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Spatial Distribution and Isolation of Microbiota Associated with Spoilage of Tomatoes Sold in Selected Markets in Nasarawa State, Nigeria

J. O. K. Abioye^{1*}, J. Y. Raymond¹ and I. A. Olayemi²

¹Department of Biological Sciences, Bingham University, P.M.B. 005, Karu, Nasarawa State, Nigeria. ²Department of Animal Biology, Federal University Technology, Minna, Niger State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author JOKA designed the study and performed the statistical analysis. Author JYR wrote the protocol and the first draft of the manuscript while author IAO managed the literature and analyses of the study. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Tomato (*Lycopersicum* spp) is widely consumed universally because of its high nutritive value, but suffers great post-harvest losses due to microbial attack. This work was carried out to investigate the causative agents of tomato spoilage and the spatial distribution of such causative agents in some selected major markets in Nasarawa State, Nigeria. Sixty tomato fruits from each of the four selected markets (total of 240 fruits) were investigated for the microorganisms responsible for tomato fruit spoilage. Eleven microorganisms, comprising of five bacterial species and six fungal species were isolated from the spoilt tomatoes. The bacterial isolates included *Micrococcus varians, Lactobacillus fermenti, Escherichia coli, Salmonella sp* and *Klebsiella sp*. The isolated fungi included *Rhizopus stolonifer, Fusarium oxysporium, Aspergillus flavus, Geotrichum candidum, Mucor mucedo,* and *Candida tropicalis.* They were all positive for the pathogenicity test. The isolated organisms were heterogeneously distributed with remarkable levels of severity across the study area. The bacterial load ranged between 2.09 X 10⁹ and 2.56 X 10⁹, while the fungal load ranged between 2.72 X10⁹ and 3.97 X 10⁹. The occurrence and magnitude of the spoilage, and

hence economic loss due to microbial attack could be attributable to the biologic, ecologic and environmental factors of the study area. Recommendations were, therefore, made for improved personal and environmental hygiene, good agricultural practice and proper treatment of tomato fruits before consumption to avert the imminent health consequences due to the spoilage microorganisms.

Keywords: Tomato; bacteria; fungi; spoilage; Nasarawa State.

1. INTRODUCTION

Tomato, scientifically known as *Solanum lycopersicum* [1], a berry plant belonging to the family *solanaceae*. It is a short lived annual plant, typically growing to about 3-5m approximately in height. The fruit is edible and brightly red colored [2].

Tomato is a widely consumed fresh fruit globally, since it contributes to a healthy well-balanced diet, rich in vitamins such as vitamins A, B, C and E; carbohydrates, minerals, water and dietary fibers [3]. Tomato is widely used as condiment or as food dietary supplement in various parts of the world and is valuable in the food industry [4].

Tomato products have some nutritional benefits for human health which gives it some unique attributes like using the tomato juice to prevent blood clot. About one cup of tomato juice per day offers the anti-clotting benefits. Other components of tomato include natural Lycopene which is a red fat soluble pigment found in vegetable commonly found in tomatoes which also add to its nutritive value [5].

Spoilage refers to any change in the condition of food in which the food becomes less palatable, or even toxic, such change may be accompanied by alteration in taste, smell, appearance or texture [6].

Susceptibility of tomato to microbial colonization is due to its intrinsic parameters such as high level of sugar, low pH (4.9-6.5) and its high water activity, all which favor the growth of microorganisms in tomato. Spoilt tomato is recognized as a source of potential health hazard to man and animals. This is due to the production of microbial toxins, capable of causing disease like respiratory infection, meningitis, gastroenteritis, diarrhoea in man following their ingestion [7].

Micro-organism can contaminate tomato as a result of poor handling practices along the tomato production chain, storage condition,

distribution, marketing practices and transportation [8].

Tomato as a plant has serious challenges to its existence which include climatic condition, pest, bacterial and fungal attack. All these challenges culminate to short shelf life, which eventually leads to economic loss of tomatoes. There has been an increase in the need to identify and isolate the microorganisms associated with their spoilage [9]. There is also a great need to control the huge annual economic loss due to microbial spoilage of tomato. Four highly patronized major markets in Nasarawa State where therefore selected for this study.

