PRESERVATIVE EFFICACY OF NEEM (*Azadirachta indica*) LEAF EXTRACT ON BANANA, BELL PEPPER AND TOMATO

BY

CYNTHIA OMOZUWA (MISS)

LSC2007065

DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE IN MICROBIOLOGY (BSCMCB)

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CERTIFICATION

This is to certify that this project was carried out by **CYNTHIA OMOZUWA (Miss)** of the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City.

12/02/2025

PROF. S.E. OMONIGHO DATE

(Project Supervisor)

PROF. F.I. AKINNIBOSUN DATE

(Head of Department)

Prof. C J Ogugbue
14-02-2025

DEDICATION

I would love to dedicate this work to God almighty for His mercy, favour and direction all through this journey.

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I express profound gratitude to God Almighty for guiding me throughout this project research work and the course of the journey through the University of Benin.

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TABLE OF CONTENT

CERTIFICATION 3

DEDICATION 4

ACKNOWLEDGEMENT 5

ABSTRACT 10

CHAPTER ONE 11

INTRODUCTION 11

1.0 Background of the Study 11

1.1 Rationale of the Study 12

1.2 Aim and Objectives of the Study 13

1.2.1 Aim 13

1.2.2 Objectives .... **Error! Bookmark not defined.**

CHAPTER TWO 14

LITERATURE REVIEW 14

2.1 Overview of the Importance of Food Preservation 14

2.2 Role of Natural Preservatives in Reducing Post-Harvest Losses 15

2.3 General Background on Neem (*Azadirachta indica*) as a Bio-Preservative 16

2.4 Botanical Description of Neem 17

2.4.1 Origin, Taxonomy, and Distribution 17

2.5 Key Chemical Components Responsible for Antimicrobial and Preservative Properties 18

2.6 Traditional and Industrial Uses of Neem 19

2.6.1 Medicine: 19

2.6.2 Agriculture: 19

2.6.3 Food Preservation: 20

2.7 Neem as an Eco-Friendly Alternative to Synthetic Preservatives 20

2.8 Characteristics of the Selected Perishable Foods 21

2.9 Bell Pepper (Capsicum annuum) 21

2.10 Common Pathogens and Spoilage Mechanisms 22

2.11 Tomato *(Solanum lycopersicum)* 22

2.12 Post-Harvest Diseases and Factors Contributing to Spoilage 23

2.13 Contributing factors to spoilage include: 24

2.14 Banana (Musa spp.) 24

2.15 Microbial Challenges during Storage 25

2.16 Natural Preservatives in Food Preservation 26

2.17 Advantages of Using Natural Preservatives 26

2.17.1 Safety 26

2.17.2 Consumer Preference 27

2.17.3 Environmental Benefits of Natural Preservative 27

2.18 Comparison of Natural and Synthetic Preservatives 28

2.19 Mechanisms of Natural Preservatives 28

2.19.1 Antimicrobial Action (Bacteriostatic and Fungistatic Effects) of Natural Preservative 29

2.20 Influence on Shelf Life and Quality Retention of Natural Preservative 29

2.21 Neem as a Preservative in Food Systems 31

2.22 Antimicrobial Properties of Neem Extracts 31

2.23 Neem's Efficacy against Spoilage Microbes in Fruits and Vegetables 32

2.24 Case Studies or Examples of Neem Usage in Food Systems 33

2.25 Factors Affecting the Efficacy of Neem Extracts 34

2.25.1 Method of Application (Immersion, Spraying, Coating) 34

2.25.2 Storage Conditions (Temperature, Humidity, and Exposure to Light) 36

2.26 Comparative Studies on Preservative Efficacy 38

CHAPTER THREE 39

MATERIALS AND METHODS 39

3.1. Selection and Processing of Neem (*Azadirachta indica*) Leaves 39

3.2. Preparation of Aqueous and Ethanolic Neem (*Azadirachta indica*) Extract 39
3.3. Sample Collection 39
3.4. Sterilisation of Prepared of Extract Solution 39
3.5. Application of Neem *(Azadirachta indica)* Extract to Fruits Samples 39
3.6. Sensory Evaluation of Fruit Samples 40
3.7. Bacteriological Analysis of Fruit Samples 40
3.7.1. Preparation of Peptone water for serial dilution 40
3.7.2. Preparation of culture media 40
3.7.3. Preparation of Inoculum 40
3.7.4. Inoculation and pour plate technique 40
3.7.5. Cultural and Morphological Characterization: 41
3.8. Gram Staining: 41
3.9. Biochemical Tests 41
3.9.1. Catalase Test: 41
3.9.2. Oxidase Test: 41
3.9.3. Indole Test: 42
3.9.4. Citrate Utilization: 42
3.9.5. Urease Production: 42
3.9.6. Hydrogen Sulphide Production: 42
3.9.7. Sugar Fermentation Tests: 42
3.9.8. Methyl Red Test: 43
3.9.9. CO2 Gas Production: 43
3.9.10. Voges Proskauer Test: 43
3.9.11. Spore Test: .... **Error! Bookmark not defined.**
3.10. Antibiotic Susceptibility Test 43
3.11 Data Analyses 44
CHAPTER FOUR 45

RESULTS **45**

CHAPTER FIVE **2**

5.0 DISCUSSION **2**

5.1 Limitations and Future Research 2

5.2 Conclusion 5

REFERENCES .... **Error! Bookmark not defined.**

ABSTRACT

Food preservation is essential for extending the shelf life and maintaining the quality of perishable goods. Neem (*Azadirachta indica*) has gained attention as a natural preservative due to its bioactive compounds with antimicrobial, antifungal, and antioxidant properties. This study aimed to evaluate the preservative potential of neem extracts (aqueous and ethanolic) on bell pepper, tomato, and banana over a 14-day storage period. Bacterial populations on fresh and neem-coated fruits were enumerated, characterized, and identified, while physical and sensory attributes were assessed. The antibiotic susceptibility patterns of isolated bacteria were also determined using the Kirby-Bauer disc-diffusion method. Results showed that ethanolic neem extracts significantly reduced bacterial loads, particularly in bell pepper (5.30±0.13 cfu/cm² by day 14), while aqueous extracts were moderately effective. Coliform and staphylococcal counts declined across all treated fruits, with ethanol-treated bananas exhibiting minimal contamination (0.04±0.00 cfu/cm² by day 9). *Salmonella* counts also decreased, with ethanol-coated banana samples showing a reduction from 39.80±0.35 to 2.20±0.01 cfu/cm² by day 14. Bacterial isolates identified include *Staphylococcus aureus, Micrococcus* spp. and *Klebsiella pneumoniae*, with control samples exhibiting a higher prevalence of pathogenic strains. Physical and sensory assessments revealed that ethanol-coated fruits retained better texture and color, while control samples deteriorated more rapidly. Antibiotic sensitivity tests indicated that *Klebsiella pneumoniae* isolated from ethanol-treated samples was most susceptible, while control samples harbored more resistant strains, suggesting a higher spoilage risk. The findings confirm the antimicrobial potential of neem extracts in reducing bacterial loads and preserving fruit quality, with ethanolic extracts demonstrating superior efficacy. However, further research is needed to optimize extract concentrations, explore additional fruit varieties, and compare neem with synthetic preservatives for commercial applications. This study highlights the potential of neem-based coatings as a sustainable alternative to chemical preservatives, aligning with global trends in eco-friendly food preservation.

CHAPTER ONE

INTRODUCTION

1.0 Background of the Study

Post-harvest losses of fruits and vegetables are a significant issue in Nigeria, with estimates suggesting up to 50% of produce is lost (Ogundele, 2022). These losses occur at various stages of the value chain, including harvesting, handling, processing, and storage (Delgado, 2021). Factors contributing to spoilage include environmental conditions like pH, temperature, and oxygen, as well as microbial contamination (Alegbeleye *et al.,* 2022). Common spoilage fungi identified in Nigerian fruits and vegetables include *Mucor, Rhizopus, Aspergillus, Penicillium,* and *Fusarium* species (Udoh *et al.,* 2015). The high moisture content of fruits and vegetables makes them particularly susceptible to microbial growth and rapid degradation (SM, 2019). Addressing post-harvest losses is crucial for improving food security, reducing the need for imports, and increasing food availability in Nigeria. Strategies to mitigate losses include improving awareness among farmers, enhancing post-harvest handling practices, and developing sustainable management ecosystems (Alegbeleye *et al*., 2022).

Food preservation is crucial for extending shelf life and maintaining the quality of perishable goods. Traditional methods like curing, freezing, canning, and pickling have been widely used (Ariyamuthu *et al.,* 2022; Sharif *et al*., 2017). In Africa, indigenous techniques such as fermentation, drying, and storage in cribs or silos are common for various produce. Modern preservation methods include pasteurization, vacuum packing, and modified atmosphere packaging (Ariyamuthu *et al.,* 2022). While synthetic preservatives are effective, they may pose health risks, leading to increased interest in natural alternatives like salt, vinegar, and honey (Sharif *et al.*, 2017). However, advanced preservation techniques are often inaccessible to resource-poor communities in Africa, necessitating reliance on traditional methods. Improved preservation and storage methods have been developed through research, but their adoption remains slow. Collaboration between traditional knowledge and modern research is recommended to enhance food preservation practices and reduce post-harvest losses (Nkwain *et al.,* 2020).

Neem (*Azadirachta indica*) has gained significant attention as a natural preservative and antimicrobial agent due to its diverse bioactive compounds. Various parts of the neem tree, including leaves, seeds, and bark, contain potent antimicrobial, antifungal, and antioxidant properties (Wylie and Merrell, 2022). Studies have demonstrated neem's effectiveness against a wide range of microorganisms, including food spoilage bacteria (Del Serrone *et al*., 2015). The plant's antimicrobial activity is attributed to compounds such as azadirachtin, nimbin, and quercetin (Seethapathy *et al.,* 2022). Neem oil has shown promising results in controlling meat spoilage, inhibiting the growth of various bacteria (Del Serrone *et al*., 2015). Furthermore, neem extracts have exhibited potential in combating drug-resistant and biofilm-forming organisms (Wylie and Merrell, 2022). With over 300 structurally diverse constituents identified, neem's bioactive compounds modulate multiple cellular pathways, contributing to its wide-ranging effects (Gupta *et al.*, 2017). These findings highlight neem's potential as a natural preservative and antimicrobial agent.

Research has shown that neem extracts can effectively extend the shelf life and maintain quality of various fruits. Studies on bananas demonstrated that 40% neem leaf extract treatment resulted in the longest shelf life (8.33 days), minimal color change, and reduced disease incidence (Siddiqua *et al*., 2018). Similar benefits were observed in mangoes, where neem leaf extract (20% and 40%) treatments led to extended shelf life, slower color changes, and reduced disease severity (Sarmin *et al*., 2018). Neem leaf powder also proved effective in preserving tomatoes, extending shelf life to 22 days compared to 15-19 days for untreated fruits. The efficacy of neem extracts has been attributed to their ability to reduce microbial spoilage, as evidenced by the isolation of fewer fungal species from treated fruits (Zewdie *et al.,* 2022). These findings suggest that neem-based treatments offer a promising, natural approach to fruit preservation.

1.1 Rationale of the Study

Conventional preservation methods like drying and use of chemical preservatives can result in nutrient loss and unwanted chemical additions, necessitating the development of "green" technologies for food preservation (Wajahat, 2023). Studies have demonstrated neem's broad-spectrum antimicrobial activity against common spoilage bacteria (Del Serrone *et al*., 2015). Neem oil's effectiveness extends to fruit and vegetable preservation, with research exploring its use in active packaging and coatings (Kumar *et al.,* 2021). In Nigeria, neem extract application on tomatoes not only preserved the produce but also increased micronutrient concentrations, including zinc, iron, selenium, and iodine (Yusuf, 2023). Despite its benefits, challenges such as photosensitivity and impacts on sensory qualities exist. However, nanoemulsions of neem oil offer improved stability and functionality, addressing some of these issues (Kumar et al., 2021). Overall, neem-based preservatives present a sustainable, locally-available alternative to synthetic chemicals for food preservation, particularly relevant for developing countries like Nigeria.

1.2 Aim and Objectives of the Study

1.2.1 Aim and Objectives

The aim of this study was to investigate the preservative potential of neem (*Azadirachta indica*) extracts with bell pepper, tomato and banana.

