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Identification and Isolation of Microorganisms Responsible for Spoilage of Tomatoes (Lycopersicon Esculentum) Fruit and Phytochemical Analysis of the Fruit in Maiduguri

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Abstract: *This study investigates the bacteria and fungi associated with the deterioration of fresh tomatoes, (Lycopersicum esculentum). A total of sixteen (16) tomato samples were obtained from four (4) different retail outlets in the Ungwan Rimi area, Kaduna. The Proximate composition of the selected tomato samples was determined using a standard protocol. The pour plate method was used to isolate bacteria and fungi from the tomato samples. The antibiogram of selected antibiotics and antifungal drugs against the bacteria and fungi isolates was determined using the disk diffusion technique. The results of proximate composition showed that sample A had a moisture content of 94.10 %, 0.74% of ash, 0.97 % of crude protein, 0.66 % of crude fat, 1.10 % crude fiber, and 2.43 % of carbohydrate while sample B showed similar percentage composition of 93.89 % of moisture content, 0.86 % of ash, 1.0 % of crude protein, 0.69 % of crude fat, 1.34% of crude fibre and 2.22 % of carbohydrate. Bacteria isolated and identified were Staphylococcus aureus, Escherichia coli, and Salmonella sp. The most prevalent bacteria isolate was Staphylococcus aureus with 50% while Salmonella sp and Escherichia coli had 25% each. The fungal isolates were Penicillium sp, Aspergillus niger, and Aspergillus flavus. Aspergillus Niger was the most prevalent with 53.8% , Penicillium sp had 30.8%, while Aspergillus flavus had the least prevalence at 15.4%. the antibacterial susceptibility of Salmonella sp showed that it was resistant to Gentamycin, moderately sensitivity to Streptomycin and Septrin, and sensitive to Chloramphenicol, Spafloxacin, Ciprofloxacin, Amoxycillin, Pefloxacin, Tarvid and Augmentine at different concentrations respectively. At different concentrations of the antibiotics, Escherichia coli was resistant to Gentamycin and Streptomycin and sensitive to Chloramphenicol, Spafloxacin, Ciprofloxacin, Amoxycillin, Pefloxacin, Tarvid, and Augmentine. Staphylococcus aureus was resistant to Rocephin, Zinacef and Streptomycin, moderately sensitive to Ampiclox, and Amoxicillin, and sensitive to Septrin, ciprofloxacin, Gentamycin, Pefloxacin, and erythromycin respectively. The antifungal susceptibility showed variations at different concentrations in its effectiveness against the test fungi isolates. The presence of these fungi, as well as the bacteria isolates, which are capable of causing food poisoning, raises concern over public health risks that may be associated with the consumption of spoilt fresh tomatoes. Proper handling, transportation, and thorough washing with clean or chlorinated water will reduce the risk of tomato spoilage associated with bacteria and fungi species.*

Keywords: *Bacteria, fungi, biodeterioration, tomatoes, antibiotics, antifungal agents.*

