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Microorganisms Associated with Tomato (*Lycopersicum Esculentum*) Rot and Effect of Neem (*Azadirachta Indica*) Extract in Rot Control in Makurdi Metropolis, Benue State, Nigeria

Igbe Abi Obed[1], Innocent Okonkwo Ogbonna[2*]

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ABSTRACT

Tomato (*Lycopersicum esculentum*) is a vital agricultural crop globally, yet it is significantly affected by various microbial pathogens leading to post-harvest rot, which threatens food security and economic stability. This study aimed to identify the microorganisms associated with tomato rot in Makurdi Metropolis and evaluate the efficacy of neem (*Azadirachta indica*) extract as a biocontrol agent. A total of 60 tomato fruits were sampled from the five major markets within the Makurdi metropolis. They were cultured on Nutrient agar, MacConkey agar, Potato Dextrose agar, Mannitol salt agar, and Eosin Methylene blue agar. The organisms identified were *Klebsiella sp, Salmonella sp, Proteus vulgaris, Enterobacter sp, Shigella sp, Staphylococcus sp, Bacillus sp, Escherichia coli, Rhizopus sp, Mucor sp, Aspergillus sp,* and *Fusarium sp.* Neem extract was used for susceptibility tests on the bacterial isolates at 10%, 50%, and 100% concentration, and it was found most effective on some bacterial isolates at higher concentrations of 100% with wider zones of inhibitions. Vegetative growth of the fungi on neem extract decreased with increase in concentrations. This finding proved the potentiality of plant extracts for controlling the fungal rot of tomato fruit. A pathogenicity test was conducted with readings taken at intervals of days and it was noticed that the microbial isolates were actually the cause of tomato rot. This study underscores the importance of identifying microbial threats to tomato crops and highlights neem extract as a promising natural alternative for managing tomato rot, promoting sustainable agricultural practices in Makurdi Metropolis and Nigeria at large.

INTRODUCTION

Tomato (*Lycopersicum esculentum*) is a widely cultivated and consumed vegetable, known for its nutritional value and economic importance. However, its high-water content makes it particularly susceptible to spoilage by various microorganisms, including bacteria and fungi (Obeng, *et al.*, 2018). The tomato fruit comprises the skin, pericarp and locular cavities. The locular cavities are filled with jelly-like parenchyma cells that surround the seeds. The cell walls are composed of alpha-cellulose, pectin, hemicelluloses and some protein (Mautante & Mala, 2024). It is rich in vitamins including vitamin A and vitamin C, carbohydrates, proteins, fats, fibres, potassium, and phytochemicals (Talvas *et al.*, 2010). It is rich in lycopene which has many beneficial health effects. Tomato is a fruit that contains the seeds and ovary of a flowering plant (Ugwu *et al.*, 2014). It is known to be a very profitable crop that provides high returns for small scale farmers in most developing countries (Lemma, *et al.*, 2014). Due to its nutritive value, taste, affordability, and accessibility, there has been an increase in demand by consumers (Behravesh, *et al.*, 2012). However, isolation and identification of microorganisms that are associated with spoilage of tomatoes have gained some research focus (Akinyele, *et al.*, 2020). In most developing countries, microbial infestation of tomatoes can occur during the harvesting period, post harvesting, handling, storage, transportation, and processing by customers (Barth, *et al.*, 2009 and Yeboah *et al.*, 2011). Baiyewu *et al.*,2018 have also reported that another means of bacterial contamination is by exposing them on benches and baskets in the open markets for customers. The relative humidity (dew) during temporary storage of tomato fruits and nature of the storage room could play a great role in their contamination (Pardaev, 2022). The proliferation of bacteria, especially in damaged tomatoes, could be considered more harmful when such contaminated tomatoes are consumed in improperly cooked food (Valadez *et al.*, 2019).