The specific objectives were to**:**

1. enumerate, isolate and identify the bacteria present on bell pepper, tomato and banana coated with neem leaf extracts over a storage period of 14 days.
2. monitor physical and sensory characteristics of bell pepper, tomato and banana coated with neem leaf extracts over a storage period of 14 days.
3. determine the antibiotic susceptibility patterns of the bacteria isolated from fresh, and treated bell pepper, tomato and banana stored over a period of 14 days.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of the Importance of Food Preservation

The global demand for fresh fruits and vegetables is increasing, but their short shelf life poses challenges for food security and sustainability (Sridhar *et al*., 2021). Various preservation techniques have been developed to extend shelf life and maintain quality. These include pressure modification methods such as high hydrostatic pressure and vacuum packaging (Li *et al*., 2017), as well as thermal, electrical, and chemical treatments (Sridhar *et al*., 2020). Advanced techniques like foam mat drying, osmotic dehydration, and irradiation have shown promise in preserving food quality and reducing postharvest losses (Sharma *et al*., 2021). Temperature management and dehydration control remain essential strategies, with controlled atmospheres, modified atmospheres, and 1-methylcyclopropene fumigation serving as effective supplements. Nanotechnology is also emerging as a potential tool for food preservation (Sridhar *et al.*, 2020). These technologies aim to address the challenges of microbial contamination, moisture control, and maintaining nutritional value in perishable produce.

Food preservation is a critical process aimed at extending the shelf life of food products by preventing spoilage caused by microbial activity, enzymatic reactions, and environmental factors (Kumar, 2019). Various techniques, both traditional and modern, are employed to maintain food safety, nutritional value, and sensory qualities. Traditional methods include drying, salting, smoking, and fermentation, while modern approaches encompass refrigeration, freezing, pasteurization, and high-pressure processing (Vaishnav *et al*., 2024). These preservation methods are essential for enhancing food security, reducing waste, and supporting sustainable food supply chains (Vaishnav *et al*., 2024). Recent advancements in food preservation technology include irradiation, pulse electric fields, and microwave volumetric heating, which aim to inhibit microbial growth and chemical deterioration while preserving food freshness, taste, color, flavor, and nutritional value (Khama, 2024). Effective preservation ensures food remains safe, nutritious, and palatable for longer periods, making it available during times of scarcity (Kumar, 2019).

Food preservation is crucial for extending shelf life and maintaining food quality. Traditional methods like curing, freezing, and pickling have been widely used (Kumar, 2019; Ariyamuthu *et al.*, 2022). However, modern techniques such as pasteurization, freeze-drying, and modified atmosphere packaging have emerged to meet contemporary needs for cost-effective preservation and consumer satisfaction (Ariyamuthu *et al*., 2022). While synthetic preservatives are effective, they may pose health risks (Sharif *et al.*, 2017). Consequently, there is growing interest in natural preservatives from plant, animal, and microbial sources, which are considered safer for human consumption and the environment (Sharif *et al*., 2017). The choice of preservation method depends on factors like nutritional content, texture, and flavor retention (Kumar, 2019). Advancements in packaging materials have significantly contributed to modern food preservation techniques (Ariyamuthu *et al.,* 2022). Overall, the field of food preservation continues to evolve, balancing effectiveness, safety, and consumer preferences.

2.2 Role of Natural Preservatives in Reducing Post-Harvest Losses

Natural preservatives are increasingly recognized as effective alternatives to synthetic chemicals for enhancing food safety and extending shelf life. These substances, derived from plants, animals, and microbes, exhibit antimicrobial properties that inhibit spoilage organisms (Teshome *et al*., 2022). For instance, plant-derived compounds such as essential oils and phenolic acids have demonstrated significant antimicrobial activity against various pathogens (Raybaudi-Massilia *et al*., 2009). The integration of natural antimicrobials into active packaging systems allows for continuous release at low concentrations, thereby preserving the quality of fresh produce without compromising sensory attributes (Ayala-Zavala *et al*., 2008). However, challenges remain regarding the sensory impact and regulatory acceptance of these natural agents, necessitating further research to optimize their application in food products (Raybaudi-Massilia *et al.,* 2009; Teshome *et al.,* 2022). Overall, natural preservatives align with consumer preferences for safer, eco-friendly food options.

Natural preservatives are gaining popularity as consumers seek clean-label products free from synthetic additives. This trend is driven by concerns about the health and environmental impacts of chemical preservatives (Mesías *et al.,* 2021; Lami *et al.,* 2024). Natural preservation methods offer sustainable alternatives that can extend shelf life, enhance food safety, and reduce post-harvest losses, particularly for perishable foods like fruits (Mesías *et al.,* 2021). Consumers perceive natural preservatives as healthier and more environmentally friendly, potentially influencing purchasing decisions. However, lack of information about these preservatives can lead to doubts about their effectiveness and concerns about price premiums. The food industry, especially the bakery sector, is adapting to this trend by reconsidering traditional preservation methods and incorporating natural alternatives to meet consumer demands for clean-label products (de Oliveira do Nascimento *et al.,* 2018).

2.3 General Background on Neem (*Azadirachta indica*) as a Bio-Preservative

Neem *(Azadirachta indica)* is a versatile plant with extensive antimicrobial properties, making it an effective bio-preservative and biopesticide. Its various parts contain bioactive compounds that exhibit antibacterial, antifungal, and insecticidal activities (Wylie and Merrell, 2022). Neem oil has shown significant inhibition of foodborne pathogens and meat spoilage bacteria, with growth reduction ranging from 30.81% to 99.70% in broth studies. As a biopesticide, neem is effective against over 550 insect pest species, offering an eco-friendly alternative to synthetic pesticides for crop protection and stored grain preservation. The plant's wide-ranging effects are attributed to over 300 structurally diverse constituents, including limonoids such as nimbolide, azadarachtin, and gedunin, which modulate multiple cellular pathways (Gupta *et al.,* 2017). These properties make neem a promising natural preservative and pest control agent in food and agricultural applications.

Neem *(Azadirachta indica)* oil has shown significant potential as a natural preservative for various food products. Its efficacy is attributed to its antimicrobial and antioxidant properties, which can inhibit the growth of spoilage microorganisms and reduce oxidative degradation (Del Serrone *et al.,* 2015; Kumar *et al*., 2021). Studies have demonstrated neem's effectiveness in preserving fresh produce like bell peppers, with ethanol extracts showing superior antimicrobial activity compared to n-hexane extracts (Oyun and Oyetayo, 2020). Neem oil has also proven effective in controlling meat spoilage, exhibiting strong antibacterial activity against various pathogens. However, challenges such as photosensitivity and impact on sensory qualities exist. To address these issues, neem oil nanoemulsions are being explored, offering improved stability and functionality (McClements *et al.,* 2021). The use of neem in food preservation aligns with the growing demand for natural, environmentally friendly alternatives to synthetic preservatives.

Neem (*Azadirachta indica*) is a versatile plant with numerous applications in medicine, agriculture, and industry. Native to India, it has been used for centuries and is now cultivated globally. Neem possesses powerful antimicrobial, antifungal, anti-inflammatory, and insecticidal properties, making it valuable in traditional and modern medicine (Dubey and Kashyap, 2014). Its active compounds, particularly azadirachtin, have been extensively studied for their biological activities. Neem products are environmentally friendly, sustainable, and compatible with beneficial insects. The plant's various parts, including leaves, bark, roots, and seeds, have specific medicinal uses and are effective against a wide range of ailments, from skin diseases to malaria. With over 65 patents derived from its uses, Neem continues to be a subject of significant scientific interest (Dubey and Kashyap, 2014).

2.4 Botanical Description of Neem

2.4.1 Origin, Taxonomy, and Distribution

Neem, a versatile tree native to the Indian subcontinent, has gained global recognition for its numerous applications (Jhariya *et al.,* 2011). It thrives in arid conditions, growing up to 30 meters tall with pinnately compound leaves. Neem's adaptability has led to its cultivation in tropical and subtropical regions worldwide, including Africa and the Caribbean (Jhariya *et al.*, 2011). The tree's various parts have been used in traditional medicine for over 2000 years, with each component possessing medicinal properties. Neem derivatives have potential applications in toiletries, pharmaceuticals, agriculture, and pest control (Koul *et al.*, 1990). The tree's ability to fix nitrogen and act as a windbreaker makes it valuable for agroforestry and soil improvement. Neem's economic importance is reflected in the growing number of neem-based industries globally, producing products such as neem oil and neem cake (Jhariya *et al.,* 2011).

Taxonomy:

Kingdom: Plantae

Order: Sapindales

Family: Meliaceae

Genus: Azadirachta

Species: *Azadirachta indica*

2.5 Key Chemical Components Responsible for Antimicrobial and Preservative Properties

Neem contains numerous bioactive compounds, these compounds, including azadirachtin, nimbin, and limonoids, are responsible for its diverse pharmacological activities (Rahmani *et al.,* 2018). Neem exhibits antimicrobial, antifungal, and insecticidal properties, as well as antipyretic, antiviral, analgesic, and hepatoprotective effects. Its therapeutic potential extends to anti-inflammatory, antimalarial, anti-allergic, antidermatic, and antiulcer applications. The plant's constituents have shown promise in cancer prevention and treatment through modulation of cell signaling pathways (Rahmani *et al.*, 2018). Neem's widespread use in traditional medicine systems like Ayurveda, Unani, and homeopathy has led to increased interest in modern medicine. Its global significance is further emphasized by its potential to address various environmental and industrial challenges (Subapriya and Nagini, 2005).

Azadirachtin: Azadirachtin is perhaps the most well-known and studied active compound in Neem. This tetranortriterpenoid is found in high concentrations in the seeds and leaves of the tree and is a potent insect repellent. Azadirachtin acts by disrupting the hormonal systems of pests, leading to their death or inability to reproduce. Its antimicrobial properties extend to bacteria and fungi, making it effective in inhibiting the growth of pathogenic microorganisms (Mordue and Nisbet, 2000).

Salannin: Salannin is a bitter-tasting compound with a strong antifungal activity. Like azadirachtin, salannin interferes with the reproductive systems of insects and microorganisms, making it an effective natural pesticide. It also possesses antibacterial properties, contributing to the preservation of food and other organic materials by preventing bacterial contamination (Haji *et al.,* 2023).

Nimbin: Another key compound found in neem is nimbin, which has antifungal, antibacterial, and antiviral properties. It is particularly known for its ability to combat skin infections and is used in traditional medicine to treat conditions like eczema, psoriasis, and acne. Nimbin also contributes to the preservative qualities of neem, as it helps inhibit the growth of harmful bacteria and fungi in both food and medicinal products (Uddin *et al.,* 2018).

Other Bioactive Compounds: Neem also contains compounds like nimbidin, quercetin, and various flavonoids that provide antioxidant, anti-inflammatory, and immunomodulatory effect. These additional compounds complement the antimicrobial and preservative properties of the tree (de Alba *et al.,* 2023).

The concentration of these bioactive components varies depending on the part of the tree and the extraction method used, but their combined effects are responsible for the diverse medicinal, agricultural, and preservative applications of neem (Zeeshan *et al.,* 2024).

2.6 Traditional and Industrial Uses of Neem

Neem has been widely recognized for its therapeutic and practical benefits. In traditional medicine, neem has been used for thousands of years as a remedy for a variety of ailments. Its applications extend to numerous aspects of health care, from skin conditions to immune system support, and it has a longstanding reputation in Ayurveda (Asghar *et al.,* 2022).

2.6.1 Medicine:

Skin Care: Neem has been used in treating skin disorders like acne, eczema, psoriasis, and fungal infections due to its antifungal, antibacterial, and anti-inflammatory properties. Neem extracts are found in many soaps, creams, and lotions aimed at promoting healthy skin (Uddin *et al.,* 2018).

Detoxification and Immune Support: The compounds in neem have detoxifying effects on the body. The bitter compounds, especially azadirachtin, help cleanse the liver and support the immune system by neutralizing toxins (Sarkar *et al.,* 2021).

Anti-inflammatory: Neem has been employed to reduce inflammation in conditions such as arthritis and muscle pain. Its natural compounds interact with inflammatory pathways in the body, making it a useful adjunct in the treatment of chronic inflammatory conditions (Uddin *et al.,* 2018).

Antimalarial and Antiviral: Neem has demonstrated efficacy against diseases like malaria and dengue, with some compounds in neem able to inhibit the replication of the malaria parasite. It is also used traditionally as a remedy for viral infections (Uddin *et al.,* 2018).