2. MATERIALS AND METHODS

2.1 Sample Collection

Sixty tomato samples consisting of both healthy and infected (that showed signs of spoilage) were randomly collected from four major markets in Nasarawa State. The four markets were Mararaba, Masaka, Auta-balefi and Keffi. The samples were collected in sterile polythene bags using sterile hand-gloves and transported to the microbiology laboratory in Bingham University for laboratory investigations. Samples collected from each market were worked upon in the laboratory within two hours after collection.

2.2 Determination of Physiochemical Parameters

2.2.1 Physical appearance

The colour, texture and sensory parameters were carried out on both spoilt and healthy tomatoes and recorded.

2.2.2 Moisture content

This was determined by using the method of Fawole and Oso [10]. One gram of healthy and spoiled tomato was weighed out separately into pre-weighed sterile crucible. Each crucible and its contents were put in a hot air oven and dried at 105 °C for 24 hours. After drying each crucible and its dried content were transferred into a desiccator and allowed to cool. After cooling the crucible and the dried content were weighed and the weight recorded. The moisture content was determined by subtracting the weight of the crucible and the dried cooled content from the weight of the crucible and tomato content before drying.

Moisture content =weight of moisture X 100% Weight of wet tomato

2.2.3 Determination of pH

The pH of both spoilt and healthy tomatoes was determined using a glass electrode pH meter (England). Each tomato sample was crushed in a sterile mortar using sterile pestle. The juice obtained after crushing was transferred into a sterile beaker into which the probe of the pH meter was inserted and the pH value was read from the pH meter and recorded. Prior to each use the pH meter was standardized with a neutral buffer solution [10].

2.3 Isolation of Microorganisms

A tomato fruit weighing averagely ten grams was properly rinsed in 100ml sterile distilled water and 1ml of the rinse water was serially diluted (tenfold) out, then 1ml from each dilution of the last four tubes were plated out using pour plate method [11]. This process was done for both healthy and spoilt tomatoes. This was to determine the surface micro flora of healthy and spoilt tomatoes.

A fruit each of spoilt and healthy tomatoes was thoroughly rinsed in three successions of sterile distilled water and then surface sterilized using 70 % ethanol. Each surface sterilized tomato fruit (about 10g) was then crushed in a sterile mortar and pestle suspended in 100ml of sterile distilled water. After mixing properly 1ml of the suspension was serially diluted (tenfold) and 1ml from each of the last four dilutions were plated out using pour plate technique, i.e. dilutions, X10⁻ 7, X10⁻⁸, X10⁻⁹, X10⁻¹⁰. Three media were used for the isolation of the microorganism, which included Nutrient agar, Mac-Conkey agar and Potato dextrose agar. Potato dextrose agar was used for fungal isolation while the other two media were used to isolate bacteria. The media were prepared following the manufactures instruction on the pack. The inoculated plates, after setting were incubated in preset incubator (England), one at 37 °C for 24 hours for bacterial isolates and the other at 25 °C for 7 days for fungal isolates. At the end of incubation inoculated plates were examined for microbial growth. Colonies on the plates were counted using colony counter (USA) and recorded both for healthy and spoilt tomatoes.

Each distinct colony (bacteria and fungi) was sub-cultured onto fresh medium to obtain the pure culture for further investigations and identification. The bacterial isolates were subjected to gram staining and biochemical test while the fungal isolates were subjected to staining with lactophenol blue followed by microscopy.

