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Research Article

Isolation, Identification and Pathogenicity Study of the Microbes Causing Tomato Post-Harvest Spoilage in Maiduguri Metropolis, Maiduguri, Nigeria

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ABSTRACT

Tomato contains high moisture, low pH and nutrients that make it very susceptible to attack by microorganisms causing more than 20% post-harvest loss. This study was carried out to isolate and identify the fungi and bacteria associated with tomato spoilage in Maiduguri metropolis and test the pathogenicity of the isolated microbes in healthy tomato varieties. Twenty-eight rotten tomato samples were collected from six markets within Maiduguri metropolis. The rotten tomatoes were cultured in different culture media suitable for fungal and bacterial growth. Identification of isolated microbes was done based on cultural characteristics, macroscopic and microscopic examination for fungi and microscopic examination, gram staining and biochemical tests for bacteria. The pathogenicity of the isolated microbes was tested in three different tomato varieties (UTC, Roman and Seria). Nineteen microbes comprising five bacteria and fourteen fungi were identified from the infected tomatoes. The fungal analysis showed that *Rhizopus stolonifer*, *Aspergillus flavus* and *Candida tropicalis* were the most frequent fungal microbes associated with the spoilt tomato samples with occurrences of 96.3%, 89.3% and 71.4% respectively. *Cladosporium spp*, *Aspergillus oryzae* and *Thielavia terricola* were the least prevalent with percent frequencies of 3.6%, 7.1% and 7.1% respectively. *Rhizopus stolonifer*, *Aspergillus fumigatus*, *Candida tropicalis* and *Aspergillus flavus* were found to be the most pathogenic in all the three tomato varieties. Developing fungicides that will target specifically *Rhizopus stolonifer*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Candida tropicalis* may greatly reduce post-harvest losses associated with tomatoes as they are found to be the major microbes associated with the tomato spoilage.

Keywords: *Tomato spoilage; Fungi; Bacteria; Pathogenicity*

INTRODUCTION

Tomato is a nutritional vegetable widely accepted worldwide and a good source of micronutrients, vitamins, organic acids, antioxidants and macronutrients (Wamache, 2005). Tomatoes are also an attractive cash crop for small- and large-scale farmers and provide a source of employment to many rural and urban Nigerians. Despite the human needs of tomato, they have serious challenges to their production. These include changes in climate conditions, pests, inadequate rainfall and microorganisms particularly bacteria and fungi that spoil them. One of the limiting factors that influence tomato economic value is its relatively short shelf life caused by pathogenic attacks. It has been estimated that 20-50 % of tomato harvested for human consumption are lost through microbial spoilage while other losses result from damage by dynamic stresses during transit, and through rough handling during loading and unloading (Kader, 1992; Okezie, 1998). Thirupathi *et al*. (2006)

estimated the magnitude of post-harvest losses in fresh tomatoes to be 25-80 %. Post-harvest decay remains a major challenge in tomato production. The magnitude of post-harvest losses varies from one country to another, one season to another and even one day to another (Mujib *et al.*, 2007).

There are numerous micro-organisms that cause post-harvest decay of tomatoes. Among these, fungi and bacteria are the most destructive. Most of the tomatoes are also damaged after harvesting because of poor handling and inadequate preservation methods (Wills *et al*., 1981). Tomatoes low pH, high moisture content and nutrient composition are factors that make them very susceptible to the attacks by fungi and bacteria which in addition to causing rot, may also make them unfit for consumption by producing mycotoxins (Stinson *et al*., 1981; Moss, 2002).

Several kinds of synthetic fungicides have been successfully used to control the postharvest decay of fruits and vegetables. However, there are major concerns. These includes, increasing consumer concern over pesticide residues on foods which are toxic and carcinogenic, predominance of fungicide resistant strains of fungi due to their excessive use and environmental pollution. Therefore, there is a need for new effective means of post-harvest disease control that poses less risk to human health and the environment.

Developing antimicrobials requires identifying the microbes responsible for the disease. The objective of the present study was therefore the isolation and identification of the bacteria and fungi associated with tomato spoilage and testing the pathogenicity of the isolated bacteria and fungi in healthy tomatoes.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade. For bacterial isolations, the media used were Nutrient agar, MacConkey agar, Blood agar and Eosin Methylene Blue agar, while for fungal isolations, the media used were Potato Dextrose agar, Sabouraud Dextrose agar and Malt Extract. All media were from Titan Biotech Ltd, India.

Equipment

The major equipment used were Orbital Incubator (S1500, Bibby Scientific Ltd. UK), Clifton shaking water bath (Nickel-Electro Ltd. UK), Zeiss microscope (Primo star ILED, Germany), Weighing balance (Scout™ pro, Ohaus, USA), Reichert Quebec dark field colony counter (Wagtech Projects, 3362, USA), Eppendorf Bio Spectrometer basic and Eppendorf AG Centrifuge (S430 Germany).

