), while the lowest enteric count was from Globe variety ($3.2 \pm 0.5 \times 10^3$ CFU/g).

As shown in Table 4.3, the highest coliform counts were gotten from Beefsteak variety ($7.6 \pm 0.5 \times 10^3$ CFU/g), followed by Roma variety ($6.4 \pm 0.5 \times 10^3$ CFU/g), while the lowest Coliform count was obtained from Globe variety ($2.6 \pm 0.5 \times 10^3$ CFU/g).

The morphological characteristics of the bacterial isolates as presented in Table 4.4 revealed seven distinct bacterial species from the three tomato varieties. The predominant isolates identified included *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Enterobacter aerogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus mirabilis.*

The biochemical characteristics of the bacterial isolates from the different Tomato varieties are presented in Table 4.5.

The morphological characteristics and presumptive identification of the fungal isolates from Tomato varieties are showing in Table 4.6. The predominant isolates identified included *Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer, Mucor* spp., *Penicillium* spp. And *Candida* spp.

Table 4.7 shows the mean Fungal Load of the Tomato varieties sold in Benin city with Beefsteak having the highest count ($1.8 \pm 0.5 \times 10^5$), followed by Roma ($9.2 \pm 0.5 \times 10^4$), while the lowest was obtained from Globe variety ($7.0 \pm 0.5 \times 10^4$)

Table 4.1: Total Aerobic Bacterial Counts (CFU/g) of Different Tomato Varieties on Nutrient Agar.

Tomato Variety	10^2 Dilution (CFU/g)	IMSCF/FAO
Beefsteak (A)	$5.0 \pm 0.5\times10^3$	**$\leq 10^5$ CFU/g
Globe (B)	$4.2 \pm 0.5\times10^3$	
Roma(C)	$4.2 \pm 0.5\times10^3$	

Values are represented as Mean ± Standard deviation .

Table 4.2: Enteric Bacterial Counts of Different Tomato Varieties on EMB Agar.

Tomato Variety	10^2 Dilution (CFU/g)	IMSCF/FAO
Beefsteak (A)	$15.0 \pm 0.5\times10^3$	**$\leq 10^1$ CFU/g
Globe (B)	$3.2 \pm 0.5\times10^3$	
Roma(C)	$9.2 \pm 0.5\times10^3$	

Values are represented as Mean ± Standard deviation.

Table 4.3: Coliform Bacterial Counts of Different Tomato Varieties on MacKonkey Agar

Tomato Variety	10^2 Dilution (CFU/g)	IMSCF/FAO
Beefsteak (A)	$7.6 \pm 0.5\times10^3$	**$\leq 10^3$ CFU/g
Globe (B)	$2.6 \pm 0.5\times10^3$	
Roma(C)	$6.4 \pm 0.5\times10^3$	

Values are represented as Mean ± Standard Deviation.

Table 4.4: Morphological Characteristics of Bacterial Isolates from Different Tomato Varieties

Tomato variety	Colour	Shape	Margin	Elevation	Surface Appearance	Organism
Beefsteak (A)	Cream on NA	Circular	Entire	Raised	Smooth, moist	*E. coli*
	Golden yellow	Circular	Entire	Convex	Smooth, glistening	*S.aureus*
	Off-white	Irregular	Undulate	Flat	Dull, rough	*B. subtilis*
	Pale pink	Circular	Entire	Raised	Smooth	*E. aerogenes*
Globe (B)	Mucoid cream	Circular	Entire	Convex	Sticky, shiny	*K. pneumoniae*
	Greenish	Irregular	Undulate	Raised	Moist, pigmented	*P. aeruginosa*
	Golden yellow	Circular	Entire	Convex	Smooth	*S. aureus*
Roma (C)	Creamy	Circular	Entire	Raised	Smooth, moist	*E. coli*
	Creamy -white	Irregular	Undulate	Flat	Swarming	*P. mirabilis*
	Off-white	Irregular	Lobate	Flat	Dry, rough	*B. subtilis*
	Greenish	Irregular	Undulate	Raised	Moist, pigmented	*P. aeruginosa*

Table 4.5: Biochemical Characteristics and Identification of Bacterial Isolates from Tomato Varieties