2.4 Pathogenicity Test

To ascertain that the isolated microorganisms caused the spoilage of tomatoe, pathogenicity test was carried out. From each pure isolated culture, inoculum was removed with sterile wire loop (for bacterial) and sterile mounted needle (for fungi) and used to infect healthy surfacesterilized tomatoes through a hole bored on each tomato using sterile cork borer. After introducing the test isolate, the hole bored was aseptically closed up with the piece removed with cork borer and the cut edge sealed up with sterile petroleum jelly. The inoculated tomatoes were incubated in preset incubators at 37 °C for 24 hours for bacterial and 25 °C for 7 days for fungi. The inoculated tomatoes were placed in desiccators having moistened cotton wool at the inner bottom of each dessicator to provide moistened environment inside, a micro-humid environment for the microbes to grow [9].

3. RESULTS

3.1 Spatial Distribution and Tomato Activities of the Bacterial and Fungal Species

Fig. 1 shows variation in the quality of tomato fruit, in terms of microbial spoilage, in the selected tomato markets in Nasarawa state. The result showed that the level of damage to the tomato fruit varied widely across the localities. The highest level of fruit spoilage was recorded in Masaka (50.00%), followed by Mararaba and Auta-balefi (46.67 and 45.0% respectively). The least infected fruits were obtained from Keffi (36.67%).

3.2 Physiochemical Parameters

The physical appearance and organoleptic parameters revealed that fresh, healthy tomatoes were bright red or orange in colour, smooth in texture, hard to touch and odourless. The spoilt tomatoes looked dull orange to dirty brown in colour, wrinkled texture, soft to touch and with offensive sour smell (Table 1, Plates 1 and 2).

3.2.1 Moisture content

The moisture content of tomato from the different markets investigated ranged between 80.7 and 90.4% (Fig. 2).

3.2.2 pH

The pH of tomatoes from different markets studied ranged between 4.5 and 5.7 (Fig.3).

3.3 Microbial Loads

The mean surface bacterial counts ranged between 1.70 X 10^9 , and 2.07 X 10^9 for the spoilt tomato and between 1.53 X 10^9 and 1.95 X 10^9 for the healthy samples.

The surface fungal count for the spoilt tomato was between 2.08 X 10^9 and 3.56 X 10^9 , while for the healthy tomato the surface fungal count ranged between 1.81 X 10^9 and 2.74 X 10^9 . For the crushed healthy samples the fungal load was zero in all the four markets.

For the crushed samples the bacterial counts ranged between 2.09×10^9 and 2.56×10^9 for the spoilt tomatoes, while in the crushed healthy tomatoes, the bacterial counts ranged between 1.05×10^9 and 1.35×10^9 (Tables 2 to 5).

On the whole, eleven microorganisms were isolated from the tomato samples examined, either from the surface of the tomatoes or after crushing them. The microorganisms included five bacterial species and six fungal species. The included bacterial isolates Micrococcus. Lactobacillus fermenti. Escherichia coli. Klebsiella sp. Salmonella sp (Table 6, Plates 3-5)while the fundal isolates included Fusarium tropicalis. oxyspoyum, Candida Rhizopus stolonifer. Asperaillus flavus. Geotrichum candidum and Mucor mucedo (Table 7, Plates 6-9).

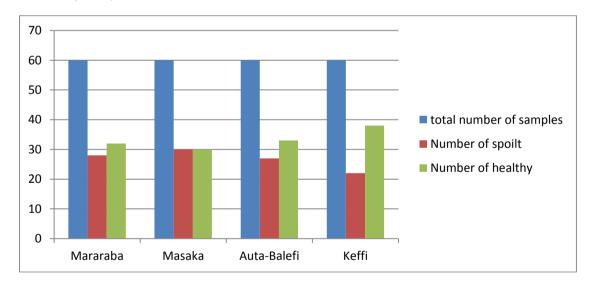


Fig. 1. spoilage signs frequency of tomato fruit in the study areas

	Healthy tomatoes	Spoilt tomatoes			
Colour	Bright red or orange	Dull orange to dirty brown			
Texture	Smooth	Wrinkled			
Touch sensory	Hard/tough	Soft			
Smell	Odourless	Foul sour smell			

Table 1.	Physical	parameters	of health	y and	spoilt tomatoes
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Plate 1. Healthy tomatoes Plate 2. Infected tomatoes