1.0 INTRODUCTION

Food spoilage refers to various changes to food in which the food becomes less palatable or even toxic to consumers these changes may be accompanied by alterations in smell taste appearance or texture (Akinmusire, 2011). According to (Obunkwu *et al*, 2018). Tomato is a widely consumed fruit eaten in both raw and processed forms. It has the botanical name *Lycopersicum esculentum* and belongs to the plant family solanaceae. It is rich in vitamins such as vitamin B, C, and E. Carbohydrates such as fructose and glucose; and trace elements like iron, copper, zinc, and dietary fiber, which are all vital nutrients in man. As reported in (Onuorah and Orji, 2015). The high water content of tomatoes makes it more susceptible to spoilage by the action of microorganisms Tomato is very important mainly for its dietary needs, it can be consumed in diverse ways; It can be cooked as vegetable, as an ingredient in many dishes and sauces, in the making of stew, fruit juices and can be eaten raw in salads. According to Onuorah and Orji, 2015, Tomatoes spoilage can be referred to as those adverse changes in the quality of tomatoes caused by the action of predominantly biological and physical factors. These changes may include changes in taste, smell, appearance or texture of the fruits. (. Estimates have shown that about one third of the produce is lost before reaching the consumer (Mbajiuka and Emmanuel, 2014). This loss has been attributed to a number of factors which include; physical (mechanical breakage, bruises), and also damages caused by microbes such as fungi and bacteria (Onuorah and Orji, 2015). Tomato spoilage usually occurs during storage, transportation and also while waiting to be processed. The microbial deterioration on tomato fruits causes reduction in its market values and nutritional qualities. The tomato fruits are rendered unsafe for consumption due to contaminations with mycotoxins that produces aflatoxins in human, following inhalation or ingestion and thus resulting to food poisoning (Bello *et al.*, 2016).Some studies have been carried out to identify both bacteria and fungi associated with the spoilage of tomato Wogu and Ofuase (2014) isolated *Bacillus subtilis, Klebsiella aerogenes, Pseudomonas aeruginosa, Salmonella typhi, Proteus mirabilis,* and *Staphylococcus aureus* from spoilt tomatoes in Benin City. A similar study also revealed high levels of *Staphylococcus* sp, *Bacillus* sp, and *Escherichia coli* in Lagos State, Nigeria (Ogundipe *et al.,* 2012). Akinmusire (2011) reported that *Rhizopus* sp were associated with the spoilage of tomatoes. Akinmusire (2011) reported that *Rhizopus* sp were associated with the spoilage of tomatoes. Wogu and Ofuase (2014) isolated *Aspergillius* sp, *Penicillum* sp, *Fusarium* sp and *Saccharomyces* sp from spoilt tomato fruits. Mbajiuka and Emmanuel (2014) also isolated *Aspergillius* spp, *Penicillum* sp and *Saccharomyces cerevisiae* from spoilt tomatoes. Ghosh (2009) reported that fungi were responsible for more tomatoes spoilage than bacteria. Wogu and Ofuase (2014) isolated *Aspergillius* sp, *Penicillum* sp, *Fusarium* sp and *Saccharomyces* sp from spoilt tomato fruits. Mbajiuka and Emmanuel (2014) also isolated *Aspergillius* spp, *Penicillum* sp and *Saccharomyces cerevisiae* from spoilt tomatoes. Ghosh (2009) reported that fungi were responsible for more tomatoes spoilage than bacteria. conveyed and marketed in wooden boxes and baskets. These baskets are often used until they become infected with

bacteria and or fungal spores. Pathogenic inoculums on these wooden boxes and baskets can initiate spoilage upon contact with healthy tomato fruits resulting in losses, which translate to a waste of the farmers' resources, a reduction in their income and ultimately their welfare. These pathogenic inoculums could also originate from infected farm tools, or during transportation. Proper isolation and characterization of these organisms in tomatoes will greatly reduce the spoilage of this perishable fruit and as such producers and consumers will be able to protect their vegetables (tomato) and also identify spoiled tomatoes that have been attacked by fungi and bacteria. Tomato is one of the most popular and widely grown plants in the world as well as in Africa. It is the second most important vegetable worldwide, in terms of the amount of vitamins and minerals it contributes to the diet (Osemwegie, *et al.,* 2010). This research aimed at identifying the various bacteria and fungi associated with the spoilage of fresh tomatoes sold in Ungwan Rimi, Kaduna.

1.2 Characterization and Identification of Bacterial Isolates from Biodeteriorated Tomatoes

The characterization of bacteria isolates from tomatoes were based on Grams staining and selected biochemical tests which include catalase test, indole production test, Voges-Proskauer (VP), Methyl red (MR) test, citrate, coagulase test, urease, Triple Sugar Iron Agar (TSI) test as described by Cheesbrough (2007).