Collection of rotten tomato samples

Rotten tomato samples were collected from six different Markets within Maiduguri metropolis. The Markets are Tashan Bama, Gamboru market, Monday market, Damboa Road, Bulunkutukasuwa and Baga Road. A total of Twenty-eight (28) samples were collected from the six different markets. Five (5) samples each were collected from Baga Road, Bulunkutu, Gamboru and Tashan Bama. Four samples were collected from Damboa Road and Monday Market. The samples were separately packaged, labelled and transported to Crop Protection Laboratory, Faculty of Agriculture, University of Maiduguri.

Isolation and identification of microorganism from rotten tomato samples

To isolate the bacteria, media including Nutrient Agar (NA), MacConkey Agar (MA), Eosin Methylene Blue Agar (EMBA), Blood Agar (BA) and Salmonella Shigella Agar (SSA) were used. Potato Dextrose Agar (PDA), Malt Extracts (ME) and Sabouraud Dextrose Agar (SDA) were used for fungal isolation.

Isolation of bacteria

The pour-plate method was carried out following the method of Harigan and McCane (1990). Using standard microbiological technique (serial dilution), a tenfold dilution of 1.0 g of the sample was carried out in 9.0 ml of sterile water (this was the aliquot). Precisely, l.0 ml of the aliquot (supernatant) was pipetted and mixed in another 9.0 ml of sterile distilled water in a test-tube. The test-tube was shaken vigorously to homogenize. The exponential dilution continued to the tenth factor (10-^{10}). 1.0 ml of the fourth factor and 1.0ml of the seventh factor were aseptically transferred and plated in duplicate sets using sterile molten nutrient agar. The plates were allowed to settle and incubated at 37^0C for 24 hours. Discrete colonies that developed after incubation were counted and enumerated as colony forming unit (cfu/g) after multiplying with the dilution factor (10^{-4} and 10^{-7}).

Subculture of bacterial isolates

Colonies from the 24 hours' incubation (plates) were aseptically picked with a sterile wire loop and transferred onto freshly prepared sterile nutrient agar plate, with a streaking technique such that discrete colonies appeared at the ends of streaked lines after incubation. The subculture plates were incubated at 37°C for 24 hours. Discrete colonies from the subculture plates were aseptically

transferred and streaked on slants and incubated for another 24 hours at 37°C.

Characterization and identification of bacterial isolates

The colonies from the different culture media were examined macroscopically for their characteristic appearance which includes colony size, shape, colour, consistency, pigmentation and texture. Gram staining revealed morphology of the organisms, characteristic group and cells arrangements. Biochemical tests were also carried out for further bacterial identification of the organisms as described by Holt *et al*. (1994). The tests include indole test, methyl red test, Voges-Proskauer (VP) test, citrate test, oxidase test, germ tube test and sugar fermentation test.

Isolation of fungi

The media were prepared according to manufacturer's recommendation and directions. Potato Dextrose Agar (PDA), Malt Extracts (ME) and Sabouraud Dextrose Agar (SDA) were prepared aseptically; 15 to 20 ml of the prepared media was poured into the plates and allowed to set at room temperature (28 ± 2^0C). Rotten tomato sample solutions were prepared and aseptically transferred to sterile petri dishes containing the different media. The petri dishes were incubated at 37^{o}C and were observed daily for 5 days.

Subculture of fungal isolates

The colonies that developed after 5 days were counted and aseptically picked with a sterile inoculation needle and sub-cultured repeatedly on PDA, SDA and ME and incubated for 5 days at 37^{o}C to obtain a pure culture. Discrete colonies were aseptically transferred and stocked on slants and incubated for another 5 days at 37^{o}C. Pure colonies were stored in the refrigerator at 4^{o}C until needed for characterization and identification.

Characterization and identification of fungal isolates

Pure colonies of the fungi isolates were identified based on macro-morphological and micro-morphological characteristics (Tafinta *et al.*, 2013). The morphological identification was done based on culture characteristic such as colony shape, the presence of septa, shape of spores and colony pigmentation. Microscopic analysis was conducted by preparing thin smears of the mycelia on a glass slide with sterile inoculating needle and stained with a drop of lactophenol cotton blue solution as adopted from Oyemeachi *et al. (*2014). Identification was made using photomicrographs of fungi from the work of Robert and Ellen (1988). Size, shape, surface feature of the conidia and hyphae arrangement constituted the microscopic identification.