Several studies have been performed on bacteria associated with tomatoes and tomato products in several countries. A survey by Ajayi and colleagues in the United States showed that *Clostridium sp.*, *Staphylococcus sp.*, and *Bacillus sp.* were the predominant bacteria isolated from canned and raw tomatoes. In India, a study conducted on tomato puree showed the presence of *Klebsiella sp.*, *Proteus mirabilis*, *Vibrio sp.*, and *Pseudomonas sp.* (Garg *et al.*, 2017). In Nigeria, Wogu and Ofuase isolated *Bacillus subtilis, Klebsiella aerogenes, Pseudomonas aeruginosa, Salmonella typhi, Proteus mirabilis,* and *Staphylococcus aureus* from spoiled tomatoes in Benin City. A similar study also showed that the content of *Staphylococcus sp.* (22.5%), *Bacillus sp.* (20%) and *Escherichia coli* (15%) in Lagos State, Nigeria

[1] Department of Biotechnology, I. M. Sechenov First Moscow State Medical University, Moscow, Russia
[2] Department of Microbiology, Joseph Sarwuan Tarka University, Makurdi Benue State, Nigeria
[*] Corresponding author's e-mail: ogbonna09@yahoo.com

Statement of the Problem

Azadirachta indica (commonly known as neem) is a tree in the mahogany family Meliaceae. Neem products are believed to be antifungal, antibacterial, antidiabetics, antiviral, contraceptive, and sedative. Hence, it is particularly prescribed for treating skin diseases such as eczema, psoriasis, etc. (Sharma and Nupur, 2014). Reports has shown that compounds from plant sources are moderately toxic and are suitable as fungicides. Neem (*Azadirachta indica*) extract has been studied for its antimicrobial properties, offering a potential natural solution for controlling tomato rot. The extract's bioactive compounds can inhibit the growth of spoilage-causing microorganisms, thus extending the shelf life of tomatoes and reducing economic losses. By investigating the microorganisms associated with tomato rot and assessing the potential of neem extract as a biocontrol agent, this study aims to advance our understanding of disease dynamics in agricultural systems and contribute to the development of environmentally friendly strategies for managing tomato diseases.

MATERIALS AND METHODS

Tomato fruit samples were collected from the five biggest markets in the Makurdi metropolis: Modern Market, Wadata Market, Wurukum Market, Northbank Market, and High-level Market. Each market sample was placed separately in a sterile plastic bag to prevent contamination. Samples were immediately transported to the laboratory, of the Department of Microbiology, Joseph Sarwuan Tarka University, Makurdi, Benue state. Culturing, Isolation, and Identification of the major pathogenic organisms in the disease portion was carried out. All materials used were sterilised by appropriate methods to remove extraneous microbial contamination. Glass wares were sterilised in a hot air oven at 120^0C for one hour. In contrast, culture media were sterilized by autoclaving at 121^0C for 15 minutes. Inoculation loops were heated till red hot in a Bunsen burner flame and allowed to cool before being used. Work surface was identified routinely by cleaning with cotton wool impregnated with ethanol before the commencement and on completion of work. The aseptic technique was maintained throughout the cause of this work.

Preparation of Stock Sample and Serial Dilution

The diseased portion was cut, crushed and fluids from the five different markets were squeezed into a conical flask labelled against each market. Five test tubes containing 9ml of distilled water (DW) were set. 1ml of tomato juice from the conical flask was transferred into the first test tube labelled against the same market, giving a dilution of 10^{-1}. This was mixed and 1ml was transferred to the next test tube (DW) marking its 10ml and giving it a dilution of 10^{-2}. This process was continued till the 5^{th} test tube giving a dilution of 10^{-5}. This same process was carried out on the samples collected from all the markets. Ethanolic extracts were prepared in the laboratory by the traditional Method. One kilogram of fresh neem leaves (*Azadirachta indica*) was collected from neem trees at Joseph Sarwuan Tarka University, Makurdi Benue State. Leaves were dried at room temperature and crushed in a mortar. Combining the crushed leaf with ethanol and subsequently soaking the mixture overnight at room temperature. The next morning, the extract was strained through a Miracloth, the liquid was kept in a water bath in the laboratory and allowed to evaporate to have a pure concentrated extract.

Microbiological Analysis

Media were prepared according to the standard preparation protocol and the media used were Nutrient agar, MacConkey agar, Potato Dextrose Agar (PDA), Mannitol Salt Agar (MSA), and Eosin Methylene Blue Agar (EMBA). Using a sterile pipette, aliquot of 0.1ml was taken from 10^{-5} dilution and was transferred into the duplicates plates of the media used i.e. Nutrient agar, MacConkey, PDA, MSA and EMBA. Pour plate method of inoculation was used to inoculate on the media according to the markets they were collected from and on what symptoms or disease inferred from these markets. Inoculated plates of the media were incubated at 37^0C for 24 hours for isolation of bacteria, and also at room temperature (25 ± 2^0C) for 48–72 hours for isolation of fungi. Representative colonies from all the media were sub-cultured on Nutrient agar for pure isolate, which were later subjected to biochemical analysis for identification.