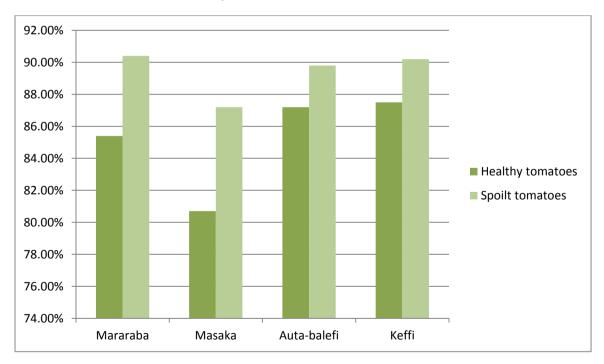


Fig. 2. Mean moisture content of healthy and spoilt tomatoes

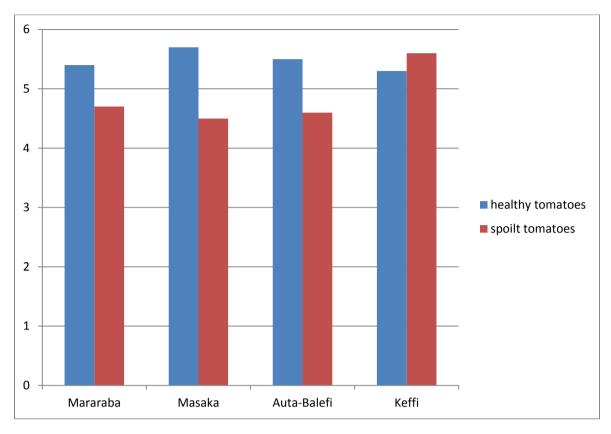


Fig. 3. Mean pH values of healthy and spoilt tomatoes

Table 2. Mean surface microbial load of healthy tomatoes in four markets

	Mararaba	Masaka	Auta-balefi	Keffi	Average loads
Bacterial isolates	1.67 X 10 ⁹	1.95 X 10 ⁹	1.57 X 10 ⁹	1.53 X 10 ⁹	1.68 X 10 ⁹
Fungal isolates	2.44 X 10 ⁹	2.74 X 10 ⁹	2.35 X 10 ⁹	1.81 X 10 ⁹	2.34 X 10 ⁹

	Mararaba	Masaka	Auta-balefi	Keffi	Average levels
Bacterial isolates	1.87 X10 ⁹	2.07 X 10 ⁹	1.62 X 10 ⁹	1.70 X 10 ⁹	1.82 X 10 ⁹
Fungal isolates	2.85 X 10 ⁹	3.56 X 10 ⁹	2.65 X 10 ⁹	2.08 X 10 ⁹	2.79 X 10 ⁹

Table 4. Mean microbial load of crushed healthy tomatoes

	Mararaba	Masaka	Auta-balefi	Keffi	Average load
Bacterial isolates	1.31 X 10 ⁹	1.35 X 10 ⁹	1.18 X 10 ⁹	1.05 X 10 ⁹	1.22 X 10 ⁹
Fungal isolates	0.0	0.0	0.0	0.0	0.0

Table 5. Mean microbial load of crushed spoilt tomatoes

	Mararaba	Masaka	Auta-balefi	Keffi	Average loads
Bacterial isolates	2.41 X 10 ⁹	2.56 X 10 ⁹	2.28 X 10 ⁹	2.09 X 10 ⁹	2.34 X 10 ⁹
Fungal isolates	3.91 X 10 ⁹	3.97 X 10 ⁹	2.86 X 10 ⁹	2.72 X 10 ⁹	3.37 X 10 ⁹