Pathogenicity test

Pathogenicity test was carried out using the technique described by Okigbo *et al*. (2009). Briefly, three varieties (Roman, Seria and UTC) of healthy tomato samples were purchased from Gambaro market, identified and authenticated at the Medicinal Chemistry Laboratory, Department of Pure and Applied Chemistry, University of Maiduguri, and assigned with voucher specimen number CHM/20/003. The samples were washed under running tap to exclude dirt from the surfaces. These were then surface sterilized in 1% NaCl for three minutes, rinsed in three changes of sterile distilled water and wiped dry using a sterile blotting paper. A sterile 5mm cock-borer was used to punch the tomatoes and the disc was removed. The same size of the cork-borer was used to cut the sections of each of the cultures of the previously isolated fungal pathogens and the discs were used to inoculate the healthy wounded tomatoes. The wound on the inoculated tomatoes were sealed using a sterile adhesive tape. The control was also set in the same manner, with sterile PDA used in place of the fungal cultures. The samples were placed in a sterile desiccator as treatment, replicated three times and stored at room temperature (25°C) in the laboratory. Disease development was checked daily for 5 days. The pathogens were isolated and identified as described earlier.

RESULTS

Outer parts of rotten tomato samples

The different number of isolates obtained from the outer part of the rotten tomato samples from different collection sites within Maiduguri metropolis is presented in table 1. For the outer parts of the rotten tomatoes, Gamboru Market produced the highest number of fungal isolates (10), followed by Monday market, while Baga Road produced the least. A total of fourteen (14) different fungi were identified from samples collected. These are *Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus terreus*, *Aspergillus ustus*, *Candida tropicalis*, *Saccharomyces cerevesiae*, *Thielavia terricola*, *Cladosporium spp*, *Zygosaccharomyces bailii*, *Aspergillus oryzae* and *Penicillium spp*. *Rhizopus stolonifer*, *Aspergillus fumigatus* and *Candida tropicalis* were seen from all the 6 different collection sites. *Penicillium spp* was only seen in Monday market while *Thielavia terricola* was seen only in Damboa Road as presented in Table 1.

Inner parts of the rotten tomato samples

The number of isolates obtained from the inner parts of rotten tomato samples collected within Maiduguri metropolis is shown in table 2. Gamboru and Bulunkutu

markets produced the highest number of fungal isolates (10 each), followed by Tashan Bama and Damboa Road with 9 and 8 of fungal isolates respectively, while Baga Road and Monday Market produced the least number of isolates. A total of fourteen (14) fungi were also identified. These are *Rhizopus stolonifer, Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Aspergillus parasiticus, Aspergillus terreus, Aspergillus ustus, Candida tropicalis, Saccharomyces cerevesiae, Thielavia terricola, Cladosporium spp, Zygosaccharomyces bailii, Aspergillus oryzae* and *Penicillium spp*. *Rhizopus stolonifer, Aspergillus fumigatus, Candida tropicalis and Saccharomyces cerevisiae* were isolated from all the 6 different collection sites as presented in table 2.

Table1. Fungi Isolated from Outer Parts of Rotten Tomatoes from Different Locations

Markets	Fungal Isolates	Number of isolates
Baga Road market	*Rhizopus stolonifer, Aspergillus fumigatus, Aspergillus flavus, Candida tropicalis*	4
Bulunkutu Market	*Rhizopus stolonifer, Aspergillus fumigatus, Aspergillus parasiticus, Zygosaccharomyces bailii, Candida tropicalis, Saccharomyces cerevisiae*	6
Damboa Road	*Rhizopus stolonifer, Aspergillus niger, Aspergillus fumigatus, Zygosaccharomyces bailii, Candida tropicalis, Saccharomyces cerevisiae, Thielavia terricola*	7
Monday Market	*Rhizopus stolonifer, Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Aspergillus oryzae, Candida tropicalis, Saccharomyces cerevisiae, Cladosporium spp, Penicillium spp*	9
Gamboru Market	*Rhizopus stolonifer, Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Aspergillus parasiticus, Aspergillus terreus, Aspergillus ustus, Zygosaccharomyces bailii, Candida tropicalis, Saccharomyces cerevisiae*.	10
Tashan Bama	*Rhizopus stolonifer, Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Aspergillus parasiticus, Candida tropicalis, Saccharomyces cerevisiae*	7

Table 2. Fungi Isolated from Inner Parts of Rotten Tomatoes Collected from Different Locations

Markets	Fungal Isolates	Number of isolates
Baga Road	*Rhizopus stolonifer, Aspergillus fumigatus, Aspergillus flavus, Candida tropicalis, Saccharomyces cerevesiae*	5
Bulunkutu Market	*Rhizopus stolonifer, Aspergillus fumigatus, Aspergillus flavus, Aspergillus parasiticus, Aspergillus terreus, Aspergillus ustus, Zygosaccharomyces bailii, Candida tropicalis, Saccharomyces cerevisiae, Penicillium spp*	10
Damboa Road	*Rhizopus stolonifer, Aspergillus niger, Aspergillus fumigatus, Zygosaccharomyces bailii, Candida tropicalis, Saccharomyces cerevisiae, Thielavia terricola, Penicillium spp*.	8
Monday Market	*Rhizopus stolonifer, Aspergillus fumigatus, Aspergillus flavus, Aspergillus oryzae, Candida tropicalis, Cladosporium spp*.	6
Gamboru Market	*Rhizopus stolonifer, Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Aspergillus parasiticus, Aspergillus terreus, Aspergillus ustus, Zygosaccharomyces bailii, Candida tropicalis, Saccharomyces cerevisiae*.	10
Tashan Bama	*Rhizopus stolonifer, Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Aspergillus parasiticus, Aspergillus oryzae, Zygosaccharomyces bailii, Candida tropicalis, Saccharomyces cerevisiae*.	9