Observation of Fungal Characteristics and Identification

The fungal isolation on PDA was identified by visual observation of their growth and microscopic examination using a drop of lactophenol cotton blue stain. The observed fungi were identified by comparison with diagram and keys documented by Harrigan and McCance (1976) as reported by Barton *et al.*, 2006. Distinguishing characteristics looked for included the general morphology and hyphae (Septate and non-septate). The shape, colour and distribution patterns were also used in identification. The staining was done according to Pelazar *et al.* (1999) as follows:

A pin head of fungi growth was picked using needle. This was placed on a slide with a drop of lactophenol blue stain. A drop of distilled water was added. The slide was covered with a cover slip and observed under the microscope at high objective of X10 power magnifications.

RESULTS AND DISCUSSIONS

Table 1 below shows the Mean of the total microbial load of tomatoes sold in different markets in the Makurdi metropolis. This investigates the levels of microbial contamination present in tomatoes from various markets within the region. It measures and compares the total microbial load, including bacteria, fungi, and other microorganisms, to assess food safety and hygiene practices. The findings revealed significant differences

in microbial loads across different markets, highlighting potential health risks for consumers and the need for improved handling and storage practices to ensure the safety of tomatoes sold in these areas.

Table 1: Mean of total microbial load of tomato sold in different markets in Makurdi metropolis

s/n	Samples	TVC (cfu/g)	TCC	Fungi
1	High level	$163x10^5$	$120x10^5$	$148x10^5$
2	Wurukum	$216x10^5$	$129x10^5$	$150x10^5$
3	North Bank	$160x10^5$	$109x10^5$	$132x10^5$
4	Modern market	$202x10^5$	$135x10^5$	$95x10^5$

Key: TVC = Total Viable count: TCC = Total Colony Count: Cfu/g = Colony forming unit/g

From the table, the bacterial count was maximum in Nutrient agar with a total viable count ranging from 160 min. to 216 max., total colony count ranging from 109 min. to 135 max. and fungi count ranging from 95 min. to 150 max.

The study on the morphological and microscopic features of fungi isolated from the tomato samples is represented in Table 2. This focuses on identifying and characterizing the fungal species found in tomatoes. It examines both the physical characteristics (morphological features) such as the fungi's colour, texture, and growth patterns, as well as their microscopic structures, including spore shape, size, and arrangement. This information helps in understanding the types of fungi present, and their potential impact on tomato quality and safety, and may provide insights into contamination sources and prevention methods. Overall, it contributes to the knowledge of fungal diversity associated with tomatoes and its implications for food safety.

Table 2: Morphological and microscopic features of fungi isolated from the tomato sample

Morphological characteristics	Microscopic examination	Suspected organism
Long hyphal growth which sporulated within two days to turn to black spores	Non-septate, branched mycelium with round-shaped sporangia	*Rhizopus sp.*
White and woolly aerial growth that darkens as it sporulates	Non-septate hyphae with straight sporangiophore with many spherical spores	*Mucor sp.*
Pink fluffy and spreading colonies which are creamy around the edges.	Septate hyphae with sickle chlamydospores at the hyphae.	*Fusarium sp.*
Velvety filament white growth that sporulates into black powdery spores.	Long septate hyphae with conidiophore bearing brown spores	*Aspergillus sp.*

From the study, the microscopic and biochemical characteristics of the bacterial Isolates which focus on identifying and characterizing bacterial strains isolated from the tomato samples are represented in table 3. The microscopic characteristics include the bacteria's shape, size, arrangement, and staining properties., which helps in their classification. The biochemical characteristics involve various tests to determine metabolic activities, such as fermentation, enzyme production, and nutrient utilization. Together, these analyses provide insights into the diversity, functionality, and potential pathogenicity of the bacterial isolates, contributing to a better understanding of their role in the ecosystem or their impact on health and disease.