1.3 Characterization and Identification of Fungal Isolates

1.4 Macroscopic Examination

Identification and classification of the fungal isolates were based on macroscopic and microscopic examination. The macroscopic examination was carried out by observing the colonial characteristics especially the colour formation of both the front and reverse sides of the plates (Obunkwu *et al*., 2018).

1.5 Microscopic Examination

Lactophenol cotton blue solution was used. A drop of the solution was placed on a clean grease-free slide. A fragment of the fungi isolate was emulsified in the solution after which the slide was covered with a cover slip, avoiding bubbles. The slide was thereafter viewed under the microscope (Obunkwu *et al*., 2018).

1.6 Standardization of Inoculum

Sets of 24 h culture of bacteria isolates was used to prepared the inocula. The bacterial was suspended in sterile normal saline and the turbidity was adjusted to 0.5 Mc Farland's standard which corresponds to 1.0 × 108 CFU/mL (Clinical Laboratory Standards Institute (CLSI), 2002)

2.0 MATERIALS AND METHODS

2.1 Experimental Site

The field experiment was conducted at the Teaching and Research Farm of the Ramat Polytechnic, Maiduguri. The site lies between latitude $11^0$5 N and longitude $13^0$09E (Kyari, et al 2014). The area is about 335m above sea level and lies within the lake Chad Basin formation,

which is an area formed as a result of down –warping during the Pleistocene period (Waziri, 2007). The average annual rainfall is around 640mm and the temperature is high ranging between 20-40^{o}C (Dalorima, 2002). The area is highly susceptible to drought with relative humidity of 13% and 65% in dry and rainy season respectively (Bashir 2014).

2.2 Collection of Samples

Four (4) samples each of the vegetable *(Lycopersicon esculentum)* with spoilage signs was purchased from four different retail stands (16 samples in total) within Maiduguri area in Borno, Nigeria. The tomatoes were placed and transported separately in sterile polythene bags to the Ramat Polytechnic Microbiology Laboratory.

2.3 Proximate Composition and Preparation of Tomato Fruit Samples

The proximate composition of the tomato fruit was analyzed according to the method described by Adebooye *et al.* (2006); Gharezi *et al.* (2012); Abdullahi *et al*. (2016) and Mohammed *et al*. (2017). The proximate parameters include percentage moisture content, ash, protein, fat, fibre and carbohydrate. However, the media preparation was accomplished using Nutrient Agar (NA) and Potato Dextrose Agar (PDA) MacConkey agar. The media were all prepared according to manufacturer's instruction. Similarly, isolation of the bio-deteriorated samples was accomplished

Using standard Microbiological technique (serial dilution), the aliquot was made by adding 25g of the tomato sample into 225ml of sterile water. A serial dilution of up to (10-4) of the aliquot was carried out using sterile test tubes. Precisely, lml of the aliquot was pipetted and mixed in another 9ml of sterile distilled water in a test-tube.

2.4 Antimicrobial Susceptibility test of Bacterial Isolates from Rotten Tomatoes

Disk diffusion method was used to determine the susceptibility of the isolates to selected antibiotics. Mueller Hinton agar was used. A sterile swab was dipped into the bacteria suspension (standardized inoculum) and it was pressed over the tube to reduce excesses and it was streaked all over the Mueller Hinton agar. Antibiotic disk was then placed on the surface of the MHA plate it was inverted and incubated at 370C for 24 h. The presence of zone of inhibition around the antibiotic disk indicated microbial inhibition and was measured to the nearest millimeter using a well calibrated meter ruler.

2.5 Preparation of Stock Solution for **Antimicrobial Susceptibility test of Fungal Isolates**

Fluconazole and ketoconazole were used. Exactly 200mg (0.2g) of these commercially available drugs was dispensed into 10ml of distilled water to make a stock solution of 200mg/mL.