Percentage occurrence of fungal isolates

The percentage occurrence of the fungi from the epicarp parts of the spoilt tomato fruits collected from the six (6) different sites is presented in table 3. *Rhizopus stolonifer* and *Aspergillus flavus* were the most prevalent fungal isolates with 96.3% and 89.3% respectively, while *Cladosporium spp*, *Aspergillus oryzae* and *Thielavia terricola* were the least prevalent with percent frequencies of 3.6%, 7.1% and 7.1% respectively. A total of 14 fungal isolates from the epicarp parts of rotten tomatoes were recorded, namely; *Aspergillus oryzae* 2 (7.1%), *Penicillium spp* 2 (7.1%),

Cladosporium spp 1 (3.6%), *Thielavia terricola* 2 (7.1%), *Aspergillus flavus* 25 (89.3%), *Aspergillus terreus* 4 (14.3%), *Aspergillus parasiticus* 8 (28.6%), *Zygosaccharomyces bailii* 12 (42.9%), *Aspergillus niger* 11 (39.3%), *Aspergillus fumigatus* 19 (67.9%), *Saccharomyces cerevesiae* 5 (17.9%), *Candida tropicalis* 20 (71.4%), and lastly *Rhizopus stolonifer* 27 (96.3%) as listed in (table 3).

The percentage frequency of the fungi from the inner part of the spoilt tomato fruits collected from the 6 (six) different markets is presented in (Table 4). *Rhizopus stolonifer* and *Aspergillus flavus* were also the most prevalent fungal isolates with 92.9% and 85.7% respectively, while *Aspergillus oryzae* and *Cladosporium spp* were the least prevalent with percent frequencies of 7.1% and 3.6% respectively. The frequency of the 14 isolated fungi from inner parts of rotten tomatoes are *Aspergillus oryzae* 2 (7.1%), *Cladosporium spp* 1 (3.6%), *Penicillium spp* 3 (10.7%), *Thielavia terricola* 2 (7.1%), *Aspergillus terreus* 4 (14.3%), *Aspergillus ustus* 5 (17.9%), *Aspergillus flavus* 24 (85.7%), *Aspergillus parasiticus* 11 (39.3%), *Zygosaccharomyces bailii* 14 (50.0%), *Aspergillusfumigatus*16 (57.1%), *Saccharomyces cerevesiae* 7 (25.0%), *Candida tropicalis* 20 (71.4%) and lastly *Rhizopus stolonifer* 26 (92.9%) , as listed in increasing order of occurrence as presented in Table 4.

Table 3. Percentage Frequency of Fungi Isolated from the Outer Parts of Rotten Tomato Samples

Fungal pathogens	B.R.	BU.M.	D.R.	M.M.	G.M.	T.B.	Total	Percentage Frequency (%)
Aspergillus fumigatus	3	2	3	4	3	4	19	67.9
Aspergillus niger	-	-	2	3	4	2	11	39.3
Aspergillus flavus	5	3	5	4	5	3	25	89.3
Saccharomyces cerevesiae	1	-	-	2	1	1	5	17.9
Aspergillus parasiticus	-	4	-	-	1	3	8	28.6
Aspergillus terreus	-	-	-	-	4	-	4	14.3
Aspergillus ustus	-	-	-	-	1	-	1	3.6
Aspergillus oryzae	-	-	-	2	-	-	2	7.1
Zygosaccharomyces bailii	-	4	5	-	3	-	12	42.9
Rhizopus stolonifera	5	5	4	3	5	5	27	96.4
Candida tropicalis	-	5	3	5	4	3	20	71.4
Thielavia terricola	-	-	2	-	-	-	2	7.1
Cladosporium spp	-	-	-	1	-	-	1	3.6
Penicillium spp	-	-	-	2	-	-	2	7.1

Key: B.R-Baga Road, BU.M.-Bulunkutu Market, D.R.- Damboa Road, M.M.- Monday Market, G.M. -Gamboru Market and T.B. -Tashan Bama

Table 4. Percentage Frequency of Fungi Isolated from the Inner Parts of Rotten Tomato Samples