2.6.2 Agriculture:

Natural Pesticide: One of the most widely recognized uses of neem in agriculture is its role as a natural pesticide. Azadirachtin, nimbin, and salannin act as insect growth regulators, preventing the development of pests and inhibiting their ability to reproduce. Neem-based pesticides are eco-friendly alternatives to chemical pesticides, as they do not harm beneficial insects or pollinators like bees (Adhikari *et al*., 2020).

Fungal and Bacterial Control: Neem's antifungal and antibacterial properties make it useful in controlling plant diseases caused by fungi and bacteria. It is commonly used as a foliar spray to prevent fungal infections in crops such as tomatoes, cucumbers, and rice (Mohideen *et al.,* 2022).

2.6.3 Food Preservation:

Neem oil and its extracts are used as preservatives in food, particularly in the preservation of cereals, grains, and legumes. The antimicrobial properties of neem help prevent spoilage caused by bacteria, fungi, and insects. In regions where refrigeration is not easily accessible, neem provides an important natural solution for extending the shelf life of food (Kumar *et al.,* 2022).

Edible Coatings: Neem has been explored as a natural coating for fresh produce, where it acts as an antimicrobial barrier that prevents the growth of spoilage microorganisms, thus extending freshness and shelf life (Passafiume *et al.,* 2022).

2.7 Neem as an Eco-Friendly Alternative to Synthetic Preservatives

Neem oil, derived from *Azadirachta indica,* offers a sustainable alternative to synthetic pesticides and preservatives in food production and storage. Its bioactive compounds, particularly azadirachtin, exhibit insecticidal, antimicrobial, and antioxidant properties (Chaudhary, 2017; Kumar *et al.,* 2022). Unlike synthetic chemicals, neem-based products are biodegradable and environmentally friendly, reducing ecological harm and pest resistance. Neem oil's effectiveness extends to over 400 harmful species, including insects, mites, and pathogenic microorganisms. Recent advancements in nanocarrier-based delivery systems have enhanced neem oil's stability and efficacy in food preservation and packaging. However, challenges such as photosensitivity and potential impacts on food sensory qualities must be addressed (Campos *et al.,* 2016). As the food industry seeks greener alternatives to synthetic preservatives, neem-based solutions offer promising applications in sustainable food preservation and packaging.

2.8 Characteristics of the Selected Perishable Foods

Perishable foods, including bell peppers, tomatoes, and bananas, are highly susceptible to post-harvest losses due to their delicate nature and vulnerability to environmental factors (Yahia *et al.*, 2004). These crops are rich in antioxidants and have significant nutritional value, but their quality can rapidly deteriorate after harvest. Post-harvest losses in fresh fruits and vegetables are estimated at 5-25% in developed countries and 20-50% in developing countries (Kader, 2005; Acedo, 2019). Proper handling and storage techniques are crucial for preserving quality and minimizing losses. These include careful manipulation of storage conditions, consideration of fruit ripening characteristics, and the use of plastic film packaging (Yahia *et al.,* 2004). Fresh-cut produce requires even more stringent packaging and storage techniques due to increased perishability and microbial activity risks. Ongoing research is necessary to improve post-harvest preservation methods for these valuable crops (Yahia *et al.,* 2004).

2.9 Bell Pepper (Capsicum annuum)

Bell peppers, also known as sweet peppers, are celebrated not only for their delightful flavor and texture but also for their exceptional nutritional value. Rich in vitamin C, they play a crucial role in supporting immune function, maintaining skin health, and offering antioxidant protection. These peppers also boast a significant content of vitamin A through carotenoids like beta-carotene, promoting good vision and skin integrity. Additionally, they provide essential dietary fiber, potassium, and magnesium, which collectively contribute to digestive health and electrolyte balance. Bell peppers are, however, highly perishable, with an optimal shelf life of 2-3 weeks under specific storage conditions. Maintaining temperatures between 7.5°C to 13°C and humidity levels of 95-98% is critical to prevent water loss and quality deterioration. Temperatures below 7°C can lead to chilling injury, particularly affecting breaker stage peppers, while higher temperatures accelerate ripening and spoilage. Modified atmosphere packaging helps mitigate water loss but may increase susceptibility to postharvest diseases due to elevated humidity levels. Studies suggest that texture degradation during storage follows an exponential model, with a maximum estimated storage life of 51 days at 5.6°C and 95% relative humidity (Hampshire *et al.,* 1987).

2.10 Common Pathogens and Spoilage Mechanisms

Bell peppers are highly susceptible to microbial infections, particularly during storage and transport, which can significantly impact their quality and shelf life. Fungal infections, such as those caused by *Botrytis cinerea* (gray mold) and *Alternaria alternata*, lead to visible lesions and tissue breakdown, accelerating decay. Bacterial rot, primarily driven by *Pectobacterium carotovorum*, thrives in moist conditions, producing enzymes that degrade cell walls and cause soft rot.

Another major threat is anthracnose, a disease caused by *Colletotrichum* species, which affects both tropical and temperate fruit crops worldwide. This disease manifests as dark, sunken lesions on the fruit surface, reducing marketability and overall quality. Anthracnose can develop pre- and post-harvest, with latent infections posing a particular risk during storage. Common causative species, including *C. gloeosporioides* and *C. acutatum*, infect a wide range of hosts such as mango, papaya, avocado, and strawberry. The disease thrives in warm, humid conditions (24-28°C) and is challenging to manage due to the complex taxonomy of *Colletotrichum* species, their diverse traits, and the limited availability of effective fungicides (Droby *et al.,* 2011; Dowling *et al.,* 2020).

2.11 Tomato *(Solanum lycopersicum)*

Tomatoes are a staple food of global significance, valued for their rich nutritional profile and economic impact. They are an excellent source of essential vitamins and minerals, particularly vitamin C, potassium, and folate, which contribute to various health benefits (Parmar *et al.,* 2021). Tomatoes and tomato-based products are especially high in lycopene, a potent antioxidant linked to a reduced risk of several cancers and cardiovascular diseases (Ali *et al.,* 2020; Li *et al.,* 2021). Lycopene, a key carotenoid predominantly found in tomatoes, has demonstrated strong antioxidant properties, with studies associating its intake with lower risks of chronic diseases (Rao and Agarwal, 2000; Agarwal and Rao, 2000; Przybylska and Tokarczyk, 2022). Beyond its antioxidant role, lycopene may also contribute to health benefits through mechanisms such as intercellular gap junction communication, hormonal and immune modulation, and metabolic regulation (Rao and Agarwal, 2000). Additionally, tomatoes are low in calories and high in water content, making them an ideal choice for weight management diets (Rolls, 2017).

Despite their economic importance, tomatoes are highly perishable, leading to significant postharvest losses, particularly in developing countries (Arah *et al.,* 2015; Arah *et al.,* 2016). These losses, which can be both quantitative and qualitative, range from 2% to 50% globally (Kader, 2005). Various factors, including pre-harvest practices such as fertilizer application, pruning, and cultivar selection, as well as postharvest handling methods like harvesting, precooling, packaging, and storage, significantly influence tomato quality and shelf life (Arah *et al.,* 2016). Effective postharvest treatments such as refrigeration, heat treatment, modified atmosphere packaging, and calcium chloride application have been shown to help maintain quality and extend shelf life (Arah *et al.,* 2016). Addressing postharvest losses in developing nations requires improved handling systems, overcoming socio-economic challenges, and fostering collaboration among producers and marketers (Kader, 2005). A comprehensive understanding and strategic management of both preharvest and postharvest factors are essential for minimizing quality deterioration and enhancing the profitability of tomato production (Arah *et al.,* 2016).

2.12 Post-Harvest Diseases and Factors Contributing to Spoilage

Tomatoes are highly susceptible to spoilage due to their soft texture and high water content, making them vulnerable to several postharvest diseases. One of the most significant diseases is anthracnose, caused by *Colletotrichum* species, which are major plant pathogens in tropical and subtropical regions (Zakaria, 2021). This disease is characterized by sunken, water-soaked lesions that expand over time, often developing into dark, necrotic spots on leaves, stems, and fruits. While some *Colletotrichum* species are host-specific, others, such as *C. gloeosporioides* and *C. acutatum*, infect a wide range of crops. Anthracnose can develop both pre- and post-harvest, causing significant economic losses by reducing fruit quality and marketability (Zakaria, 2021). Another critical postharvest disease is bacterial spot, caused by *Xanthomonas* species, which severely affects tomato and pepper production worldwide (Larrahondo-Rodríguez *et al.,* 2022; Potnis *et al.*, 2015). This disease presents as dark brown or black lesions on leaves, stems, and fruits, leading to defoliation and a decline in market value (Larrahondo-Rodríguez *et al.,* 2022). Additionally, gray mold, caused by *Botrytis cinerea*, thrives in high humidity environments, with spore germination requiring at least 80% relative humidity (RH) and optimal infection occurring above 85% RH. Without proper post-harvest management, these diseases can significantly impact tomato shelf life and profitability.

2.13 Contributing factors to spoilage include:

High temperatures significantly impact tomato post-harvest quality, reducing shelf life and increasing fungal susceptibility (Thole *et al.,* 2021). Chilling exposure stimulates ethylene production and increases decay susceptibility, with prolonged exposure inhibiting color development and altering respiration (Cheng and Shewfelt, 1988). The chilling threshold for green tomatoes is around 7.5°C, with longer exposure causing delayed and uneven ripening. Ethylene treatment before storage can prevent chilling injury and prolong marketable life, while post-storage treatment becomes less effective with increased storage time. More mature tomatoes and those treated with ethylene before storage lose less weight but have lower vitamin C content (Chomchalow *et al.,* 2002). These findings highlight the importance of temperature management and ethylene control in maintaining tomato quality post-harvest.

2.14 Banana (*Musa* spp.)

Bananas are among the most widely consumed fruits worldwide, valued for their sweet taste, portability, and nutritional benefits. They are a rich source of carbohydrates, with starch gradually converting into sugars such as sucrose, glucose, and fructose during ripening. In addition, bananas provide essential minerals like potassium, calcium, and magnesium, which play crucial roles in heart health, nerve function, and muscle contraction. Potassium, in particular, helps regulate water balance and acid-base homeostasis in the blood and tissues, supporting cardiovascular and nervous system function. Bananas are also an excellent source of vitamin B6 and dietary fiber, promoting metabolism and digestive health. They contain resistant starch and dietary fibers that escape absorption in the small intestine and are fermented in the large intestine, making them a suitable option for diabetics (Netshiheni *et al.,* 2019).

The ripening of bananas is an ethylene-driven process characterized by significant biochemical changes (Thompson *et al.,* 2019). The most visible transformation is the shift from green to yellow, caused by the breakdown of chlorophyll (Müller and Kräutler, 2011). Internally, starch conversion to sugars increases sweetness from approximately 2% to 20%, a process regulated by ethylene-mediated enzymes and influenced by hormones such as indole-3-acetic acid and abscisic acid (Cordenunsi-Lysenko *et al.,* 2019).

Additionally, fruit softening occurs due to the degradation of cell wall components and starch (Maduwanthi and Marapana, 2017; Cordenunsi-Lysenko *et al.,* 2019). Other ripening-associated changes include increased malic acid content and modifications in the fruit's volatile profile (Maduwanthi and Marapana, 2017). A unique aspect of banana ripening is the accumulation of fluorescent chlorophyll catabolites, which cause the fruit to exhibit blue luminescence (Müller and Kräutler, 2010). Understanding these physiological and biochemical processes is crucial for improving postharvest quality and minimizing losses in this globally significant crop (Cordenunsi-Lysenko *et al.,* 2019).

2.15 Microbial Challenges during Storage

Microbial spoilage significantly impacts bananas during storage and transportation. Common microbial threats include:

Crown Rot: Crown rot is a significant postharvest disease of bananas, causing substantial losses and early fruit ripening (Triest and Hendrickx, 2016). The primary causative agents are *Colletotrichum musae, Fusarium species,* and *Lasiodiplodia theobromae. C. musae* is particularly aggressive, capable of causing extensive rot with low inoculum levels (Finlay and Brown, 1993). The disease begins with mycelium development on the crown surface, followed by internal progression, leading to softening and blackening of fruit tissue (Triest and Hendrickx, 2016).