2.6 Antimicrobial Susceptibility test of Fungal Isolates from Rotten Tomatoes

The agar well diffusion method was used to determine the susceptibility of the isolates to fluconazole and ketoconazole. Sabouraud Dextrose Agar was used as described by Oghenejobo *et al.* (2013). The susceptibility tests were carried out using the concentration of 200mg/ml for the fungal isolates. One (1) mL of the the standardized inoculum of each test organism was used to flood plates and excess aseptically drained, the plates were allowed to dry at 370C temperature in a sterilized incubator. Adopting the agar well diffusion method, a sterile cork

borer (6mm) was used to bore holes in the agar plates. The bottoms of the wells (holes) was then sealed with the appropriate molten agar. Using a micropipette, a drop of each of the diluted drug concentrations prepared was introduced into wells bored on the surface of Sabouraud Dextrose Agar seeded with prepared fungi. The plates were incubated for 4 days at 280C. The presence of zone of inhibition around the wells indicated antimicrobial inhibition by the drug concentration used and was measured to the nearest millimeter using a venier caliper.

3.0 Result and Discussions

2.1 RESULTS

Table 1 shows the proximate composition of the tomato samples. The proximate composition showed that sample A had moisture content of 94.10 %, 0.74% of ash, 0.97 % of crude protein, 0.66 % of crude fat, 1.10 % crude fiber and 2.43 % of carbohydrate while sample B had 93.89 % of moisture content, 0.86 % of ash, 1.0 % of crude protein, 0.69 % of crude fat, 1.34% of crude fibre and 2.22 % of carbohydrate.

Table 1 Average Proximate Composition of Selected Tomato Samples.

Parameters (%)	Sample: A	B	Tc-	Pv-
Moisture content	94.10±0.4020	93.89±0.431	0.296	2.446
Ash	0.74±0.009	0.86±0.047	9.593	3.182
Crude Protein	0.97±0.021	1.0±0.081	23.334	2.446
Crude Fat	0.66±0.011	0.69±0.012	6.148	2.446
Crude Fibre	1.10±0.008	1.34±0.027	31.112	2.776
Carbohydrate	2.43±0.021	2.22±0.026	0.645	2.446

Table 2 Shows the bacterial load of spoilt fresh tomatoes. The total bacterial count ranged from 8.0X105 to 1.7 X106 (CFU/g) and total coliform count ranged from 8.0X104 to 1.16 X105.Table 3 shows the total viable fungi count of spoilt fresh tomatoes. This is expressed in CFU/g. It ranged from 1.5X105 to 3.0X105.

Table 2: Total Viable Bacteria Count of Spoilt Fresh Tomatoes

Sample	Total Bacterial Count (CFU/g)	Total Coliform CFU/g
A1	1.8710^5	9.2×10^4
	1.610^6	7.0×10^5
A2	1.310^5	8.0×10^4
	1.010^5	6.6×10^5
B1	1.510^6	1.0×10^5
	8.010^5	7.7×10^5
B2	1.9210^5	8.7×10^4
	1.610^6	6.5×10^5
C1	1.2510^6	9.2×10^4
	9.010^5	7.6×10^5
C2	2.010^5	1.16×10^5
	1.710^6	8.6×10^5
D1	$1.15X10^5$	8.7×10^4
	$9.6X10^5$	6.5×10^5
D2	$1.4X10^5$	1.1×10^5
	$1.1X10^5$	8.2×10^5

Table 7 shows the percentage occurrence of fungi isolates in spoilt fresh tomato. Aspergillus *niger* was the most prevalent with 53.8% , Penicillium sp had 30.8%, while *Aspergillus flavus* had the least prevalence of 15.4%. The antibacterial susceptibility profile of selected antibiotics against the Gram negative bacteria isolates are presented in the table. The antibacterial susceptibility indicated that *Salmonella* sp was resistant to Gentamycin, moderately sensitivity to streptomycin and Septrin, and sensitive to Choramphenicol, Spafloxacin, Ciprofloxacin, Amoxycillin, Pefloxacin, Tarvid and Augmentine at different concentrations. Escherichia coli was resistant to Gentamycin and Streptomycin, sensitive to Choramphenicol, Spafloxacin, Ciprofloxacin, Amoxycillin, Pefloxacin, Tarvid and Augmentine at different concentrations. Table 8b shows the antibacterial susceptibility profile of selected antibiotics against the Gram positive bacteria isolates. *Staphylococcus aureus* was resistant to Rocephin, Zinacef and Streptomycin, moderately sensitive to Ampiclox, and Amoxicillin, and sensitive to Septrin, ciprofloxacin, Gentamycin, Pefloxacin, and erythromycin.