Organism	Gram stain	Catalase	Coagulase	Indole	Citrate	Urea	Oxidase
E. Coli	-	+	-	+	-	-	-
S. aureus	+	+	+	-	+	+	-
B. subtilis	-	+	-	-	+	-	+
E. aerogenes	-	+	-	-	+	-	-
K. pneumoniae	-	+	-	-	+	+	-
P. aeruginosa	-	+	-	-	+	-	+
P. mirabilis	-	+	-	+	+	+	-

Table 4.6: Morphological Characteristics and Presumptive Identification of Fungal Isolates from Tomato Varieties Sold in Benin City

Tomato Variety	Colour	Texture/surface appearance	Elevation	Margin	Organism
Beefsteak (A)	Blackish- green to yellow	Powdery, dry and velvety with dense mycelia	Flat to slightly raised	Irregular	*Aspergillus niger*
	White, cottony growth covering entire plate	Fluffy aerial mycelia	Raised	Filamentous	*Rhizopus stolonifer*
	Greyish -white colonies	Woolly and fast growing	Raised	Irregular	*Mucor* spp.
Globe (B)	Blue-green colonies	Velvety and compact	Flat	Circular	*Penicillium* spp.
	White to creamy smooth colonies	Moist, yeast-like	Convex	Entire	*Candida* spp.
Roma (C)	Greenish colonies with yellowish reverse	Powdery and spreading	Flat	Irregular	*Aspergillus flavus*
	White fluffy mycelia	Cottony aerial hyphae	Raised	Filamentous	*Rhizopus stolonifer*

Table 4.7: Mean Fungal Load of Tomato Varieties Sold in Benin City

Tomato Variety	**Mean Fungal Load (CFU/g)**
Beefsteak (A)	$1.8 \pm 0.5 \times 10^5$
Globe (B)	$7.0 \pm 0.5 \times 10^4$
Roma (C)	$9.2 \pm 0.5 \times 10^4$

Values are represented as Mean ± standard deviation.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 BACTERIOLOGICAL FINDINGS

The results of this study revealed that all tomato varieties examined: Beefsteak, Globe, and Roma, harboured varying levels of bacterial contamination. The Beefsteak variety had the highest total aerobic bacterial count ($5.0 \pm 0.5 \times 10^3$ CFU/g), Enteric Bacterial counts($15.0 \pm 0.5 \times 10^3$ CFU/g) and for Coliform Bacterial Counts ($7.6 \pm 0.5 \times 10^3$ CFU/g). It was followed by the Roma variety which had counts for total aerobic bacterial counts as ($4.2 \pm 0.5 \times 10^3$ CFU/g), Enteric Bacterial counts as ($9.2 \pm 0.5 \times 10^3$ CFU/g) and Coliform Bacterial Counts as ($6.4 \pm 0.5 \times 10^3$ CFU/g). Globe had the lowest counts for total aerobic bacterial counts ($4.2 \pm 0.5 \times 10^3$ CFU/g), Enteric Bacterial Counts ($3.2 \pm 0.5 \times 10^3$ CFU/g) and Coliform bacterial counts as ($2.6 \pm 0.5 \times 10^3$ CFU/g). This variation can be attributed to differences in the surface structure and moisture content of the tomato varieties, which influences microbial attachment and growth. The high bacterial count in Beefsteak tomatoes could also result from their softer texture and larger surface area, which increases susceptibility to contamination during handling and display in open markets.

The bacterial isolates identified included *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. The presence of *E. Coli* is particularly significant as this organism is a classic indicators of fecal contamination and poor hygienic practices during post-harvest handling (Obi and Polson, 2020). Similar findings were reported by Odu and colleagues (2018), who observed *E. Coli* and *Klebsiella species* as dominant contaminants of tomatoes sold in Port Harcourt markets. Likewise, Adeolu *et al.* (2019) noted *Staphylococcus aureus* as one of the predominant bacteria isolated from tomato fruits in Lagos, reflecting contamination from human contact during sorting and sale.