Soft Rot: Recent studies have identified *Nigrospora* species as emerging pathogens causing postharvest rot in various fruits. *Nigrospora sp.* was reported to cause black rot in kiwifruit in South Korea, characterized by sunken, watery lesions on the fruit pericarp (Kwon *et al.,* 2017). Similarly, *Nigrospora sphaerica* was identified as the causal agent of postharvest rot disease in kiwifruit in China, resulting in soft, milk-white flesh and sour odor (Li *et al.,* 2018).

Surface Mold: Moisture content is a crucial parameter affecting citric acid production by *Aspergillus niger* during koji fermentation of banana peels.

Banana storage and transportation require careful management of temperature, humidity, and ethylene exposure to maintain quality and extend shelf life. Optimal storage temperatures range from 13-15°C, as lower temperatures can cause chilling injury while higher temperatures accelerate ripening. High humidity (98%) is beneficial, as low humidity can reduce green life by up to 7 days. Controlled atmosphere storage with elevated CO_2 (up to 5%) and reduced O2 (2%) can prolong green life, especially at 13-15°C. Ethylene exposure temperature significantly affects ripening, with 15°C exposure extending shelf life compared to 20°C (Lobo *et al.,* 2005). Proper air circulation in palletized stowage and avoiding ethylene-producing commodities during shipping are crucial (Snowdon, 2010). Additionally, the effectiveness of 1-MCP treatment is temperature-dependent, with the greatest impact on shelf life and firmness observed when fruit is ripened at 16°C (Moradinezhad *et al.,* 2010).

2.16 Natural Preservatives in Food Preservation

Natural preservatives have gained attention as safer alternatives to synthetic additives in food preservation. These substances, derived from plants, animals, and microorganisms, can inhibit microbial growth, extend shelf life, and enhance food safety (Teshome *et al.,* 2022). Plant-based preservatives, particularly from herbs and spices, are of significant interest due to their antimicrobial properties (Teshome *et al.*, 2022; Yu *et al.*, 2021). Natural preservatives work by increasing microbial cell membrane permeability, interrupting protein synthesis, and disrupting cell metabolism. While effective in preserving food, they may influence sensory properties such as flavor and texture (Yu *et al.,* 2021). To enhance their applicability, strategies like combining preservatives or using active packaging systems have been explored (Yu *et al.,* 2021). The growing consumer demand for healthier, more natural food options has driven research into these preservatives, as they are perceived to be safer and less likely to cause health complications compared to synthetic alternatives.

2.17 Advantages of Using Natural Preservatives

The use of natural preservatives in food preservation offers several benefits that appeal to both consumers and manufacturers, particularly as the global market leans toward healthier and environmentally-friendly food production methods. The advantages of natural preservatives can be broadly categorized into safety, consumer preference, and environmental benefits (Sharif *et al.*, 2017).

2.17.1 Safety

One of the most compelling reasons for the shift towards natural preservatives is their safety profile. Many synthetic preservatives, such as artificial colorings, flavors, and preservatives (e.g., sodium benzoate, Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT)), have raised concerns about their long-term health effects. Some of these synthetic chemicals have been linked to allergic reactions, toxicity, and even carcinogenicity (Dong *et al*., 2024). In contrast, natural preservatives generally come from edible plant-based sources that have been used in food for centuries and are regarded as safe for consumption in appropriate quantities. Natural substances like vinegar, rosemary extract, and citrus bioflavonoids do not carry the same health risks as their synthetic counterparts and are often recognized as safer alternatives by regulatory agencies such as the Food and Drug Administration (FDA) and European Food Safety Authority (EFSA) (Carocho *et al*., 2014).

2.17.2 Consumer Preference

The growing consumer demand for clean-label products has led to increased interest in natural preservatives and ingredients in food production (Asioli *et al.,* 2017; Nascimento *et al.,* 2018). This trend is driven by health concerns, desire for transparency, and preference for minimally processed foods (Asioli *et al.,* 2017). The bakery industry, in particular, is exploring natural alternatives to chemical additives to extend shelf life while maintaining product quality and safety (de Oliveira do Nascimento *et al*., 2018). Consumer awareness of food ingredients has prompted manufacturers to develop products with clean-label appeal, focusing on natural, organic, and "free-from" claims (Asioli *et al.*, 2017; Kamila de Oliveira do Nascimento *et al.,* 2018). Recent studies on fruit preservation indicate a growing consumer preference for natural preservatives over artificial ones, driven by health and environmental concerns. However, the higher cost of these natural alternatives remains a barrier to widespread adoption (Lami *et al*., 2024).

2.17.3 Environmental Benefits of Natural Preservative

Natural preservatives are gaining popularity as alternatives to synthetic preservatives due to environmental and health concerns. Synthetic preservatives, often petroleum-based, contribute to environmental degradation and chemical waste (Yu *et al.*, 2021). In contrast, natural preservatives are often biodegradable and derived from renewable plant resources, making them more sustainable (Panou and Karabagias, 2023). These natural alternatives, including plant extracts, essential oils, and organic acids, can effectively inhibit microbial growth and extend shelf life in various food products. They work by increasing microbial cell membrane permeability and disrupting protein synthesis and cell metabolism (Yu *et al*., 2021). However, natural preservatives may affect food sensory properties and have limitations in mechanical and barrier properties (Yu *et al.,* 2021; Panou and Karabagias, 2023). To address these challenges, strategies such as combining different preservatives, using active packaging systems, and encapsulation are being explored.

2.18 Comparison of Natural and Synthetic Preservatives

Natural preservatives are gaining popularity due to consumer preferences for cleaner, safer food products (Carocho *et al*., 2014). While synthetic preservatives are often more effective and cost-efficient, they may pose health risks and alter food organoleptic qualities. Natural preservatives, derived from plants and herbs, offer potential health benefits through antioxidant and antimicrobial properties (Kumar and Rajput, 2023). They work synergistically to provide balanced preservation without significantly changing food taste or texture. However, natural preservatives generally have shorter shelf lives and may be more expensive to produce. The food industry faces challenges in balancing consumer demands for eco-friendly products with the need for effective preservation, especially as formulations become more susceptible to microbial spoilage. As research progresses, new technologies like nanoencapsulation and "smart" additives may help address these challenges (Carocho *et al.,* 2014).

2.19 Mechanisms of Natural Preservatives

Natural preservatives offer effective alternatives to synthetic additives for enhancing food safety and extending shelf life. These compounds, derived from plants, animals, and microorganisms, exhibit antimicrobial properties against various pathogens and spoilage microbes (Teshome *et al*., 2022). Their mechanisms of action include membrane disruption, inhibition of ATP-ase activity, and enzyme inactivation (Pisoschi *et al.,* 2018). Natural preservatives can limit microbial resistance and meet consumer demands for healthier foods (Teshome *et al*., 2022). However, their effectiveness may be influenced by food composition, processing methods, and storage conditions (Teshome *et al*., 2022). In meat products, natural preservatives inhibit microbial growth by increasing cell membrane permeability and interrupting protein synthesis and cell metabolism. To enhance their applicability, strategies such as combining different preservatives or using active packaging systems have been explored. While natural preservatives can effectively extend shelf life, they may also impact food sensory properties (Yu *et al.,* 2021).

2.19.1 Antimicrobial Action (Bacteriostatic and Fungistatic Effects) of Natural Preservatives

Natural preservatives are increasingly sought after as alternatives to synthetic additives in food preservation. These compounds, derived from plants, animals, and microorganisms, exhibit antimicrobial properties through various mechanisms. They can disrupt microbial cell membranes, interfere with DNA/RNA replication, affect protein synthesis, and alter cellular metabolism (Dong *et al.,* 2024). Common natural preservatives include polyphenols, essential oils, flavonoids, lysozyme, and antimicrobial peptides (Yu *et al.,* 2021; Dong *et al.,* 2024). Their antimicrobial action involves membrane rupture, ATP-ase activity inhibition, leakage of essential biomolecules, and enzyme inactivation (Pisoschi *et al.,* 2018). While effective in extending shelf life and inhibiting microbial growth, natural preservatives may influence food sensory properties. To enhance their applicability, strategies such as combining different preservatives or using active packaging systems are being explored (Yu *et al.,* 2021). Natural preservatives offer a promising solution to address microbial resistance and mitigate potential negative effects of synthetic compounds (Pisoschi *et al.,* 2018).

2.20 Influence on Shelf Life and Quality Retention of Natural Preservative

In addition to their antimicrobial effects, natural preservatives also contribute to the overall quality retention of food products, helping to preserve their sensory attributes, nutritional value, and freshness over time. The mechanisms that influence shelf life and quality retention include:

Prevention of Oxidation: Oxidation is a major cause of food spoilage, particularly in lipid-rich foods, leading to rancidity and reduced nutritional value (Ramalho and Jorge, 2006). Antioxidants are used to prevent oxidation and extend shelf life (Mitterer-Daltoé *et al*., 2021). While synthetic antioxidants like BHA, BHT, and Tertiary Butylhydroquinone (TBHQ) are effective, concerns about their safety have increased interest in natural alternatives (Valenzuela *et al.,* 2003). Natural antioxidants, such as tocopherols, ascorbic acid, rosemary extracts, and green tea extracts, are considered safer and can enhance endogenous antioxidant systems (Valenzuela *et al*., 2003; Permana *et al.,* 2022). These natural compounds, rich in polyphenols, have been shown to improve oxidative stability in edible oils and prevent degradation of polyunsaturated fatty acids (Permana *et al.,* 2022). Foods preserved with natural antioxidants may be considered functional foods, offering potential health benefits beyond basic nutrition (Valenzuela *et al*., 2003).

Moisture Retention: Natural preservatives like honey and sugar create osmotic environments that inhibit microbial growth, helping to maintain food moisture balance and prevent spoilage. Osmotic dehydration, a process that removes water from food, is an effective method for extending the shelf life of fruits and vegetables (Kaur *et al.,* 2022). Honey, in particular, has been shown to retard microbial growth in tomato jam, independent of water activity, compared to sucrose (Kapira *et al.,* 2023). This antimicrobial effect is due to the creation of an osmotic environment that draws moisture out of microbial cells. Natural preservatives, including plant-derived and animal-derived products, can inhibit the growth of spoilage microorganisms and food-borne pathogens by increasing cell membrane permeability and interrupting protein synthesis and cell metabolism (Yu *et al.,* 2021). Understanding these mechanisms can help in developing effective food preservation strategies using natural substances.

Flavor and Aroma Protection: Natural preservatives offer a safe alternative to synthetic additives for extending food shelf life and maintaining quality. Plant-derived compounds, such as those found in herbs and spices, possess antioxidant and antimicrobial properties that can inhibit microbial growth and prevent chemical changes in food (Kumar and Rajput, 2023). These natural preservatives can be extracted from various sources, including plants, animals, and microorganisms, with herbs and spices receiving particular attention (Teshome *et al.,* 2022). Common natural preservatives include plant extracts, chitosan, bacteriocins, bioactive peptides, and essential oils (Olatunde and Benjakul, 2018). Their effectiveness in food applications depends on factors such as food composition, processing methods, and storage conditions. While natural preservatives are generally considered safe and meet consumer demands for healthier foods, their extraction and purification processes may affect their functionality (Teshome *et al*., 2022). Overall, natural preservatives show promise in enhancing food safety and extending shelf life across various food products.

Nutrient Preservation: Some natural preservatives like vitamin C (ascorbic acid) and vitamin E (α-tocopherol) play crucial roles in maintaining food quality and nutritional value. These antioxidants protect against free radicals, preventing rancidity and extending shelf life (Torres *et al.,* 2008). Ascorbic acid, being water-soluble, is often modified to increase its miscibility with lipids, while vitamin E is typically used as acetate or succinate for improved stability (Torres *et al.,* 2008). Natural preservatives, including herbs and spices, possess antimicrobial properties that inhibit bacterial and fungal growth (Kumar and Rajput, 2023). Citric acid and chestnut flowers have shown effectiveness in preserving nutraceutical preparations, maintaining phenolic compounds and controlling microbial growth (Fernandes *et al.,* 2020). However, ascorbic acid's instability poses challenges in food applications, necessitating strategies for stabilization and improved delivery (Yin *et al.,* 2022). Overall, natural preservatives offer potential health benefits and can effectively maintain food quality, often outperforming synthetic alternatives (Fernandes *et al.,* 2020; Kumar and Rajput, 2023).