Fungal pathogens	B.R.	BU.M.	D.R.	M.M.	G.M.	T.B.	Total	Percentage Frequency (%)
Aspergillus fumigatus	-	4	3	3	3	3	16	57.1
Aspergillus niger	3	-	2	-	2	1	8	28.6
Aspergillus flavus	5	3	5	4	4	3	24	85.7
Saccharomyces cerevisiae	1	1	-	1	2	2	7	25.0
Aspergillus parasiticus	-	5	-	-	1	5	11	39.3
Aspergillus terreus	-	2	-	-	2	-	4	14.3
Aspergillus ustus	-	3	-	-	2	-	5	17.9
Aspergillus oryzae	-	-	-	1	-	1	2	7.1
Zygosaccharomyces bailii	-	3	5	-	2	4	14	50.0
Rhizopus stolonifer	5	5	4	3	4	5	26	92.9
Candida tropicalis	5	5	3	-	3	4	20	71.4
Thielavia terricola	-	-	2	-	-	-	2	7.1
Cladosporium spp	-	-	-	1	-	-	1	3.6
Penicillium spp	-	2	1	-	-	-	3	10.7

Key: B.R.-Baga Road, BU.M.- Bulunkutu Market, D.R. Damboa Road, M.M. Monday Market, G.M. Gamboru Market and T.B. Tashan Bama

Characterization and identification of bacteria isolates

The bacteria present in the rotten tomato samples were identified based on their cultural and morphological characteristics, gram reaction, motility, sugar fermentation and lastly biochemical reactions as shown in Table 5.

The total bacterial isolates from the six (6) different locations in Maiduguri metropolis are as presented below. The fourth serial dilution had numerous isolates at various locations (table 6), the seventh serial dilution showed that tomato fruits samples from Monday market have the highest bacterial count of 272×10^{-7} (Table 7).

Table 8 showed the % distribution of bacterial isolates from the rotten tomatoes found in the six (6) different markets. Eleven (11) of the 39 organisms isolated were *Escherichia coli,* nine (9) *Staphylococcus aureus,* eight (8) *Pseudomonas aeruginosa*, *Proteus mirabilis* were six (6) while five (5) were isolates of *Klebsiella aeruginosa* strain with percentages (%) of 28.21, 23.08, 20.51, 15.38 and 12.82 respectively.

Table 5. Bacterial Characterization and Identification

Characteristics	Isolate Descriptions				
	Escherichia coli	*Klebsiella aeruginosa*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Proteus mirabilis*
Shape	Small	Large	Large	Medium	Large
Margin	Entire	Entire	Entire	Smooth	Entire
Colour	Pink	White	Creamy	Yellow	White
Cell type	Rod	Rod	Rod	Cocci	Rod
Cell arrangement	Single	Single	Single	Cluster	Single
Gram Reaction	–	–	–	+	–
Motility test	–	+	+	–	+
Glucose	AG	AG	A	A	A
Lactose	+	+	–	+	–
Mannose	+	+			–
Sucrose		+			
Coagulase	–	–	–	–	+
Catalase	+	+	+	+	+
Oxidase	–	–	+	+	–
Indole	–	–	–	–	–
Urease	–	+ slow			–
Citrate test	–	+			+
Lysine decarboxylase	+	+			+
Beta galactosidase	+	+			–

Key: + = Positive - = Negative A = acid production only. AG = acid and gas production

Table 6. Total Bacterial Count Found in Different Markets Using Fourth Serial Dilution

		Nutrient Agar 10^{-4}				
Locations		*Escherichia coli*	*Klebsiella aeruginosa*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Proteus mirabilis*
(i)	Tashan Bama	Numerous	Nil	Numerous	219×10^{-4}	Nil
(ii)	Baga Road	49.0×10^{-4}	Nil	32.0×10^{-4}	14.0×10^{-4}	Nil
(iii)	Bulunkutu Market	380×10^{-4}	Nil	219×10^{-4}	116×10^{-4}	Nil
(iv)	Damboa Road	Numerous	216×10^{-4}	Numerous	Numerous	310×10^{-4}
(v)	Monday Market	Numerous	201×10^{-4}	Numerous	Numerous	312×10^{-4}
(vi)	Gamboru Market	332×10^{-4}	Nil	214×10^{-4}	111×10^{-4}	Nil

Table 7. Total Bacterial Count Found in Different Markets Using Seventh Serial Dilution

Nutrient Agar 10^{-7}						
Locations		*Escherichia coli*	*Klebsiella aeruginosa*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Proteus mirabilis*
(i)	Tashan Bama	136×10^{-7}	Nil	101×10^{-7}	12×10^{-7}	2.1×10^{-7}
(ii)	Baga Road	37.3×10^{-7}	Nil	18.7×10^{-7}	13.6×10^{-7}	Nil
(iii)	Bulumkutu Market	9.3×10^{-7}	Nil	Nil	16.0×10^{-7}	Nil
(iv)	Damboa Road	6.1×10^{-7}	3.2×10^{-7}	9.4×10^{-7}	4.9×10^{-7}	Nil
(v)	Monday Market	272×10^{-7}	8.4×10^{-7}	141×10^{-7}	96×10^{-7}	5.2×10^{-7}
(vi)	Gamboru Market	12.0×10^{-7}	Nil	Nil	Nil	Nil

Table 8. Percentage Distribution of Bacterial Isolates from Rotten Tomatoes

S/no	Organisms	No. of organisms isolated	%
1.	*Escherichia coli*	11	28.21%
2.	*Pseudomonas aeruginosa*	8	20.51%
3.	*Proteus mirabilis*	6	15.38%
4.	*Klebsiella aeruginosa*	5	12.82%
5.	*Staphylococcus aureus*	9	23.08%
Total		**39**	**100%**

Pathogenicity test on the varieties of fresh tomato samples

Three (3) varieties of fresh tomato fruits (Seria, UTC and Roman) were used for the pathogenicity test; inoculated singly with the identified microbes for 96 hours. Decay weight loss was used as parameters to determine the pathogenic microbes responsible for the tomato spoilage.