The presence of *Klebsiella* and *Enterobacter species* further suggests environmental and soil-related contamination, as these organisms are commonly found in soil and water (Adebayo *et al*., 2021). The occurrence of both Gram-positive and Gram-negative bacteria aligns with the findings of Egbenya *et al*. (2020), who reported diverse bacterial flora on tomatoes sold in Ghanaian markets, highlighting the influence of multiple contamination sources such as air, handling, and washing water.

Overall, the bacterial load levels recorded in this study are within the acceptable limits (10^5 CFU/g) for Total aerobic counts for fresh produce and exceeded the acceptable limits for Coliform counts for fresh produce (10^3 CFU/g) as recommended by the International Commission on Microbiological Specifications for Foods (ICMSF, 2018), indicating potential public health risks associated with the consumption of raw or improperly washed tomatoes and FAO (2000) also indicating microorganisms and agents causing parasitic diseases should be equal to zero.

5.2 MYCOLOGICAL FINDINGS

The fungal analysis revealed that all tomato varieties were contaminated with different fungal species, though at varying proportions. The Beefsteak tomatoes recorded the highest fungal load ($1.8 \pm 0.5\times10^5$ CFU/g)), followed by Roma ($9.2 \pm 0.5\times10^4$ CFU/g) and Globe ($7.0 \pm 0.5\times10^4$ CFU/g). This pattern is consistent with the bacterial load distribution, suggesting that the physicochemical properties of the Beefsteak variety favour microbial colonization.

The fungi presumptively identified included *Aspergillus niger, Mucor* spp., *Candida* spp., and *Rhizopus stolonifer*. These organisms are well-documented spoilage agents of fruits and vegetables and are often associated with post-harvest deterioration (Olawale *et al*., 2019). The predominance of *Aspergillus niger* in the samples supports findings by Adedeji *et al.* (2018), who reported its frequent isolation from spoilt tomatoes due to its ability to thrive under humid storage conditions. *Mucor* and *Rhizopus* species, on the other hand, are known to cause soft rot and black mould diseases in tomatoes, leading to significant post-harvest losses (Akanbi *et al*., 2020).

The detection of *Penicillium* species*,* though less frequent, is of health concern due to their potential to produce mycotoxins such as ochratoxin and citrinin, which pose toxicological risks to consumers (Bankole and Adebanjo, 2019). The relatively lower fungal load in Roma and Globe tomatoes may be attributed to their firmer skin texture and reduced moisture retention, which inhibit fungal penetration.

The fungal presence observed in this study is comparable to the findings of Ogbu *et al.* (2021), who recorded *Aspergillus, Penicillium,* and *Fusarium* as the major post-harvest pathogens of tomatoes in Nsukka markets. This widespread similarity underscores the critical role of environmental exposure and poor storage in promoting fungal proliferation.

5.3 CONCLUSION

The detection of both pathogenic and spoilage microorganisms in the examined tomato varieties indicates substantial post-harvest contamination, likely arising from poor handling, inadequate sanitation, and unsanitary display environments in Benin City markets. The co-occurrence of faecal indicator bacteria such as *E. Coli* and *Salmonella* with toxigenic fungi like *Aspergillus* underscores a dual microbial threat, both infectious and toxicological.

These findings highlight the urgent need for improved post-harvest hygiene, including the use of clean water for washing, proper storage, and reduced hand contact during market display.

Public health education targeting tomato vendors and consumers is also essential to minimize risks associated with microbial contamination and foodborne illnesses.

5.4 RECOMMENDATIONS

From the findings of this study on the microbial analysis of different tomato varieties Beefsteak, Globe, and Roma, sold in Benin City, several recommendations are proposed to enhance the microbial safety, quality, and shelf life of tomatoes as well as to reduce health risks associated with their consumption.

5.4.1 Handling and Hygiene Practices

Improved Market Sanitation

Vendors and retailers should ensure that tomatoes are displayed and sold under hygienic conditions. Market authorities should enforce regular cleaning of market stalls and discourage the practice of placing tomatoes directly on bare ground or contaminated surfaces.

Personal Hygiene of Handlers

Vendors should maintain high personal hygiene standards, including frequent hand washing, wearing clean aprons, and avoiding handling tomatoes when ill. The use of clean, non-contaminated water for washing and rinsing tomatoes should be strictly observed to prevent cross-contamination.