2.21 Neem as a Preservative in Food Systems

Neem *(Azadirachta indica)* has gained attention for its potential as a natural food preservative due to its antimicrobial, antioxidant, and insecticidal properties (Kumar *et al.,* 2022). The tree's bioactive compounds, particularly azadirachtin, have shown efficacy against various pathogens, including drug-resistant and biofilm-forming organisms (Wylie and Merrell, 2022). Neem oil and its nanoemulsions have been explored for food preservation and packaging, offering improved stability and functionality compared to traditional applications (Kumar *et al.,* 2021). The seed extract's ability to repel insects and modify their growth makes it an effective preservative for stored grains. As consumer demand for natural preservatives increases, plant-derived antimicrobials like neem are being investigated as alternatives to synthetic chemicals in food preservation (Hintz *et al.,* 2015). However, challenges such as photosensitivity, rapid degradation, and potential impacts on food sensory qualities need to be addressed for widespread implementation (Kumar *et al*., 2021).

2.22 Antimicrobial Properties of Neem Extracts

Neem extracts from various parts of the tree have demonstrated significant antimicrobial properties against a wide range of pathogens. Leaf extracts have shown the strongest antimicrobial activity, followed by bark and seed extracts. These extracts are effective against both bacteria and fungi, including drug-resistant and biofilm-forming organisms (Wylie and Merrell, 2022). The antimicrobial activity is attributed to bioactive compounds such as azadirachtins, quercetin, and β-sitosterol in leaves, and margolone, margolonone, and isomargolonone in bark (Seethapathy *et al*., 2022). Neem oil has shown potential as a natural preservative in meat products, inhibiting the growth of various spoilage bacteria (Dhakar *et al.,* 2023). The broad-spectrum antimicrobial activity of neem extracts suggests their potential as alternative or adjuvant treatments to conventional antibiotics, warranting further research into their mechanisms of action and clinical efficacy (Wylie and Merrell, 2022; Seethapathy *et al.,* 2022). Research has demonstrated the efficacy of plant extracts, particularly neem *(Azadirachta indica)*, in inhibiting the growth of foodborne pathogens and spoilage microorganisms. Neem extracts have shown antibacterial activity against both Gram-positive and Gram-negative bacteria, including *Listeria monocytogenes, Staphylococcus aureus,* and *Escherichia coli* (Imran *et al.,* 2021). The antimicrobial properties of plant extracts are attributed to bioactive compounds like phenolics and terpenes (Pinto and Ayala-Zavala, 2024). Plant extracts also exhibit antifungal properties, effectively inhibiting the growth of common foodborne fungi such as *Aspergillus* and *Penicillium*. These natural compounds offer a promising alternative to synthetic preservatives, as they can control microbial growth without introducing harmful chemicals into the food chain (Imran *et al.,* 2021). The effectiveness of plant extracts in food preservation highlights their potential as natural antimicrobial agents in the food industry.

2.23 Neem's Efficacy against Spoilage Microbes in Fruits and Vegetables

Plant extracts, particularly from neem *(Azadirachta indica),* have shown promising results in extending the shelf life and maintaining the quality of fruits and vegetables post-harvest. Neem-based treatments have been effective in reducing microbial spoilage caused by various pathogens, including *Aspergillus spp., Fusarium oxysporum,* and *Botryodiplodia theobromae.* These natural preservatives have demonstrated efficacy in controlling decay and maintaining biochemical characteristics in citrus fruits (Malik *et al*., 2016). Studies on tomatoes revealed that neem leaf powder treatment extended shelf life and reduced post-harvest decay compared to untreated fruits. The use of plant extracts and essential oils as alternatives to synthetic fungicides has gained attention due to their antimicrobial properties and environmental friendliness. However, challenges remain, including potential phytotoxicity and the need for further research on extraction technologies and safety assessments (El Khetabi *et al.,* 2022).

2.24 Case Studies or Examples of Neem Usage in Food Systems

Several case studies and real-world examples highlight the effectiveness of neem as a preservative in food systems.

Neem in Post-Harvest Management of Mangoes: Recent studies have demonstrated the effectiveness of plant-based coatings, particularly neem oil and extracts, in extending the shelf life and maintaining the quality of mangoes post-harvest. Neem-based treatments have shown significant benefits in reducing weight loss, preserving firmness, and inhibiting microbial growth (Gupta and Jain, 2014; Shrestha *et al*., 2018; Passafiume *et al.,* 2022). In comparison to untreated fruits, neem-coated mangoes exhibited higher levels of ascorbic acid, total soluble solids, and sugars, while maintaining lower pH levels (Gupta and Jain, 2014; Shrestha *et al.,* 2018). The efficacy of neem treatments was attributed to the presence of azadirachtin, an active compound with antimicrobial properties (Gupta and Jain, 2014). While other plant-based coatings such as starch and beeswax also showed positive effects on mango preservation, neem extracts consistently outperformed them in various quality parameters (Bibi and Baloch, 2014; Shrestha *et al.,* 2018).

Neem in Preservation of Tomatoes: Recent studies have demonstrated the efficacy of neem *(Azadirachta indica)* extracts in preserving tomatoes and extending their shelf life. Neem leaf extracts significantly reduced weight loss and fungal growth on tomatoes, extending their shelf life up to 24 days (Okolo *et al*., 2022). The antimicrobial activity of neem was particularly effective against common tomato pathogens, including *Aspergillus niger, Fusarium oxysporum,* and *Rhizopus stolonifer* (Okolo *et al*., 2022; Chime and Aiwansoba, 2023). Neem-treated tomatoes exhibited improved quality retention, with reduced decay and better firmness compared to untreated fruits (Masood *et al*., 2016; Zewdie *et al.,* 2022). In controlled atmosphere storage, neem leaf extract was found to be as effective as traditional fungicides in preserving tomato quality. These findings suggest that neem extracts could serve as a promising bio-preservative for tomatoes, offering an environmentally friendly alternative to synthetic preservatives (Masood *et al*., 2016; Zewdie *et al.,* 2022).

Neem as an Alternative to Synthetic Pesticides in Stored Grains: Neem extracts have shown promising results in protecting stored grains from insect pests and fungal contamination. Studies have demonstrated that neem leaf extract and oil are effective against various storage pests, including the Khapra beetle *(Trogoderma granarium)* (Kteo and Mohammed, 2019; Rafi *et al.,* 2022). Neem oil application on wheat grains resulted in high mortality rates of *T. granarium,* reduced egg hatching, and decreased grain weight loss. Additionally, neem-treated grains showed fewer fungal isolates compared to untreated grains, indicating its potential in controlling fungal growth (Kteo and Mohammed, 2019). Botanicals like neem offer a biodegradable, non-residual alternative to synthetic pesticides for grain preservation (Vijayan *et al.,* 2023). These plant-based products can address issues associated with synthetic pesticides, such as resistance development and health hazards (Chugh *et al.,* 2023). However, further research is needed to assess the practical applicability and biosafety of botanical insecticides in commercial storage conditions.

2.25 Factors Affecting the Efficacy of Neem Extracts

Neem extracts have shown promising potential as natural preservatives in food systems, with their efficacy influenced by various factors. Studies have demonstrated that neem leaf extract at 20% concentration, combined with cool storage, can effectively inhibit pathogens and extend the shelf life of mango fruits (Gupta and Jain, 2014). The effectiveness of natural antimicrobials, including neem, is affected by factors such as concentration, extraction method, food composition, processing techniques, and storage conditions (Teshome *et al.*, 2022). Plant extracts like neem can be particularly useful in preserving fresh foods, such as fresh-cut salads and marinated chilled products, when combined with other technologies like processing and packaging (Sultanbawa, 2015). However, the extraction and purification processes may impact the structure and functionality of these natural preservatives, highlighting the importance of selecting appropriate methods to maintain their active ingredients (Teshome *et al*., 2022).

2.25.1 Method of Application (Immersion, Spraying, Coating)

The method of applying Neem extracts to food products is another critical factor influencing their efficacy. The method used determines the uniformity of coverage, the quantity of extract applied, and the interaction between the extract and the food's surface. Different methods are suited for different types of food and desired outcomes.

Immersion

Neem extracts have shown promise in food preservation and packaging due to their antimicrobial and antioxidant properties (Kumar *et al.,* 2021). Immersion of fruits and vegetables in neem solutions can effectively extend shelf life and maintain quality, as demonstrated with tomatoes (Ewekeye *et al.,* 2024) and papayas. Neem leaf and fruit extracts at 5% concentration were particularly effective in preserving papaya fruits for up to 12 days, controlling fungal growth and maintaining external quality. However, challenges remain, including potential impacts on food sensory qualities and the need for standardization of plant extracts for food use (Negi, 2012; Kumar *et al.,* 2021). Nanoformulations of neem oil may offer improved stability and efficiency in food preservation applications (Kumar *et al.,* 2021). While plant extracts like neem show promise as natural preservatives, further research is needed to address safety concerns and develop cost-effective isolation procedures (Negi, 2012).

Spraying

Spraying is a widely used and efficient method for applying edible coatings to fruits and vegetables, offering an eco-friendly alternative to chemical pesticides (Shahbaz *et al.,* 2022; Andrade *et al*., 2012). These coatings, often incorporating herbal extracts like neem, aloe vera, and lemongrass, can extend shelf life by 15-35 days at low temperatures, compared to 8-10 days for uncoated produce (Rajial and Varma, 2024). The coatings form a protective barrier that prevents water loss, controls ripening, delays oxidative browning, and reduces microbial growth. Spraying allows for uniform application and is suitable for large-scale industrial use (Andrade *et al.,* 2012). These natural coatings are safe for human consumption and environmentally friendly, addressing concerns about chemical pesticide use (Shahbaz *et al.,* 2022). By improving food safety, appearance, and shelf life while reducing packaging waste, edible coatings offer significant benefits to both consumers and the environment (Rajial and Varma, 2024).

Coating

Edible coatings have emerged as an effective solution for extending the shelf life and maintaining the quality of fruits and vegetables. These coatings, which can be derived from various sources including polysaccharides, proteins, and lipids, form a protective barrier that limits moisture loss and gas exchange. Recent research has focused on incorporating herbal extracts, such as neem, tulsi, and ginger, into edible coatings due to their antimicrobial and beneficial biochemical properties (Rajial and Varma, 2024). These coatings can significantly extend storage time, with some studies reporting 15-35 days at low temperatures compared to 8-10 days for uncoated produce (Rajial and Varma, 2024). Edible coatings offer advantages over other preservation techniques, including reduced packaging waste and improved food safety. They can also help maintain color, texture, and moisture while controlling microbial growth (Lin and Zhao, 2007).

Optimal Method for Application

Neem extracts and edible coatings have shown promising results in extending the shelf life and maintaining the quality of various fruits and vegetables. Studies have demonstrated that neem leaf extract dipping combined with beeswax coating significantly extended the shelf life of tomatoes by reducing weight loss, decay, and changes in acidity and soluble solids (Zewdie *et al*., 2022). Similarly, neem extract dipping improved the storage life and quality of guava fruits. For fresh-cut mangoes, neem oil added to edible coatings effectively reduced firmness loss, color changes, and microbial growth (Passafiume *et al.,* 2022). The application method and concentration of neem extracts can vary depending on the fruit type and desired outcome. Edible coatings, including those incorporating herbal extracts like neem, form a protective layer that limits moisture loss and gas exchange, thereby enhancing preservation. These studies highlight the potential of neem-based treatments in postharvest fruit preservation.

2.25.2 Storage Conditions (Temperature, Humidity, and Exposure to Light)

Neem extract has shown promising potential as a natural preservative for food products due to its antimicrobial and antioxidant properties (Kumar *et al*., 2021). Studies have demonstrated its effectiveness in extending the shelf life of fruits like Nagpur mandarin when used as a coating in combination with cold storage. However, the efficacy of neem-based preservatives can be influenced by various factors, including storage conditions, food composition, and processing methods (Teshome *et al.*, 2022). To address challenges such as photosensitivity and rapid degradation, researchers have explored advanced delivery systems like nanoemulsions and microencapsulation techniques (Liu and Xu, 2019; Kumar *et al.,* 2021;). These approaches have shown improved stability and functionality compared to traditional neem extracts. Microencapsulated neem preservatives, for instance, exhibited better resistance to environmental factors like UV light and water spray (Liu and Xu, 2019). Overall, careful consideration of storage conditions and preservation techniques is crucial for maintaining the efficacy of neem-based preservatives in food applications.