Table 3: percentage Occurrence of Fungi Isolates from Spoilt Fresh tomatoes

Fungi isolates	A	B	C	D	Occurrence	Percentage
Aspergillus niger	1	2	2	2	7	53.8
Aspergillus flavus	1	Nil	Nil	1	2	15.4
Penicillium sp	1	1	1	1	4	30.8
Total	3	3	3	4	13	100

2.2 DISCUSSION

The proximate composition indicates a high moisture content of sample A and B, which favours microbial growth. This results are similar to the report of Chuku *et al*. (2008). The bacteria isolated from the tomatoes sample were *Escherichia coli*, *Salmonella* sp and *Staphylococcus aureus*. The bacteria isolated in this study is similar to that of Ogundipe *et al.(* 2012) and Wogu and Ofuase (2014) who also isolated these bacteria as organisms associated with tomato spoilage. The occurrence of bacteria species could be as a result of feacal contamination due to poor hygienic practices by the farmers and /or the sellers. The fungi isolated from the tomatoes samples were *Aspergillus niger, penicillium* sp and *Aspergillus flavus.* These three organisms were frequently occurring fungi isolated from all samples and this is in agreement with the work of Mbajiuka and Emmanuel (2014) who reported *Aspergillus niger, penicillium* sp and *Aspergillus flavus* also as frequent fungal pathogen associated with the spoilage of tomato. These fungi are usually found in the environment, their spores can be carried on air and thus can infect exposed tomato fruit, as well as farm tools. Antibacterial susceptibility profile of selected antibiotics against the bacteria isolates indicated *Salmonella* sp was sensitive to Choramphenicol, Spafloxacin, Ciprofloxacin, Amoxycillin, Pefloxacin, Tarvid and Augmentine at different concentrations. Escherichia coli was sensitive to Choramphenicol, Spafloxacin, Ciprofloxacin, Amoxycillin, Pefloxacin, Tarvid and Augmentine and *Staphylococcus aureus* was sensitive to Septrin, ciprofloxacin, Gentamycin, Pefloxacin, and erythromycin, thus making this antibiotics effective against the bacterial isolates at different concentrations. The antifungal susceptibility of fluconazole and ketoconazole showed 13mm and 18mm zones of inhibition against Penicillium sp, 7mm and 12mm against Aspergillus *niger,* and 10mm and 14mm against *Aspergillus flavus* respectively. The implications of microbial contamination and growth on tomatoes, causes spoilage, decreased sensory appeal and also decreased shelf life, leading to

loss and wastage of products which have significant economic consequences as reported by Obunkwu *et al*. (2018).

2.3 Conclusion

The proximate analysis of the spoilt fresh tomato samples indicated varying percentages in composition. The bacteria and fungi associated with tomato spoilage were *Escherichia coli*, *Salmonella* sp, Staphylococcus *aureus* and *Aspergillus niger Penicillium* sp, *Aspergillus flavus* respectively. The antibiogram of selected antibiotics and antifungal agents, against the bacteria and fungi isolates indicated some been sensitive and resistant to antibiotics and antifungal agents used.

2.4 Recommendation

It is recommended that;

i. The thorough washing of harvested tomatoes with clean or chlorinated water, proper cleaning and sanitation of ware houses and disinfection of packaging containers, proper handling of the vegetable during harvest should be done to prevent bruises and scars or other mechanical injuries.

ii The inhibition of bacterial and fungal growth by lowering storage temperature through storage under refrigeration of less than 100C but not freezing and the use of appropriate antimicrobial agents when stored by drying is encouraged

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