Use of Clean Packaging Materials

Traditional woven baskets and reused sacks that harbour microorganisms should be replaced with easily washable or disposable materials such as plastic crates or sanitized trays to minimize microbial build-up.

Minimization of Physical Damage

Since bruising and cracking favour microbial colonization, handlers should avoid rough transportation and overloading of tomatoes. Gentle handling and the use of padded crates are recommended to maintain the integrity of the fruit surface.

5.4.2 Storage and Transportation

Temperature Management

Tomatoes should be stored at moderate temperatures (12–15°C) with adequate ventilation to slow microbial growth and reduce spoilage rate. Refrigeration, although effective, should be applied carefully to avoid chilling injury in certain varieties.

Segregation of Varieties by Shelf Life

This study revealed that Beefsteak tomatoes, due to their high moisture and soft texture, spoil faster than Globe and Roma varieties. Therefore, Beefsteak tomatoes should be prioritized for immediate sale and consumption, while Roma varieties may be reserved for longer storage or transport.

Use of Natural Preservatives and Sanitizers

The use of food-safe organic acids (e.g., acetic or citric acid) or plant-based antimicrobial rinses can reduce microbial load before marketing. These methods are cost-effective and environmentally friendly alternatives to synthetic preservatives.

5.4.3 Consumer-Level Recommendation

Proper Washing Before Consumption

Consumers should thoroughly wash tomatoes under running potable water before eating raw or processing them into sauces and stews. Washing with mild vinegar or salt solution can further reduce microbial contamination.

Avoidance of Visibly Spoiled Fruits

Tomatoes showing signs of mould growth, discoloration, or off-odours should not be consumed, as these may harbour pathogenic bacteria or mycotoxin-producing fungi.

Adequate Cooking

Cooking tomatoes at sufficient temperatures can effectively destroy most pathogenic microorganisms and reduce health risks associated with bacterial or fungal contamination.

5.4.4 Policy and Public Health Recommendations

Regular Microbial Surveillance

Government health agencies such as NAFDAC and the Environmental Health Department should implement regular microbial quality monitoring of fresh produce sold in markets to ensure compliance with food safety standards.

Public Health Education

Continuous enlightenment campaigns should be organized to educate farmers, vendors, and consumers about the importance of hygienic handling, proper storage, and foodborne disease prevention.

Development of Local Food Safety Regulations

There is a need for localized microbial safety benchmarks tailored to fresh produce in Nigerian markets. Such policies should set acceptable microbial load limits and outline penalties for non-compliance.

5.4.5 Contributions to Knowledge

Identification of Microorganisms with Public Health Relevancy

Microorganisms isolated such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Aspergillus* and *Fusarium* species highlights potential food safety risks associated with the consumption of raw or poorly handled tomatoes. This reinforces the need for improved hygiene and monitoring systems.

Combined Bacterial and Fungal Analysis

Unlike many previous studies that focused primarily on bacterial contamination, this research integrates both bacterial and fungal analyses. This therefore provides a broader perspective on the microbial ecology of tomatoes in open markets.

Comparative Analysis of Variety-type Susceptibility

This study establishes the differences in microbial load among three tomato varieties, demonstrating that varietal characteristics such as texture, moisture content, and surface structure influence microbial colonization and spoilage rates.

Provision of Baseline Microbial Data for Benin City

This research provides updated and location-specific data on the microbial quality of commonly consumed tomato varieties (Beefsteak, Globe, and Roma) sold in Benin City. Prior to this study, limited comparative data existed specifically for these varieties within Benin city.

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APPENDIX I

3.4 MATERIALS

3.4.1 CHEMICALS AND REAGENTS

Distilled water, Ethanol (95% and 70%), Hydrogen peroxide, Kovacs's reagent, Lactophenol cotton blue, Gram staining reagents (Crystal violet, Iodine, Safranin), Immersion oil and Oxidase reagent (tetramethyl-phenylenediamine- dihydrochloride) were used in this study.

3.4.2 EQUIPMENTS

Incubator**,** Autoclave, Weighing balance (electronic), Sterile Petri dishes, Microscope,Inoculating loop, Blender**,** Vortex mixer**,** Bunsen burner, Cotton wool and aluminium paper corks, Test tubes and rack, Micropipette and Colony counter were used in this study.