Seria

The result of the pathogenicity test showed that all the fourteen (14) fungi inoculated into the healthy tomato fruits caused significant weight loss on the tomato fruits but in varying degrees. *Rhizopus stolonifer, Aspergillus fumigatus, Aspergillus flavus and Candida tropicalis* were the most pathogenic, causing percentage weight loss of 60.50%, 36.96%, 32.68% and 28.05% respectively after 96 hours of incubation at room temperature, and the least pathogenic fungi were *Zygosaccharomyces bailli, Aspergillus parasitucus, Aspergillus oryzae and Thielavia terricola*, causing percentage weight loss of 7.73%, 12.01%, 12.32% and 12.45% respectively after 96 hours of incubation at same room temperature. However, the control for Seria showed only 2.39% weight loss after the 96 hours of incubation as shown in table 10.

UTC

All the fourteen (14) organisms caused spoilage of healthy UTC tomato fruits but at different degrees, *Rhizopus stolonifer, Candida tropicalis, Aspergillus fumigatus and Aspergillus flavus* were the most pathogenic, causing percentage weight loss of 61.47%, 40.38%, 41.01% and 38.40% respectively after 96 hours of incubation at room temperature, while the least pathogenic organisms were *Aspergillus parasitucus ,Zygosaccharomyces bailli, Thielavia terricola and Aspergillus oryzae*, causing percentage weight loss of 12.96%, 14.56%, 15.39% and 15.60% respectively after 96 hours of incubation at the same room temperature. The control for UTC showed only 3.50% weight loss after 96 hours of room temperature incubation (table 11).

Roman

Rhizopus stolonifer, Candida Tropicalis, Aspergillus fumigatus and *Aspergillus Flavus* were found to be the most pathogenic in Roman Healthy tomato fruits with percentage weight reductions of 79.75%, 46.15%, 41.11% and 38.09% respectively after incubation at room temperature for 96 hours with *Aspergillus parasitucus, Zygosaccharomyces bailli* and *Thielavia terricola* having the least percentage weight reduction of 21.83%, 23.01% and 23.73% respectively after room temperature incubation of 96 hours. However, all the fourteen (14) fungi isolated were responsible for causing tomato spoilage when tested on healthy Roman tomato sample. The control showed 4.70% percentage weight reduction when tested with fresh Roman tomato sample after 96 hours of room temperature incubation (12).

Table 10. Percentage Weight Loss Caused by Fungi on Healthy Seria Tomato

	Microorganisms	0 hours	24 hours	48 hours	72 hours	96 hours
	Control	37.7±10.86	37.43±10.76	37.36±10.75	37.00±10.37	36.80±10.37 (2.39%)
1.	*Aspergillus fumigatus*	68.5±12.2	67.18±12.47	64.70±13.53	59.00±20.43	43.18±13.04 (36.96%)
2.	*Aspergillus flavus*	72.4±11.0	71.62±10.67	69.38±11.47	56.94±16.87	48.74±18.32 (32.68%)
3.	*Rhizopus stolonifer*	57.98±12.88	56.16±12.39	48.98±12.66	39.06±11.92	22.90±12.17 (60.50%)
4.	*Aspergillus ustus*	72.94±6.92	69.70±7.22	65.84±12.84	61.98±31.44	58.56±11.60 (19.71%)
5.	*Zygosaccharomyces bailii*	73.76±6.06	72.98±5.96	72.22±6.06	69.92±8.98	68.06±10.84 (7.73%)
6.	*Saccharomyces cerevisiae*	62.48±9.98	61.40±8.72	59.70±8.80	55.40±7.48	52.33±7.07 (16.25%)
7.	*Aspergillus parasiticus*	66.90±16.50	66.42±16.45	64.04±16.32	60.62±16.34	58.86±16.81 (12.01%)
8.	*Thielavia terricola*	61.36±5.32	60.34±4.79	59.56±6.95	55.56±6.95	53.72±8.78 (12.45%)
9.	*Penicillium spp*	47.38±11.09	47.22±10.48	44.54±9.76	40.66±9.86	38.99±9.88 (17.71%)
10.	*Candida tropicalis*	70.08±16.14	69.98±12.28	66.37±12.04	62.58±11.44	50.42±11.09 (28.05%)
11.	*Aspergillus niger*	55.16±11.76	47.38±11.09	46.12±11.07	44.84±11.35	40.84±11.25 (25.96%)
12.	*Aspergillus oryzae*	58.92±9.57	57.94±9.34	57.08±9.18	54.62±9.62	51.66±10.40 (12.32%)
13.	*Cladosporium spp.*	65.02±12.41	64.42±12.34	63.76±12.47	60.64±12.41	54.48±16.10 (16.21%)