Cultural characteristic	Gram stain	Cell shape	Motilitv Indole	Catalase	Oxidase	coagulase	Urease	Citrate	MRVP	spore	Glu	Fru	Lac	Gal	Suc	Mal	Man	Identification
Flat, smooth, circular, creamy, opeaque with entire margin	+	Sperical cells arranged singly and paired	- +	+	-	-	+	-	+ -	-	A/G	-	-	A/G	+	A/0	-	Micrococcus varians
Translucent, milky flat colonies with irregular margin	+	Slender rods		+	-	-	+	+	+ -	-	A/G	-	A/G	-	A/G	A/O	-	Lactobacillu s fermenti
Circular, smooth,convex, colourless with entire margin turns red on MacConkey agar	-	Short rod shaped	+ -	-	-	-	-	-	+ -	-	A/G	-	A/G	-	A/O	-	-	Esherichia coli
Large , opaque, raised colonies, with entire margin, colonies turned red on MacConkey agar	-	Short thick rods		+	-	-	+	+	-+	-	A/O	-	+	-	-		-	Klebsiellasp p
Pale, large opaque colonies with entire margin	-	Short rods	+ -	+	-	-	-	+	+ -	-	A/G	-	-	-	-	-	-	Salmonella spp
Round creamy, distint, colonies with entire margin	+	Large spherical cells, septatepseu dohyphae and pseudomyce lium well developed		-	-	-	-	-	-	-	A/G	-	-	-	A/G	A/G	-	*Ċandida tropicalis

Table 6. Isolation, characterization and identification of bacterial isolates

Round creamy, distint, colonies with entire margin	+	Large spherical	 -	-	-	-	-	-	-	A/G	-	-	-	A/G	A/G	-	*Candida tropicalis
		cells,															
		septatepseu															
		dohyphae															
		and															
		pseudomyce															
		lium well															
		developed															

*= fungal isolate.



Plate 3. Micrococcus varians



Plate 4. Klebsiella colonies on MacConkey agar

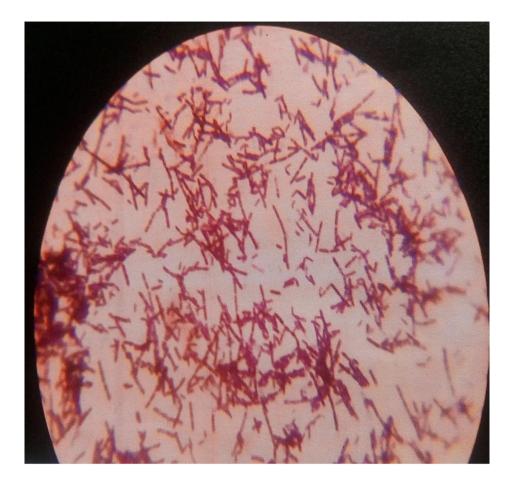


Plate 5. Lactobacillus fermenti

Cultural characteristics	Microscopic features	Identification
Creamy, large colonies with clustered growth.	Macroconidia were fusiform, curve, pointed at both ends, borne on branched, septateconidosphore.	Fusarium oxysporium
Round translucent to creamy colonies with entire margin.	Septatepseudohyphae and well developed pseudomycelium.	Candida tropicalis
Distinct large brownish white, mouldy colonies with black centre.	Long, nonseptate hyphae arising from rhizoids with brownish black sporangia containing zygospore.	Rhizopus stolonifer
Large white filamentous colonies which became covered with smoky green spores	Friuting head made up of a swollen receptacle on conidophore on which sterile bearing chains of conidiospores are borne.	Aspergillus sp
Mouldy, large to medium size colonies with flat surface	Septate thick hyphae with dichotomous branching. Conidia are cylindrical, barrelshaped or ellipspidal.	Geotrichum candidum
Large whitish black mouldy colonies which later turned black as the culture matured	Coelocytic branched hyphae with vertical ones bearing glabose sporangia containing sporangiospores.	Mucor mucedo

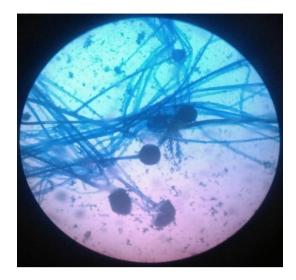


Plate 6. Rhizopus stolonifer

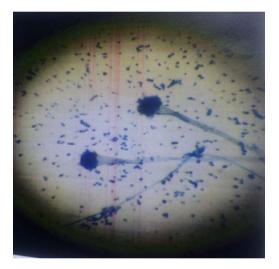


Plate 7. Aspergillus flavus



Plate 8. Geotrichumcandidum



Plate 9. Fusarium oxysporum on PDA (Grey colonies)