3.4.3 MEDIA AND CULTURE SUPPLIES

Nutrient agar, MacKonkey agar, Eosine Methylene Blue Agar, Potato Dextrose Agar, Simmon's Citrate Agar, Triple Sugar Iron Agar and Urease Agar were used in this study.

3.5 BIOCHEMICAL TESTS

3.5.1 CATALASE TEST

This test is used to detect the pressence or absence of catalase enzyme. The catalase enzyme catalyzes the breakdown of hydrogen peroxide to release free oxygen gas and formation of water. A few drops of freshly prepared 3% was added onto bacterial isolate smeared on a slide. When gas bubbles are produced, it indicates the pressence of the catalase enzyme.

3.5.2 COAGULASE TEST

Coagulases are enzymes that clot blood plasma and they are produced by Staphylococcus aureus . The enzyme Protease converts fibrinogen to Fibrin resulting in blood clotting. A bacterial smear was made on a sterile glass slide and human plasma was added to the smear and rocked gently for some minutes. A clumping or agglutination of the plasma indicates the pressence of coagulase.

3.5.3 INDOLE TEST

The test was used to determine which of the isolates has the ability to split indole from tryptophan present in buffered peptone water. The test is used to differentiate Gram-negative Bacilli especially those of the Enterobacteriaceae. Peptone water was prepared and about 10ml of it was dispensed into test tubes using a sterile graduated cylinder. A sterile inoculating loop was used to pick a well isolated colony of bacteria and inoculated into the test tubes. Thereafter, the tubes were incubated at 37°C for 48 hours. After incubation period, 0.5ml of Kovacs's reagent was added to the inoculation test tubes. A red ring in the surface layer within 10 minutes indicates a positive Indole reaction.

3.5.4 UREASE TEST

The bacterial isolates were inoculated into slants of urea medium and incubated at 37°C for 24-48 hours. Red-pink colour indicates the pressence of urease cultures.

3.5.5 OXIDASE TEST

A piece of filter paper was wet with a few drops of the dilute (1%) solution of oxidase reagent (tetramethyl-phenylenediamine-dihydrochloride) which was prepared by standard procedure. A bit of growth from the nutrient agar slant was obtained using sterilized platinum wire loop and smeared on the wet piece of paper. Development of an intense purple colour by the cells within 30 seconds indicates a positive result.

3.5.6 CITRATE UTILIZATION TEST

This test is based on the ability of some organisms to utilize Citrate as a sole carbon source. It was carried out by inoculating the test organism in test tubes containing

Simmon's Citrate medium and incubated at 37°C for 24-48 hours. A deep blue colour indicates a positive result.

3.5.7 TRIPLE SUGAR IRON AGAR TEST

Triple Sugar Iron Agar Medium is recommended for identification of Gram-negative enteric bacilli on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulphide production. 64.42 grams (the equivalent weight of dehydrated medium per litre) was suspended in 1000ml of distilled water. It was heated to boiling to dissolve the medium completely. It was then sterilized in the autoclave at 115°C for 15 minutes. It was mixed well and distributed into test tubes and allowed to cool to room temperature. An isolated pure colony was inoculated on the agar. The tubes were then incubated aerobically at 35-37°C for 18-24 hours. Acid slant/ Acid butt indicate dextrose and sucrose fermentation or dextrose and lactose fermentation or all the three sugars- dextrose, lactose and sucrose fermentation. An alkaline/ acid butt indicates Dextrose fermentation only. Pressence of alkaline butt/ alkaline butt means absence of fermentation. Bubbles or cracks present signifies gas production. When black precipitate is present, it signifies H2S gas production.

3.5.8 GRAM STAINING PROCEDURE

A smear of the bacterial culture was made on a clean slide and heat-fixed. The slide was flooded with crystal violet (primary stain) for 1 minute and then rinsed with water. Lugol iodine (mordant) was added for 1 minute and then rinsed. The slide was decolorized with 95% alcohol for 10–30 seconds, then rinsed immediately. Counterstaining was done using safranin for 1 minute, then rinsed and air-dried. The slide was observed under a microscope at 100x (oil immersion lens).