Table 3: Microscopic and Biochemical Characteristics of the Bacterial Isolates

Gram reaction	Cat	Cit	Ure	Ind	MR	VP	Suspected Organism
-ve rod	+	+	+	-	-	-	*Klebsiella sp.*
-ve rod	+	+	-	-	-	-	*Salmonella sp.*
-ve rod	+	+	+	+	+	-	*Proteus vulgaris*
-ve rod	+	+	-	+	-	-	*Enterobacter sp.*
-ve rod	+	-	-	-	-	-	*Shigella sp.*
+ve cocci	+	-	+	-	-	-	*Staphylococcus sp.*
+ve rod	+	+	+	-	-	+	*Bacillus sp.*
-ve rod	+	+	-	+	+	-	*Escherichia coli*

Key: '+' = positive result; '-' = negative result

Table 4 and 5 summarizes the susceptibility test of Neem extract in different concentrations on bacterial isolates using Amoxicillin as control and the Pathogenicity test of bacterial isolates on healthy tomatoes respectively. Antibacterial effects of neem extract at various concentrations against specific bacterial isolates were identified and recorded. The effectiveness of neem extract is compared to that of amoxicillin, a commonly used antibiotic, serving as a control. The results indicate how different concentrations of neem extract inhibit bacterial growth, providing insights into its potential as a natural antimicrobial agent. The pathogenicity of isolated bacteria was evaluated by inoculating healthy tomato plants with these strains. The effects on tomatoes health, including disease symptoms and overall plant vigour, were observed. Findings from this test help determine which bacterial isolates are harmful to tomatoes, contributing to understanding plant-bacterial interactions and potential agricultural impacts.

Table 4: Susceptibility Test of Neem extract in different concentrations on Bacterial Isolates using Amoxicillin as control

Bacterial isolates	**Zone of inhibition per concentration (mm)**			
	10%	**50%**	**100%**	**Control (Amoxycillin)**
Klebsiella sp	-	-	12	15
Salmonella sp	8	12	14	17
Proteus vulgaris	-	6	11	15
Enterobacter sp	11	13	17	19
Shigella sp	11	16	18	21
Staphylococcus sp	13	14	19	22
Bacillus sp	-	8	13	18
Escherichia coli	10	14	16	19

Key: '-' Shows no zone of inhibition

Table 5: Pathogenicity Test of Bacterial Isolates on Healthy Tomato

Bacterial isolates	**Zone of pathogen spread per day (mm)**						
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Klebsiella sp	-	-	3	5	9	13	15
Salmonella sp	-	-	4	8	12	15	16
Proteus vulgaris	-	2	6	11	13	16	18
Enterobacter sp	-	3	7	13	15	17	20
Shigella sp	-	4	8	10	14	15	19
Staphylococcus sp	-	-	3	7	12	14	18
Bacillus sp	-	2	6	9	12	15	17
Escherichia coli	-	-	2	7	10	13	15

Key: - Shows no zone of spoilage

From the table, they were no or little infection on the first day and there were subsequent spread of infection and spoilage from the second day to maximum infection on the tenth day.

CONCLUSION

This study was carried out to examine the tomato rot causing microorganisms and the effect of neem extract on rot control in the Makurdi metropolis. The organisms identified were *Klebsiella sp, Salmonella sp, Proteus vulgaris, Enterobacter sp, Shigella sp, Staphylococcus sp, Bacillus sp, Escherichia coli, Aspergillus sp, Rhizopus sp, Mucor sp,* and *Fusarium sp.* Neem extract was used to conduct susceptibility tests on the bacterial isolates and it was found most effective on some bacterial isolates at higher concentrations. A pathogenicity test was conducted with readings taken at interval of days and it was noticed that the microbial isolates were actually the cause of tomato rot.

From the result of this study, it is found that tomato rot is caused by some microorganisms such as *Klebsiella sp, Salmonella sp, Proteus vulgaris, Enterobacter sp, Shigella sp, Staphylococcus sp, Bacillus sp, Escherichia coli, Aspergillus sp, Rhizopus sp, Mucor sp,* and *Fusarium sp.* And neem extract at 50% and 100% concentration can be used to control the rot caused by some of these organisms.

Therefore, the extracts are potentially simple environmentally safe alternative for use as botanical fungicides, and could be exploited to manage post-harvest diseases of tropical fruits effectively. Neem extract, derived from the seeds, leaves, and bark of the neem can be used as natural pesticide due to its insecticidal properties. It can also be used in various traditional and modern medicines for their anti-inflammatory, antibacterial, antifungal, and antiviral properties, and as natural food preservatives.

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