Temperature

Temperature significantly affects the stability and efficacy of neem extracts in food preservation. High temperatures can degrade azadirachtin A, the active compound in neem (Jenkins *et al*., 2003). However, neem kernel extract stored at 50°C for 2 weeks still reduced infestations of *Callosobruchus maculatus* in cowpeas, possibly due to the oil properties (Jenkins *et al.,* 2003). Refrigeration is recommended for preserving azadirachtin, with storage in brown bottles preferred over plain bottles. Neem extracts at 5% concentration effectively extended the shelf life of papaya fruits to 12 days at 30.4°C (Freitas *et al.,* 2018). For mangoes, 20% neem leaf extract combined with cool storage completely inhibited pathogens, minimized weight loss, and maintained fruit quality (Gupta and Jain, 2014). These findings suggest that while temperature affects neem extract stability, proper storage and application methods can enhance its effectiveness in food preservation.

Humidity

Humidity plays a crucial role in food preservation, particularly for fresh-cut fruits and vegetables. High relative humidity in packaging can promote microbial growth and spoilage (Ayala-Zavala *et al.,* 2008). However, controlled humidity levels are essential for maintaining food quality and stability. Neem extracts have shown promise as antimicrobial coatings for fruits, with studies demonstrating their effectiveness in preserving papaya (Freitas *et al.,* 2018) and fresh-cut mango (Passafiume *et al.,* 2022). These neem-based coatings can extend shelf life by inhibiting microbial growth and reducing quality degradation. However, their efficacy may be compromised in high-humidity environments. To address this, innovative approaches like using cyclodextrins as carriers for antimicrobial compounds in high-humidity packaging have been proposed (Ayala-Zavala *et al.,* 2008). Proper storage conditions, including temperature and relative humidity control, are crucial for maintaining the effectiveness of neem-based and other antimicrobial treatments in food preservation (Freitas *et al.,* 2018; Passafiume *et al.,* 2022).

Exposure to Light

Research has shown that exposure to light, particularly ultraviolet (UV) and sunlight, significantly degrades azadirachtin, the primary bioactive compound in neem extracts, reducing its antimicrobial and antifeeding properties (Stokes and Redfern, 1982). Studies indicate that azadirachtin's half-life under UV light is as short as 48 minutes, while sunlight exposure results in a half-life of 3.98 days (Johnson *et al.,* 2003). To preserve neem extract efficacy, dark storage conditions are preferable, with refrigeration or room temperature storage in brown bottles showing better results than plain bottles. Photostabilizers such as 8-hydroxy quinoline and tert-butylhydroquinone can significantly extend azadirachtin's half-life under both UV and sunlight exposure (Johnson *et al.,* 2003). Recent research has explored neem oil nanoemulsions as a more stable and efficient alternative for food preservation and packaging applications (Kumar *et al*., 2022).

2.26 Comparative Studies on Preservative Efficacy

The growing demand for natural food preservatives has led to increased interest in plant-based antimicrobials as alternatives to synthetic preservatives (Pinto *et al.,* 2023). Neem (*Azadirachta indica*) has emerged as a promising candidate due to its broad-spectrum antimicrobial properties, with potential applications in food safety, dentistry, and against drug-resistant pathogens (Wylie and Merrell, 2022). Natural preservatives, including essential oils, offer advantages such as addressing microbial resistance and reducing negative side effects associated with synthetic compounds. These natural alternatives work through various mechanisms, including membrane disruption and enzyme inactivation Essential oils, in particular, have gained attention for their antimicrobial efficacy and potential to meet consumer demands for "green" products. However, further research is needed to optimize formulations, assess safety, and address regulatory aspects to fully realize the potential of plant-based antimicrobials in food preservation (Pinto *et al.,* 2023).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Selection and Processing of Neem (*Azadirachta indica*) Leaves

Freely cut neem leaves, detached from the stems, were spread out on tarpaulin sheets for 5 days to dry. After drying completely, the leaves were pulverized into a fine powder.

3.2. Preparation of Aqueous and Ethanolic Neem (*Azadirachta indica*) Extract

1000 mL of deionised water and ethanol, 100g of neem (*Azadirachta indica*) leaf powder was added to a 2000 mL capacity conical flask; and this was done separately for both aqueous (sterile deionised water) and ethanolic (75%) extract. The conical flasks were corked securely and placed in a dark air-tight cabinet for 2 days. After 48 hr, the conical flasks were extracted and the contents were concentrated using a rotary evaporator to dry. The dried extract was then transferred into a sterile container awaiting use (Nasrine *et al.,* 2023)

3.3. Sample Collection

Bananas, bell peppers and tomatoes were purchased wholesale from trucks in Benin City, Edo State. The fruits were matured, whole and without any visible injuries or infection. On getting to the laboratory, the fruits were wiped clean with dry sterile wipes. Thereafter, they were transferred into sterile plastic dishes with perforated lids. Samples were sorted and designated and kept for 15 days of storage and were analyzed at intervals for 3 days.

3.4. Sterilization of Prepared of Extract Solution

The aqueous and ethanolic extract solutions were prepared respectively by measuring 50g of the evaporated powder into 500 mL of sterile deionised water. The mixture was agitated and then transferred into a 1000mL capacity glass bottle with cover and sterilized at 121^{o}C for 15 min in an autoclave (Nasrine *et al.,* 2023).

3.5. Application of Neem *(Azadirachta indica)* Extract to Fruits Samples

The aqueous and ethanolic extracts were applied to the appropriately designated fruit samples by brushing using a sterile finely bristled brush; different brushes were used for the different extracts. Samples were then placed in doorless cupboards and analyzed at 3 days intervals for the next 14 days.

3.6. Sensory Evaluation of Fruit Samples

Colour, smell, texture, taste, flavour and overall appearance were evaluated daily for 14 days. The parameters were grading:

1 = very good

2 = good

3 = bad

4 = very bad

Evaluation of taste and flavour was terminated at day 5 (Peryam and Pilgrim, 1957).

3.7. Bacteriological Analysis of Fruit Samples

3.7.1. Preparation of Peptone water for serial dilution

Peptone water was prepared according to the manufacturer's instruction (15g to 1000 ml of deionized water) and dispensed into dilution bottles, that is, 45mL into the dilution bottle and then 9 mL each into the rest of the dilution bottle up to 10^6 This was prepared for all the samples to be analysed. The bottles were closed then sterilised in the autoclave for 15 min at 121^{o}C.

3.7.2. Preparation of culture media

The culture media used in this study include: Nutrient Agar, *Salmonella-Shigella* Agar, Mannitol Salt, MacConkey Agar. Each medium was prepared according to the manufacturer's instructions. They were sterilised in the conical flask inside an autoclave for 15 minutes at 121^{o}C

3.7.3. Preparation of Inoculum

Sterile swab-stick was used to swab approximately 6cm on either side of the fruit sample carefully. Thereafter, the tips were aseptically cut into the 45 mL dilution bottles (10^{-1}). Samples were serially diluted up to 10^{-6}.

3.7.4. Inoculation and pour plate technique

Petri dishes were inoculated with 1mL aliquot from the 10^{-3} and 10^{-6} dilution bottles. Cooled molten agar medium (20mL) was aseptically poured into the appropriately labelled Petri dishes. Plates were rocked gently and allowed to cool and solidify. After which, the plates were inverted and incubated for 24-48 hr at 37^{o}C.

3.7.5. Cultural and Morphological Characterization:

Cultural characteristics were observed on the plates, and morphological characteristics were determined using Gram staining and slides were viewed with the aid of a microscope. Biochemical tests were carried out based on the method of Cheesbrough (2006).

3.8. Gram Staining:

A thin smear each of the isolates was made on different slides with the aid of a wire loop, after which they were heat fixed and allowed to cool. Then the different smears were covered with crystal violet stain for 60 sec and rapidly washed with sterile, deionized water. Subsequently, the smears were covered with Lugol's iodine for 30 sec and rapidly washed off with sterile, deionized water. The smears were decolorized rapidly with acetone and ethanol mixture (1:2) and washed off immediately with sterile, deionized water. Then the smears were covered with safranin for 60 sec and rinsed immediately with sterile, deionized water. The stained smears were air-dried. After drying, a drop of oil immersion was placed on the stained smears and viewed with the aid of a microscope (x 100 objective lens).

3.9. Biochemical Tests

Bacterial isolates kept on nutrient agar slants were propagated in Tryptic soy broth for 24h, and then sub-cultured on nutrient agar plates by streak plate technique. The plates were incubated for 18-24 hr before commencing biochemical tests.

3.9.1. Catalase Test:

Discreet colonies of each of the isolates were transferred with a wired loop onto a clean, grease-free slide and emulsified in a drop of hydrogen peroxide (H_2O_2). Bubbles of gas formed indicated a positive result according to Cheesbrough (2006).

3.9.2. Oxidase Test:

A piece of filter paper was placed in a clean Petri dish and 2-3 drops of freshly prepared oxidase reagent was added. With the aid of a loop, discreet colonies of the isolates were collected separately and smeared on the filter paper. A positive result gave a purple-blue coloration after 10 sec while a negative result gave no such color after 10 sec according to Cheesbrough (2006).

3.9.3. Indole Test:

Organisms were grown in tryptophan broth for 48hr. The broth was prepared by measuring out 0.1g of tryptophan, 0.05g of creatinine into 100 ml of peptone water. To each test bottle, 5mL of tryptophan broth was dispensed, and then aseptically inoculated with test bacterium using a wire loop, or from cell suspension. After 48hr, 0.5ml of Kovac's indole reagent (isoamyl alcohol + para dimethylaminobenzaldehyde DMAB + concentrated HCl) was pipetted into the broth and swirled gently. The formation of a pink – red coloured ring at the surface indicated a positive test, i.e, production of indole by the bacterium.

3.9.4. Citrate Utilization:

Streaks of all isolates were done on the surface of Simmons citrate agar in test tubes and incubated at 37^{o}C for 48hr. Development of deep blue colour within 24-48 hr was considered as a positive test.

3.9.5. Urease Production:

Inoculum from discrete colonies were streaked on the surface of Christiansen's urea agar base containing 40% sterile urea and incubated for 48hr. A change of colour from light brown to pink indicated a positive test.

3.9.6. Hydrogen Sulphide Production:

This was determined using the triple sugar iron agar (TSI), which is a multi-test medium. It tests the ability of bacteria to ferment glucose, sucrose and lactose, in addition to testing the formation or production of hydrogen sulphide, and carbon (IV) oxide. Colonies were streaked on TSI agar slants and incubated for 72hr with the corks of the bottles set loosely. Formation of blackening sections in the medium indicated a positive test for hydrogen sulphide.

3.9.7. Sugar Fermentation Tests:

The sugars tested in this study include glucose, lactose, maltose, sucrose, mannitol and xylose. The fermentation medium (100mL) was prepared using peptone water (1.5g), 1g of appropriate sugar, 0.5g of disodium phosphate, and 0.72mL of phenol red indicator. Sterilization was at 121^{o}C for 5 min (the sugars become burnt and caramelized in excess of 5 min). Phenol red colours an alkaline medium red, and turns gold in an acidic medium; sodium phosphate serves to increase the pH of the broth medium to an alkaline state. The medium was inoculated with 50µL from cell suspension (MacFarland $1x10^8$) and incubated for 24hr. Utilization of the sugar leads to formation of acid that decreases the pH of the medium and makes the phenol red turn gold.

3.9.8. Methyl Red Test:

This tests how low the pH of the medium has been reduced to. The effective test pH range is 4.5 and below. Methyl red is red in an acidic medium, and gold in an alkaline medium. A drop of overnight bacterial broth medium was introduced to a drop of methyl red indicator on a clean, grease-free slide. A positive result was indicated by the mixture turning red while gold/yellow was negative

3.9.9. CO_2 Gas Production:

Durham tubes were inserted facedown into the glucose fermentation test tubes before sterilization at 121^oC for 5 min. If the bacterium produces gas during fermentation of the sugar, the gas produced displaces the liquid medium within the durham tube and takes up the vacated space. This is indicated by visible clear space within the Durham tube.

3.9.10. Voges Proskauer Test:

This test determines if an organism produces acetyl methyl carbinol from the fermentation of glucose. To a test tube containing 48h glucose broth culture, 6 drops of 5% α-naphthol solution was added. The tube was shaken well to aerate its contents, after which 2 drops of 40% potassium hydroxide was added, and the tube mixed well. The formation of a pink or red colour at the upper part of the medium indicated a positive result.