Table 8. Level of bacterial infection in the tomato samples

	Mararaba	Masaka	Auta-balefi	Keffi
Micrococcus varians	28 (46.67)	30 ((50.0)	27 (45.0)	22 (36.67)
Lactobacillus	28 (46.67)	30 (50.0)	27 (45.0)	22 (36.67)
fermenti				
Escherichia coli	25 (41.67)	29 (48.33)	23(38.33)	18 (30.0)
Klebsiellasp	23 (38.33)	28 (46.67)	22 (36.67)	18 (30.0)
Salmonella sp	22 (36.67)	24 (40.0)	22 (36.67)	12 (20.0)

Values in parenthesis are percentages, * Values outside parenthesis are number of tomatoes infected

Table 9. Level of fungal infection in the tomato samples

	Mararaba	Masaka	Auta-balefi	Keffi
Fusarium oxysporum	8 (28.57)	12 (20.0)	0 (0.00)	0 (0.00)
Candida tropicalis	15 (25.0)	20 (33.33)	15 (25.0)	10 (16.67)
Rhizopus stolonifer	28 (46.67)	30 (50.0)	27 (45.0)	22 (36.67)
Aspergillus flavus	28 (46.67)	30 (50.0)	25 (41.67)	18 (30.0)
Geotrichum candidum	25 (41.67)	28 (46.67)	21 (77.78)	10 (16.67)
Mucor mucedo	20 (33.33)	25 (41.67)	15 (25.0)	12 (20.0)

*Values in parenthesis are percentages, * Values outside parenthesis are number of tomatoes infected



Plate 10. Healthy tomato inoculated with the isolates (after incubation)

3.4 Pathogenicity Test

The result of the pathogenicity test showed that all the inoculated healthy tomatoes developed signs of spoilage, and all the microorganisms isolated and inoculated into the healthy tomatoes grew at the sites of inoculation within 5 to 7 days of incubation as shown in Plate 10.

4. DISCUSSION

Fresh tomato fruits are naturally protected from microorganisms by their outer epidermal layer. This protection can be hindered and the fruit become infected by microorganisms during field cultivation, harvesting, post-harvest handling, including transportation. The work done here shows that microorganisms, both bacteria and fungi are responsible for the great loss of postharvested tomato fruits. About 44.58% (107 out of 240) loss is attributed to microbial spoilage in this study.

The physicochemical parameters obtained in this work support the above in that despite the natural tough outer covering of tomatoes, the high moisture content (80.7-90.4%), and slightly acidic pH (4.5-5.7) lend them easily to microbial spoilage.

The eleven microorganisms isolated from spoilt tomato fruits in this work included five bacterial species and six fungal species. *Micrococcus varians* and *Lactobacillus fermenti* were the most dorminant bacteria in the spoilage of tomato fruits, while *Rhizopus stolonifer, Aspergillus flavus* and *Geotrichum candidum* were the most dominant fungi in the spoilage.

The non-isolation of fungi in the crushed healthy tomato fruits indicated that fungal spoilage could strictly be post-harvest. Also isolation of some bacteria in the crushed healthy tomato fruits suggests that bacterial infection of tomato fruits could set in from the soil during cultivation and post-harvest time.