3.5.9 INTERPRETATION

– Bacterial cells with thick peptidoglycan layer will retain the primary stain and appear purple in colour. This means Gram-positive.

- Bacterial cells with thin peptidoglycan layer will retain only the counter stain and appear pink or red in colour. This means Gram-negative.

APPENDIX II

Fig 1: Beefsteak Tomato

Fig 2: Globe Tomato

Fig 3: Roma Tomato

Fig 4: Bacterial growth on Nutrient Agar

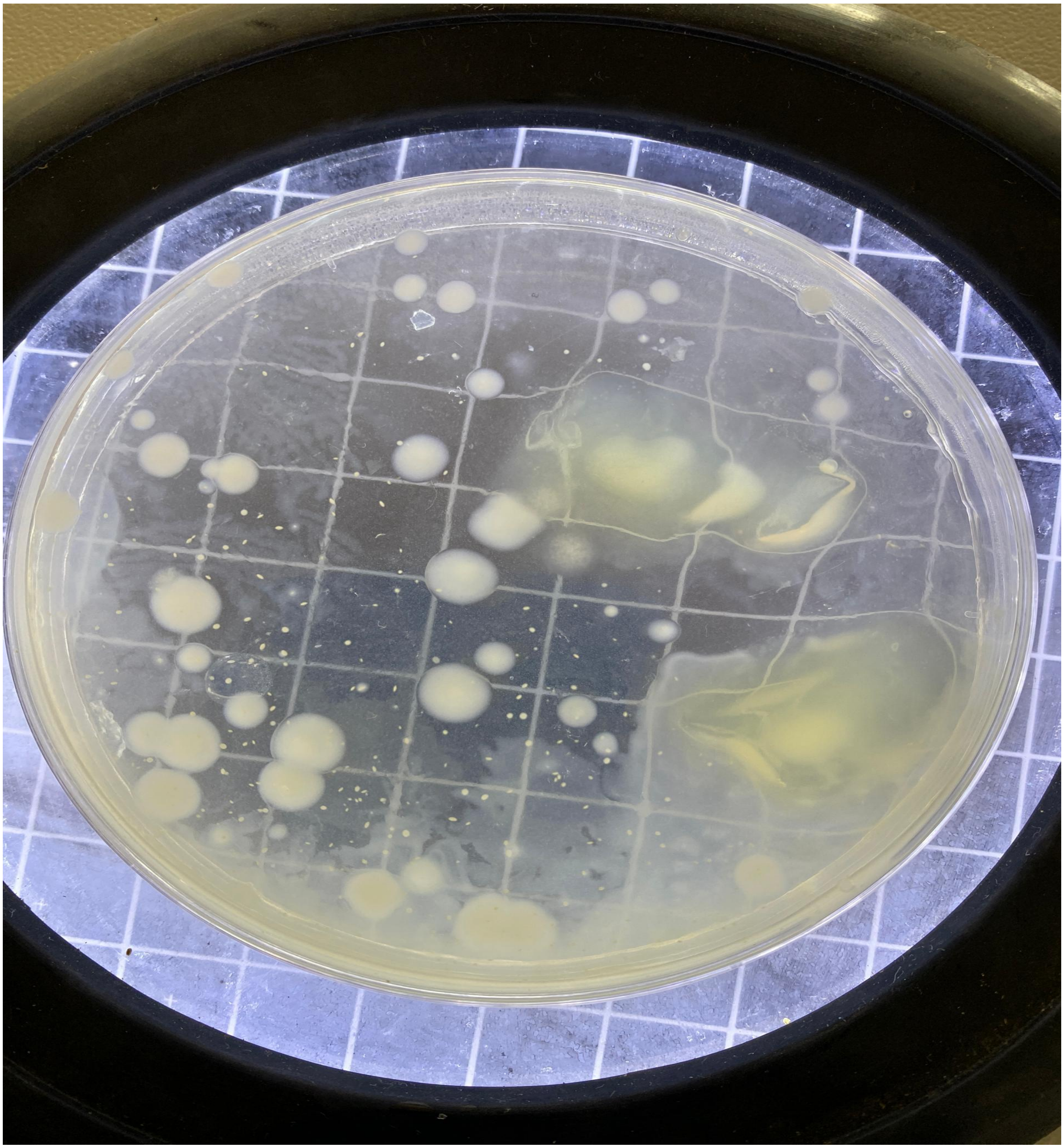

Fig 5: Bacterial growth on EMB Agar

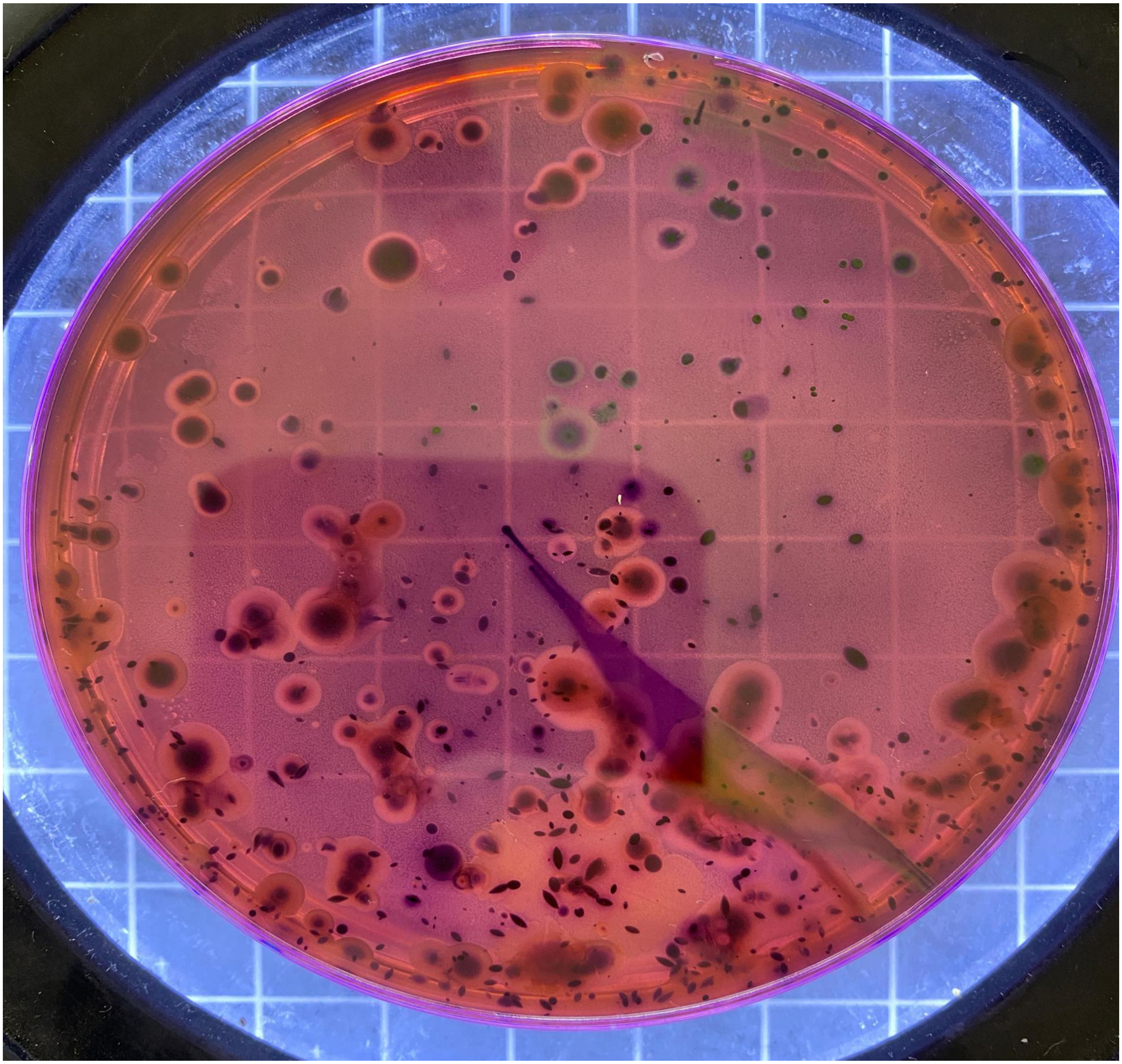

Fig 6: Bacterial growth on MacKonkey agar

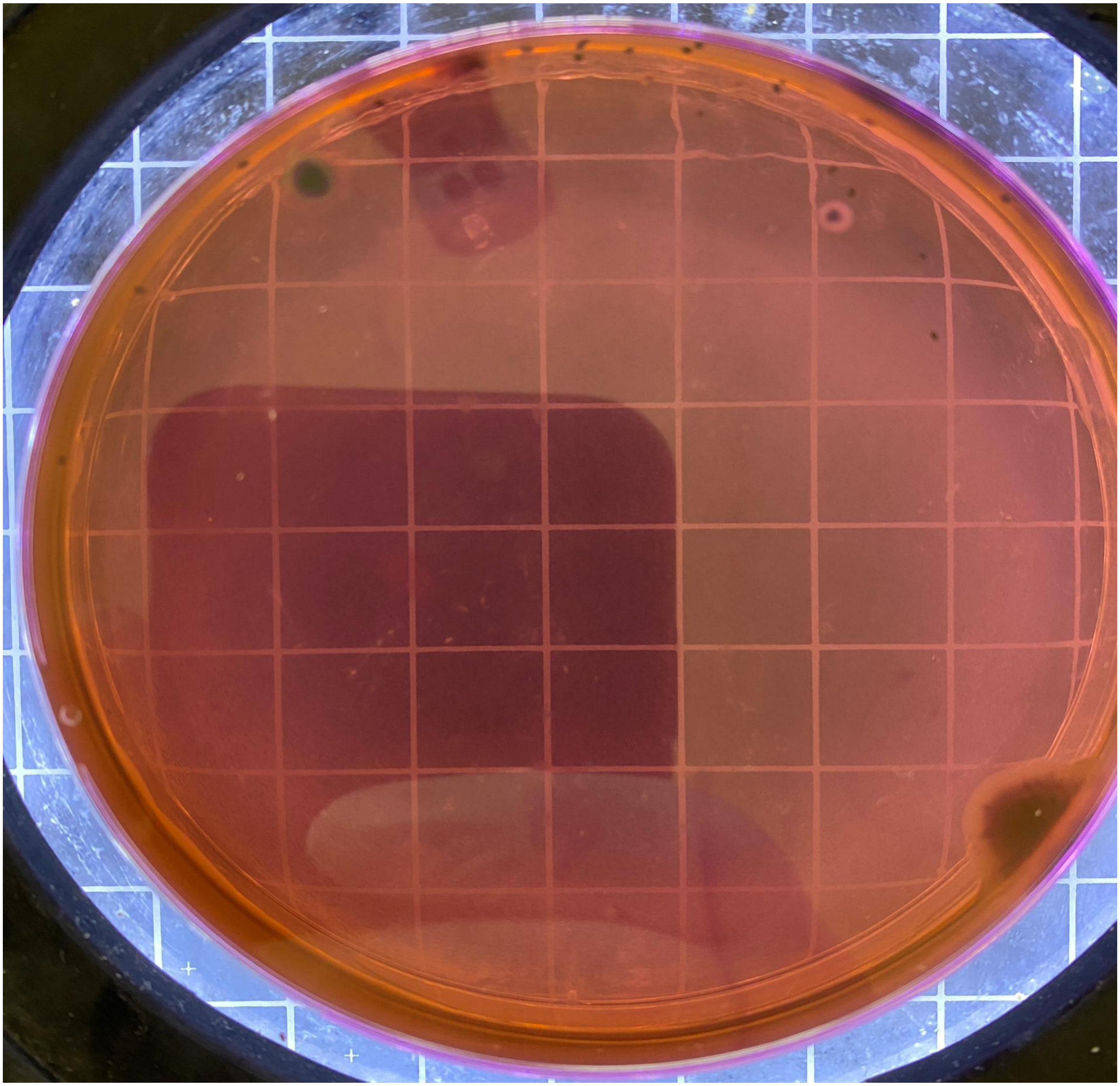

Fig 7: Fungal growth on PDA agar