Table 11. Percentage Weight Loss Caused by Fungi on Healthy UTC Tomato

	Microorganisms (Fungi)	0 hours	24 hours	48 hours	72 hours	96 hours
	Control	37.43±7.01	37.18±6.95	37.00±6.81	36.90±6.81	36.12±6.80 (3.50%)
1.	*Aspergillus fumigatus*	71.0±18.4	68.16±18.36	61.76±18.44	52.78±20.96	41.88±23.71 (41.01%)
2.	*Aspergillus flavus*	62.2±5.48	59.56±7.04	51.12±8.48	45.58±9.91	38.32±10.44 (38.40%)
3.	*Rhizopus stolonifer*	58.03±10.45	55.58±10.82	44.46±12.18	33.88±15.92	22.36±12.83 (61.47%)
4.	*Aspergillus ustus*	54.84±11.47	53.9±11.35	52.08±11.82	44.26±11.78	39.84±12.70 (27.35%)
5.	*Aspergillus parasiticus*	41.08±8.68	40.66±8.76	39.02±10.00	37.24±13.72	35.1±15.08 (14.56%)
6.	*Zygosaccharomyces bailii*	63.64±6.80	62.26±6.32	56.08±8.34	50.24±9.06	45.32±6.38 (28.79%)
7.	*Saccharomyces cerevisiae*	44.12±7.97	43.20±7.86	42.64±7.88	40.48±8.97	38.40±8.53 (12.96%)
8.	*Thielavia terricola*	40.80±6.70	40.14±6.60	39.42±6.17	36.08±5.11	34.52±5.63 (15.39%)
9.	*Penicillium spp*	47.38±11.09	44.20±11.02	40.42±9.80	36.14±11.18	33.92±9.80 (28.41%)
10.	*Candida Tropicalis*	77.26±14.42	76.06±12.14	69.42±12.04	62.09±12.02	46.06±11.80 (40.38%)
11.	*Aspergillus niger*	55.56±10.82	54.64±10.26	51.84±12.0	40.48±10.10	38.80±10.28 (30.17%)
12.	*Aspergillus Oryzae*	44.74±7.58	43.8±7.63	42.86±8.40	38.38±11.57	37.76±11.39 (15.60%)
13.	*Cladosporium spp*	45.16±4.60	44.44±4.83	43.76±5.10	42.08±6.14	35.94±8.21 (20.42%)

Table 12. Percentage Weight Loss Caused by Fungi on Healthy Roman Tomato

	Microorganisms (Fungi)	0 hours	24 hours	48 hours	72 hours	96 hours
	Control	67.87±7.27	66.8±3.91	66.2±3.42	65.6±3.91	64.68±3.96 (4.70%)
1.	*Aspergillus fumigatus*	116.7±29.8	115.18±29.8	114.04±29.84	99.72±24.30	68.72±22.72 (41.11%)
2.	*Aspergillus flavus*	97.6±14.77	96.68±14.67	93.96±13.96	80.26±15.82	60.42±16.29 (38.09%)
3.	*Rhizopus stolonifer*	104.08±41.37	101.2±40.99	66.46±21.49	40.18±17.54	21.26±15.19 (79.75%)
4.	*Aspergillus ustus*	114.26±41.46	112.3±39.04	100.44±30.05	88.18±35.66	81.12±39.66 (29.00%)
5.	*Aspergillus parasiticus*	79.64±22.50	78.36±22.53	71.18±23.32	66.14±22.61	60.74±25.32 (23.73%)
6.	*Zygosaccharomyces bailii*	77.80±8.24	76.24±8.62	69.74±9.42	62.71±12.28	56.32±8.20 (27.61%)
7.	*Saccharomyces cerevisiae*	92.44±22.96	91.24±24.46	86.14±24.46	74.26±27.84	72.26±27.61 (21.83%)
8.	*Thielavia terricola*	73.00±11.93	72.26±12.08	70.46±11.94	63.3±13.32	56.20±16.85 (23.01%)
9.	*Penicillium* spp.	61.12±14.27	59.59±10.68	51.06±10.08	46.31±12.76	42.32±10.22 (30.76%)
10.	*Candida tropicalis*	70.12±12.84	68.32±10.90	58.80±11.8	48.38±10.38	37.06±11.02 (46.15%)
11.	*Aspergillus niger*	61.12±14.27	56.74±10.60	51.8±9.10	49.76±5.85	40.34±6.55 (34.00%)
12.	*Aspergillus oryzae*	52.74±10.17	51.34±9.56	49.5±9.54	39.88±8.56	38.22±8.71 (27.53%)
13.	*Cladosporium spp*	99.64±19.26	98.14±19.29	96.14±19.27	74.52±8.50	64.68±16.92 (35.09%)