3.10. Antibiotic Susceptibility Test

The Isolated bacteria were first grown in rich tryptone soya broth for 24hr, after which they were subcultured onto nutrient agar and incubated for 24h. Sterile preparation of $1x10^8$ MacFarland Standard concentration of each bacterial isolate was made and 0.5ml of inoculum was spread on Mueller-Hinton agar plates with a sterile glass rod. The inoculum was allowed to dry for 10 min before sterile discs impregnated with various antibiotics of specific concentrations (10µg pefloxacin, 10µg gentamycin, 30µg ampiclox, 20µg zinnacef, 30µg amoxicillin, 25µg rocephin, 10µg ciprofloxacin, 30µg streptomycin, 30µg septrin, 10µg erythromycin, 20µg levofloxacin, 10µg cefotaxime, 10µg sparfloxacin, 10µg augmentin, 10µg tarivid, 12µg azithromycin) were placed on the agar surface with the help of sterile forceps. The plates were incubated for 24hr after which readings of zones of inhibition were taken by measuring from the middle of the disc to the end of the inhibition zone upwards. This gave the radius of the inhibition zone, so the actual zone was taken to be twice the measured value. The results were interpreted according to the CLSI (2023) standard.

3.11 Data Analyses

Tests were conducted in duplicates to obtain means and standard deviations or errors, and were reported as mean ± Standard deviation. Results were processed statistically with Microsoft Excel 2023 and SPSS 19.00.

CHAPTER FOUR

RESULTS

The total heterotrophic bacterial counts (cfu/cm^2) for banana, bell pepper, and tomato coated with neem (*Azadirachta indica*) extracts over 14 days are presented in Table 4.1. Ethanol extracts initially had higher counts but reduced significantly, especially in bell pepper (5.30±0.13 on day 14). Aqueous extracts also reduced bacterial loads, while control samples, particularly banana, peaked at 18.30±0.07 cfu/cm^2.

Table 4.2 details coliform counts, showing ethanol-treated banana had lower contamination (0.04±0.00 cfu/cm^2 by day 9) compared to steadily increasing control samples. In bell pepper samples, ethanol and aqueous extracts suppressed coliform growth better than controls, where counts peaked at 21.00±1.41 cfu/cm^2 by day 14.

The staphylococcal counts are presented in Table 4.3. It was observed that all fruit samples coated with ethanol extracts recorded significantly reduced counts. Tomato had the least contamination (0.20±0.00 cfu/cm^2 on day 14), while counts in control samples increased, with bell pepper reaching 5.10±0.07 cfu/cm^2 by day 14.

Table 4.4 presents *Salmonella* counts, counts on ethanol-coated banana samples reduced from 39.80±0.35 - 2.20±0.01 cfu/cm^2 by day 14. Aqueous extracts were effective in bell pepper, while counts in control samples increased sharply, with bell pepper reaching 125.50±6.36 cfu/cm^2.

Tables 4.5a-c document physical observations, indicating ethanol-coated samples retained better texture and color. By day 14, banana in the control group had significant contamination (14 sites), while ethanol-treated samples had fewer. Bell pepper control samples deteriorated faster, turning red earlier.

Table 6 provides sensory evaluations, where ethanol-coated banana had higher appearance scores (3.16±0.09), while aqueous-treated bell pepper had the lowest taste score (1.56±0.30). Control samples scored lower across parameters, affirming better preservation by neem extracts.

Following cultural, morphological and biochemical characterization, the following bacterial isolates were identified: *Staphylococcus aureus*, *Micrococcus* spp., *Listeria monocytogene, Lactobacillus* spp., *Micrococcus* spp., *Staphylococcus epidermidis, Acinetobacter baumannii* and *Klebsiella pneumoniae.* Fewer bacterial species were obtained from ethanol-coated samples. Results are shown in Table 4.8.

The distribution of bacterial isolates in all samples are shown in Table 4.9. It was observed that control samples exhibited the highest diversity of bacteria, including *Klebsiella pneumoniae* and *Acinetobacter baumannii.*

The antibiotic susceptibility patterns of bacterial isolates are presented Table 4.10. It was observed that most bacteria exhibited resistance, however *Klebsiella pneumoniae* was most susceptible in ethanol-treated samples. Resistant strains in control samples suggest higher spoilage risk.

Table 4.1: Total Heterotrophic Bacterial Counts of Fruits Coated with Neem (*Azadirachta indica*) Leaf Extracts (cfu/cm^2) Stored over a 14 Day Period

Fruits	Storage Duration (Days)				
	0	4	7	10	14
Banana					
Aqueous	-	5.75±0.007	16.00±0.004	14.70±0.04	15.50±0.04
Ethanolic	-	42.40±0.11	14.30±0.007	13.00±0.007	13.10±0.06
Bell Pepper					
Aqueous	-	46.00±0.98	13.60±0.02	11.20±0.05	12.60±0.11
Ethanolic	-	62.40±0.33	19.20±0.05	6.00±0.11	5.30±0.13
Tomato					
Aqueous	-	0.30±0.02	0.40±0.01	0.60±0.01	0.70±0.007
Ethanolic	-	9.10±0.37	1.80±0.01	1.80±0.01	2.3±0.02
Control					
Pepper	0.70±0.04	4.00±1.07	13.60±1.07	41.20±4.01	81.90±7.07
Banana	0.40±0.08	6.00±0.36	19.00±2.01	31.90±3.10	81.30±3.07
Tomato	0.20±0.00	3.20±2.01	15.20±0.00	11.10±3.02	61.20±2.12

- = Not investigated.

Table 4.2. Mean Coliform Counts of Coated Fruits with Neem (*Azadirachta indica*) Leaf Extracts (cfu/cm^2) Stored over a 14 Day Period

Extracts	Storage Duration (Days)				
	0	**4**	**7**	**10**	**14**
Banana					
Aqueous	-	0.00±0.00	0.60±0.01	0.40±0.00	1.70±0.06
Ethanolic	-	12.40±0.87	7.70±0.04	0.04±0.00	5.80±0.01
Pepper					
Aqueous	-	19.40±0.21	3.40±0.02	9.00±1.41	21.00±1.41
Ethanolic	-	17.70±0.09	3.40±0.04	0.2±0.00	4.2±0.01
Tomato					
Aqueous	-	0.00±0.00	0.10±0.007	0.10±0.007	0.20±0.00
Ethanolic	-	4.60±0.15	0.70±0.007	0.20±0.00	0.90±0.007
Control					
Pepper	0.10±0.007	8.00±0.14	5.20±0.05	11.00±30.35	7.90±0.02
Banana	0.20±0.00	10.00±0.00	2.40±0.01	2.50±0.007	2.20±0.01
Tomato	0.80±0.02	2.00±0.00	2.50±0.00	3.10±0.07	5.00±0.04

- = Not investigated.

Table 4.3: Total Staphylococcal Counts of Banana, Bell Pepper and Tomato Coated with Neem (*Azadirachta indica*) Leaf Extract (cfu/cm^2) Stored over a 14 Day Period

Extracts	Storage Duration (Days)				
	0	4	7	10	14
			Banana		
Aqueous	-	1.80±0.11	0.50±0.007	1.60±0.04	1.20±0.01
Ethanolic	-	9.10±0.02	0.30±0.007	1.30±0.02	0.8±0.01
			Pepper		
Aqueous	-	1.90±0.07	1.30±0.007	1.70±0.02	1.50±0.02
Ethanolic	-	5.30±0.03	0.20±0.00	0.30±0.007	0.40±0.01
			Tomato		
Aqueous	-	0.30±0.007	0.00±0.00	0.50±0.007	0.10±0.007
Ethanolic	-	1.40±0.04	0.10±0.007	0.30±0.007	0.20±0.00
			Control		
Pepper	3.20±0.14	5.10±0.07	11.60±0.02	13.3±0.007	15.10±0.07
Banana	2.30±0.007	6.60±0.00	10.80±0.01	12.40±0.04	14.1±0.07
Tomato	0.20±0.00	4.00±0.00	7.85±0.02	10.80±0.01	17.00±0.08

- = Not investigated.

Table 4.4. Mean of Salmonella count in the Fruits Coated with Neem (*Azadirachta indica*) Leaf Extracts (cfu/cm^2) Stored over a 14 Day Period

Extracts	Storage Duration (Days)				
	0	4	7	10	14
			Banana		
Aqueous	-	0.50±0.007	0.50±0.007	1.40±0.02	1.00±0.01
Ethanolic	-	39.80±0.35	2.30±0.03	2.40±0.02	2.20±0.01
			Pepper		
Aqueous	-	25.00±0.63	7.1±0.06	5.30±0.07	2.40±0.02
Ethanolic	-	21.60±0.28	3.70±0.03	1.30±0.02	1.00±0.007
			Tomato		
Aqueous	-	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Ethanolic	-	8.50±0.06	1.80±0.04	0.00±0.00	0.00±0.00
			Control		
Pepper	0.10±0.007	2.00±2.83	30.00±1.41	50.50±12.02	125.50±6.36
Banana	0.10±0.007	2.00±0.00	6.00±0.00	10.00±2.83	45.00±7.07
Tomato	0.40±0.01	0.60±0.00	1.20±0.02	4.00±0.02	4.80±0.09

- = Not investigated.

Table 4.5a. Physical Observation of Banana coated with Neem (*Azadirachta indica*) Leaf Extracts Stored over a 14 Day Period

Characteristics	Storage Duration (Days)											
	0			6			10			14		
Texture	1	1	1	3	3	3	5	5	3	-	-	-
Colour	G	G	G	Y	Y	Y	B	B	Y	-	-	-
Smell	1	1	1	1	1	1	4	4	3	-	-	-
Taste	1	1	1	2	2	2	-	-	3	-	-	-
Contamination site	0	0	0	2	3	3	12	14	9	-	-	-

Key:
1= Very good
2= Good
3= Fair
4= Bad
5= Very bad
G = Green, Y= Yellow, B = Blue
0 = No contamination site

Table 4.5b: Physical Observation of Bell Pepper coated with Neem (*Azadirachta indica*) Leaf Extracts Stored over a 14 Day Period

Characteristics	**Storage Duration (Days)**											
	0			6			10			14		
Texture	1	1	1	2	2	3	3	2	4	3	2	5
Colour	G	G	G	G	G	G/R	G/R	G/R	R	R	G/R	R
Smell	1	1	1	1	1	1	1	1	2	2	1	3
Taste	1	1	1	1	1	1	1	1	2	2	1	-
Contamination site	0	0	0	1	4	5	6	6	9	10	7	17

Key:
1= Very good
2= Good
3= Fair
4= Bad
5= Very bad
G = Green, G/R = Green/Red, R = Red

0 = No contamination site

Table 4.5c: Physical Observation of Tomato coated with Neem (*Azadirachta indica*) Leaf Extracts Stored over a 14 Day Period

Characteristics	Storage Duration (Days)											
	0			6			10			14		
Texture	1	1	1	1	1	2	1	1	3	1	1	5
Colour	R	R	R	R	R	R	R	R	R	R	R	R
Smell	1	1	1	1	1	1	1	1	2	2	2	5
Taste	1	1	1	1	2	2	1	1	2	2	1	-
Cont. site	0	0	0	0	0	0	0	0	1	0	1	7

Key:
1= Very good
2= Good
3= Fair
4= Bad
5= Very bad
R = Red
0 = No contamination site

Table 4.6: Evaluation of Sensory Characteristics of Banana, Bell Pepper and Tomato Coated with Neem Leaf Extract Over a Period of 14 Days by Panel of Judges

Samples	Appearance	Taste	Flavour	Texture	Smell
BANANA					
Aqueous	3.04±0.17	2.72±0.23	2.92±0.18	3.12±0.54	2.68±0.27
Ethanolic	3.16±0.09	2.92±0.18	2.96±0.09	3.20±0.36	2.92±0.00
Control	2.88±0.23	2.48±0.11	2.16±0.36	2.92±0.09	2.52±0.27
BELL PEPPER					
Aqueous	2.40±0.14	1.56±0.30	1.08±0.18	2.52±0.09	1.52±0.18
Ethanolic	2.48±0.23	1.72±0.30	1.12±0.27	2.20±0.18	1.84±0.27
Control	2.56±0.17	2.00±0.24	2.04±0.09	3.08±0.36	2.16±0.88
TOMATO					
Aqueous	1.12±0.11	1.16±0.17	1.12±0.27	1.68±0.34	1.20±0.09
Ethanolic	1.28±0.23	1.40±0.20	1.12±0.27	1.60±0.09	1.32±0.18
Control	1.88±0.11	2.05±0.19	2.00±0.00	2.64±0.63	2.12±0.00