The microorganisms isolated in this work have also been reported in similar works done by several authors. *Rhizopus stolonifer, Aspergillus flavus* were reported by Omolaran *et al.*,[12] . John *et al.*, [13] reported *Rhizopus, Fusarium* and *Geotrichum* from Jos, Plateau State. Also Onourah and Orji [14] in Awka reported tomato spoilage by *Aspergillus, Rhizopus, Geotrichum* and *Fusarium*. Onyemeachi *et al.*, [15] in Onitsha reported tomato fruits spoilage by *Rhizopus,* Aspergillus, Fusarium and Candida implicated. Barth et al., [16] also reported Rhizopus, Aspergillus, Fusarium in tomato fruits spoilage. [15] isolated Escherichia coli, Klebsiella sp. and Salmonella sp. as bacteria agents, while they isolated fungal agents which included Candida tropicalis, Aspergillus sp. Fusarium oxysporium and Rhizopus stolonifer.

The two notorious bacterial species, Micrococcus and Lactobacillus were also reported by some authors in similar works Agbabiaka *et al.*, [17]. These two bacterial species could be of soil origin to cause the infection of tomato fruits. Other bacterial isolates in this work, i.e. *Escherichia coli, Klebsiella sp* and *Salmonella* were also reported for tomato fruits spoilage by some authors [15] and Olokun *et al.*, [18]. These bacterial could get in contact and infect tomato fruits through contact by handlers and faeces used to manure their crops, including tomatoes.

There was spatial heterogenicity in the levels of microbial spoilage of tomatoes across the four selected markets in Nasarawa State, especially in Masaka and Keffi. Similar findings have been reported in Umudike, Abia State by Mbajuika and Enya [19] where various bacteria and fungi were reported to be responsible for tomato fruits spoilage. The considerable variation in level of tomato spoilage in the different markets may be due to differences in certain biologic and environmental conditions that affect tomato quality in the four sites. Such factors may include differences in microclimate condition that influence the occurrence and thriving of microbial agents, differences in tomato varieties sold in the markets and the level of hygiene obtainable in these areas. Observations made during sample collection showed that Masaka and Mararaba, where microbial virulence was highest, were slum settlements with high population densities, thus resulting in considerable environmental deterioration that could promote the abundance of pathogenic microbes. On the other hand, Keffi enjoys low human density and relatively high environmental cleanliness.

Although, more fungal species were encountered compared to their bacterial counterparts, the latter showed higher widespread distribution and to a large extent, disease virulence. This finding, indicating bacteria as more involved in tomato spoilage than fungi, is contradictory with the report of [16] who suggested that fungi are responsible for most tomato spoilage than bacteria. The contradictory results obtained in this present study may be due to better adaptability of the bacterial species to the ecologic and environmental conditions enhancing tomato spoilage generally in the study area.

The result of the pathogenicity test confirmed the implication in tomato spoilage of the microorganisms isolated in this work, as all of them infected the fresh healthy tomato fruits inoculated and within a short space of time of three to four days.

The health implication of our findings in this work is that many consumers of tomatoes are at risk of infection and disease, as most of the isolated organisms are known to produce toxic materials like bacterial toxins and aflatoxins from the fungi as highlighted by [9].

5. CONCLUSION

The findings of this study show the occurrence of high microbial species diversity actively involved in tomato fruit spoilage in the selected sites of Nasarawa State. However, it appears that certain perhaps ecologic, biologic factors. and environmental. significantly affect the wide spread distribution and virulence of the microbial agents against tomatoes in the study area. Significant attention should be given to the control of major microbial agents responsible for the spoilage of tomatoes in the areas, especially, Micrococcus varians, Lactobacillus fermenti, Escherichia coli, Rhizopus stolonifer and Aspergillus flavus for improved tomato productivity and shelf-life in the study areas.

6. RECOMMENDATION

Efforts should be made to improve the environmental conditions in the study area. Level of personal and environmental hygiene must be improved in the localities. Consumers of tomatoes should be informed about the unsafe condition of raw tomatoes and the need to prevent infections from their consumption. Tomato fruit must be properly washed and disinfected before consuming it raw.

Adequate cooking is very necessary to reduce or eliminate the microbes associated with tomato spoilage before being consumed. Good Agricultural practices should be encouraged among tomato farmers in order to reduce or prevent post-harvest loss of tomatoes. The use of human waste as manure in tomato farms should be discouraged in order to avoid faecal contamination of the produce.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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