DISCUSSION

The major cause of spoilage of vegetables and other perishable goods are microorganisms, which can either be bacteria, fungi or both. Microscopy, macroscopy, gram staining and biochemical tests reveals the presence of several fungi and bacteria in our test samples. A total of fourteen (14) fungi were isolated from the decayed tomato samples. *Rhizopus stolonifer*, *Aspergillus flavus*, *Candida tropicalis* and *Aspergillus fumigatus* had the highest percentage of occurrence in both inner and outer parts of the rotten tomatoes across the six different markets, while *Aspergillus ustus*, *Aspergillus oryzae* and *Penicillium spp* had the lowest percentage occurrence. This is however different from what was reported by many researchers (Akinmusire, 2011; Bello *et al*., 2016, Ibrahim *et al*., 2011) who reported *Aspergillus niger* as the most frequent in occurrence in their studies. However, results of this study agreed with the work of Samuel and Orji (2015), who also found *Rhizopus stolonifer*, *Aspergillus flavus*, *Aspergillus fumigatus* as fungi with the highest occurrence in the rotten tomato samples.

The present study also isolated five (5) bacteria from the rotten tomato samples collected from the six markets. *Escherichia coli* was the most occurring with 28,21% followed by *Staphylococcus aureus* with 23.08%, while *Klebsiella aerogenes* and *Proteus mirabilis* were the least occurring recording 15.38% and 12.82% respectively. Isolation of bacteria in rotten tomato samples have previously being reported (Chukwu *et al*., 2008; Oyemaechi *et al*., 2014), specifically, the presence of *Escherichia coli*, *Protease klebsiella* and *Staphylococcus aureus* bacteria in rotten tomato samples collected from markets.

Microbial contamination of fresh tomato fruits can be due to high water content in the tomatoes, storage and environmental conditions, handling condition, fungal and bacteria load by tomato traders, quality of the tomato sample and by exposing the tomato fruits to faecal contaminated water or organic manure (Gosh 2009; Oyemaechi *et al*., 2014). Bruises and damages inflicted on fruits during harvesting, cross contamination with other products, contact with infection during transportation can also promote the proliferation of microorganisms which result in fruits decay (Al-Hindi *et al*., 2011; Mathew, 2011).

The isolated microbes were aseptically inoculated into three (3) different varieties of healthy tomato samples (Seria, UTC and Roman) in a pathogenicity study. This is a usual practice to determine if the microbes are responsible for the decay in fruits (Samuel and Orji 2005; Omolaran *et al*., 2016). All the

fungal isolates caused varying degrees of decay in the three (3) different tomato varieties. Among all the fungi inoculated *Rhizopus stolonifer*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Candida tropicalis* were the most pathogenic showing the highest deterioration of all the treated three varieties of tomatoes within 96 hours of inoculation, causing a decay weight loses ranging between 32.68% and 60.50% in Seria, 38.40% and 61.47% UTC, and 46.09% and 79.75% in Roman. *Rhizopus stolonifer* and *Aspergillus flavus* have also been reported to be responsible for the decay of orange fruit (Tafinta *et al*., 2013), avocado and pears (Chukwu, 2005), and tomatoes (Lydia 2015). Among the three varieties of tomato fruits studied for the pathogenicity, Roman appeared to be the worst affected by the pathogenic fungi followed by UTC and lastly Seria.

In order of susceptibility the Roman tomato varieties appeared to be the most affected by *Rhizopus stolonifer*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Candida tropicalis*. The variety when inoculated with *Rhizopus stolonifer*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Candida tropicalis* was disintegrated within 48 hours of inoculation, while Seria and UTC tomato varieties disintegrated completely after 96 hours. Pathogenicity test with the isolated bacteria (data not shown) showed that none of the identified bacteria initiated the decay process. Many studies have clearly indicated that in fruits decay, particularly tomato, bacteria are only opportunistic, thus fungi remain the most pathogenic in causing tomato spoilage (Lemma, 2002; Omolaran *et al*., 2016).

Conclusion: Fourteen fungi and five bacteria were isolated from rotten tomato sample collected from six different markets within Maiduguri metropolis. *Rhizopus stolonifer*, *Aspergillus fumigatus* and *Candida tropicalis* were found to be the most pathogenic when tested on three different varieties of fresh tomatoes. Developing fungicides that will target specifically the *Rhizopus stolonifer*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Candida tropicalis* may greatly reduce post-harvest losses associated with tomatoes in Nigeria as they are found to be the major microbes associated with tomato spoilage.

AUTHORS' CONTRIBUTIONS

The research is a collaborative work between all the authors. The conception, design and substantial revision of the work were the contributions of AS, MAM and AG. JBN contributed with the experimental work and AID performed the experimental work, collected and analysed the data and produced the draft of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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