Hedonic scale:
1 = Very good
2 = Good
3 = Bad
4 = Very bad

Table 8: Cultural, Morphological and Biochemical Characteristics of Bacteria Isolated from Banana, Bell Pepper and Tomato Coated with Neem Leaf Extract Over a Period of 14 Days

Cultural characteristics											
	1	2	3	4	5	6	7	8	9	10	11
Colour	Milky	Milky	Golden yellow	Milky	Milky	Lemon	Coffin	Cream	Milky	Milky	Golden
Shape	irregular	round	round	round	spike	round	round	round	round	round	round
Elevation	flat	flat	elevated	elevated	flat	elevated	elevated	flat	flat	flat	elevated
Margin	serrate	smooth	smooth	smooth	rough	smooth	smooth	smooth	rough	rough	smooth
Opacity	dull	shiny	shiny	shiny	dry	shiny	shiny	shiny	dull	dull	shiny
size	~5 - 12mm	~6 mm	~1.5- 2mm	~1.5- 2mm	30 mm	~1.5- 2.5 mm	~2.5 mm	~2 -2.5 mm	~1 mm	~1 mm	~0.5 - 1.5 mm
Morphological Characteristics											
Gram stain	+	+	+	+	+	+	+	+	-	-	-
shape	Cocci	Cocci	Coccobacili	Cocci	Short rod	Cocci	Cocci	Short rod	Coccobacili	Coccobacili	Cocci
arrangement	cluster	cluster	cluster	cluster	pairs	cluster	cluster	pairs	cluster	cluster	cluster
Biochemical test											
KOH	-	-	-	-	-	-	-	-	+	+	+
Oxidase	-	+	-	-	-	+	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	-	+	+	+	-	+	+	+	+	+
Lactose	+	-	-	+	+	-	-	+	N/A	+	+
Maltose	+	+	-	+	+	+	+	+	N/A	+	+
D- Mannitol	+	-	+	-	+	-	-	+	-	+	+
Xylose	+	-	-	+	-	-	-	-	+	+	+
Urease	-	-	-	-	-	-	-	-	-	+	+
Citrate	-	-	-	-	-	-	-	-	+	+	+
Indole	-	-	-	-	-	-	-	-	-	-	-
H2s	-	-	-	-	-	-	-	-	-	-	-
MR	+	-	+	+	-	-	+	-	-	-	-
VP	+	-	+	+	-	-	+	-	-	+	+
Gas (CO2)	-	-	-	-	-	-	-	-	-	+	+
	Staphylococcus aureus	*Micrococcus* spp.	*Listeria monocytogene*	*Staphylococcus aureus*	*Lactobacillus* spp.	*Micrococcus* sp.	*Staphylococcus epidermidis*	*Lactobacillus* spp.	*Acinetobacter baumannii*	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*

Key: + = positive, - = negative, N/A = variable

Table 9: Distribution of Bacteria Isolated from Banana, Bell Pepper and Tomato Coated with Neem Leaf Extract Over a Period of 14 Days

Samples	*Staphyloco ccus aureus*	*Micrococcus* spp.	*Listeria monocytogene*	*Staphyloco ccus aureus*	*Lactobacillus* spp.	*Micrococcus* sp.	*Staphylococcus epidermidis*	*Lactobacillus* spp.	*Acinetobacter baumannii*	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*
PAQ1	-	-	-	+	-	-	+	-	-	-	-
PAQ2	-	-	-	+	-	-	+	-	-	+	-
BAQ1	-	-	-	+	-		+	-	-	-	-
BAQ2	+		+	+	-	+	+	-	-	-	-
TAQ1	-	+	-	-	-	-		-	-	+	+
PETH1	-	-	-	+	-	-	+	-	-	+	-
PETH2	-	-	-	+	-	-	-	-	-	-	-
BETH1	+	-	+	+	-	+	-	+	-	-	-
BETH2	+	-	+	+	-	+	-	+	-	-	-
TETH1	-	-	-	+	-	-	-	-	-	-	-
TETH2	-	-	-	+	-	-	-	-	-	-	-
PC1	+	+	+	+	-	+	+	-	+	+	+
PC2	+	+	+	+	-	+	+	-	-	+	+
BC2	+	+	-	+	-	+	-	+	-	+	-
TC1	-	+	-	+	-	+	-	-	-	-	-
TC2	-	-	+	+	-	-	-	-	-	-	-

Key: + = positive, - = negative

Paq = Bell Pepper Aqueous, PEth = Bell Pepper Ethanol, PC = Bell Pepper Control, Baq = Banana Aqueous, BEth = Banana Ethanol, BC = Banana Control, Taq = Tomato Aqueous, TEth = Tomato Ethanol, TC = Tomato Control

Table 10: Antibiotics sensitivity test of Bacteria Isolated from Banana, Bell Pepper and Tomato Coated with Neem Leaf Extract Over a Period of 14 Days

Antibiotics	RD	CTZ	S	AZM	AMX	CPX	E	LEV	CN	CEF	MAR INDEX
Gram positive											
Staphylococcus aureus	I	S	I	R	R	S	I	S	S	R	0.3
Micrococcus spp.	R	I	R	R	I	S	S	R	I	I	0.4
Listeria monocytogenes	R	R	S	S	S	S	S	S	S	R	0.3
Staphylococcus aureus	R	R	I	I	R	S	S	S	S	R	0.4
Lactobacillus spp.	I	I	I	R	R	R	R	R	S	R	0.5
Micrococcus sp.	S	R	R	R	R	R	R	S	S	S	0.5
Staphylococcus epidermidis	R	R	I	S	I	I	S	S	S	I	0.2
Lactobacillus spp.	S	R	R	R	S	S	S	S	S	I	0.3

Gram-negative	LEV	CF	SP	CPX	AM	AU	CN	PEF	CEX	AZ	MAR INDEX
Acinetobacter baumannii	R	R	S	R	I	I	I	R	I	I	0.4
Klebsiella pneumoniae	S	I	S	I	R	R		S	S	S	0.2
Klebsiella pneumoniae	S	S	S	S	S	I	S	S	S	S	0

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Key: R = Resistant, S = Susceptible, I = Intermediate

CHAPTER FIVE

5.0 DISCUSSION

The study investigated the antimicrobial effects of neem (*Azadirachta indica*) leaf extracts on the preservation of banana, bell pepper, and tomato. The results indicated that neem extracts, particularly ethanol-based formulations, significantly reduced microbial load and extended shelf life compared to control samples. The study further revealed that ethanol extracts exhibited greater antimicrobial efficacy than aqueous extracts, corroborating previous findings that highlight solvent-based extraction as a key factor in antimicrobial potency (Nwokafor *et al.,* 2020). This suggests that ethanol serves as a more effective medium for extracting bioactive compounds from neem leaves, thereby enhancing their antimicrobial potential.

The differential response of fruits to neem extracts was evident in this study. Bananas, with a total heterotrophic coount of 5.75±0.007 and 42.40±0.11 for aqueous and ethanolic respectively exhibited the least preservation with either extract, likely due to their ripening process, which can counteract preservative effects (Murmu and Mishra, 2018; Rahman *et al.,* 2020). The natural metabolic changes that occur during ripening, such as increased respiration and ethylene production, may interfere with the antimicrobial effects of neem extracts. However, bell peppers displayed better preservation, remaining intact until day 6 before signs of deterioration appeared. This suggests that bell peppers may have intrinsic properties that allow them to retain the beneficial effects of neem extracts for a longer period. Tomatoes, particularly those treated with aqueous neem extract, demonstrated the longest shelf-life extension. This aligns with previous studies suggesting that neem-based treatments can prolong fruit freshness by delaying ripening and microbial spoilage through their antifungal and antibacterial properties (Pesis, 2005; Sanwal and Payasi, 2007).

The antimicrobial activity observed in this study aligns with findings from Wang *et al*. (2010), who demonstrated that neem seed kernel extracts effectively inhibited postharvest fungal infections in fruits. Similarly, Zakawa *et al.* (2018) reported significant reductions in fungal rot in mangoes treated with neem leaf extract, highlighting the broad-spectrum antimicrobial properties of neem. The strong antibacterial properties of neem ethanol extracts were particularly notable in reducing total heterotrophic bacterial counts in bell pepper and banana samples. This aligns with Mahfuzul-Hoque *et al.* (2007), who found that neem had strong antibacterial effects on foodborne pathogens. The ability of neem to target a wide range of spoilage organisms emphasizes its potential as an effective natural preservative. The reduction in coliform counts in this study further supports neem's efficacy in mitigating microbial contamination in fresh produce, making it a suitable alternative to synthetic preservatives.

The present findings align with Li *et al.* (2025), who found that different concentrations of neem leaf extract significantly influenced the antioxidant activity and physiochemical traits of peach fruits during storage. The ability of neem extracts to preserve not only the microbial quality but also the nutritional and sensory properties of fruits further underscores their significance. Ethanol-based extracts, as seen in this study, were more effective, likely due to the enhanced solubility of active compounds in organic solvents. This increased solubility ensures better penetration of bioactive molecules into fruit tissues, thereby prolonging their shelf life. Additionally, Sarmin *et al.* (2018) demonstrated that neem extracts significantly delayed ripening and microbial spoilage, consistent with the findings for bell pepper samples stored over a 14-day period. The prolonged freshness observed in this study is thus supported by existing literature on neem's ability to enhance postharvest fruit quality.

Furthermore, Chime and Aiwansoba (2023) found that neem extract effectively preserved tomato fruits, supporting the present study, where tomatoes treated with ethanol extracts exhibited reduced microbial loads and maintained better physical characteristics over time. This supports the broader application of neem-based treatments in extending the usability of different fruit types. Compared to other plant-based preservatives, neem extract demonstrated a competitive antimicrobial effect. Malik *et al.* (2016) reported that neem extract outperformed other botanical extracts, such as castor oil, in preserving citrus fruits. The superior efficacy of neem may be attributed to its rich phytochemical composition, which includes triterpenoids and flavonoids—bioactive compounds known for their potent antimicrobial properties (Coventry and Allan, 2001). These properties likely explain its effectiveness in extending fruit shelf life beyond conventional methods.

The extended shelf life of banana, bell pepper, and tomato observed in this study is comparable to previous findings. For instance, Sarmin *et al.* (2018) found that mangoes treated with 10% neem extract had a significantly prolonged shelf life, similar to the shelf-life extension seen in this research. This further reinforces the role of neem as a viable alternative to synthetic chemical preservatives. Likewise, neem-based treatments have shown promise in mitigating postharvest losses in various fruit types, indicating its potential for broader application in food preservation.

These findings hold significant implications for postharvest fruit preservation. Neem extracts, particularly ethanol-based formulations, present a viable, eco-friendly alternative to synthetic preservatives. Their antimicrobial and antioxidant properties contribute to prolonged freshness, reducing food waste and enhancing food security. Additionally, the use of neem aligns with global trends favoring natural preservatives over chemical alternatives, promoting safer and more sustainable food preservation methods. The findings also support the development of neem-based coatings as potential commercial solutions to fruit preservation, as suggested in previous research (Islam *et al.,* 2018). Future studies should explore the optimal concentrations and application methods of neem extracts to maximize their effectiveness across different fruit varieties and storage conditions.

5.1 Limitations and Future Research

Despite promising results, certain limitations exist. The study focused on a limited range of fruit varieties, and future research should explore a broader spectrum of perishable produce. Additionally, optimization of neem extract concentrations and formulation methods could enhance efficacy. Further studies should also examine the sensory attributes of neem-treated fruits over extended storage periods. Comparative studies with synthetic preservatives would also provide further insight into neem's effectiveness in commercial applications.

5.2 Conclusion

Neem leaf extract demonstrates substantial antimicrobial potential for fruit preservation, corroborating previous research. Ethanol-based extracts are particularly effective in reducing microbial loads and extending shelf life. This study adds to the growing body of evidence supporting neem as a natural preservative, with potential applications in sustainable food preservation strategies. Future research should focus on large-scale applications and consumer acceptance of neem-treated fruits in the market.

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