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Fungal and Bacterial Species Associated with the Deterioration of Fresh Tomato Fruits (*Lycopersium esculentum* M.) Sold in Aliero Market, Kebbi State

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Abstract—*Lycopersium esculentum* M. are economic vegetables that deteriorated due to the nutrients contain which allowed the growth of microorganisms on it. Bacterial and fungal species were identified using poured plate method and Biochemical tests. Three (3) categories of tomato fruits samples were collected, varying different degrees of spoilage; highly rotten, slightly rotten and fresh tomatoes. A total of sixty (60) tomatoes were randomly collected within Aliero central market. From the results obtained, seven fungal species and four (4) bacterial species were isolated namely; *Saccharomyces cerevisiae*, *Candida* species, *Alternaria alternate*, *Trichoderma* species, *Penicillium digitatum*, *Mucor* species and *Aspergillus* species and bacterial species identified were; *Bacillus* species, *Escherichia coli*, *Staphylococcus aureus* and *Proteus* species. The mean colony counts of bacteria ranges from 2.9 x 10^3 to 6.0 x 10^2 cfu/g and spore formed per unit for fungal species ranged from 3.0 x 10^3 to 5.6 x 10^2 sfu/g. In conclusion, these spoilage of fresh tomato usually occurs during storage and transit as well as during processing. Since many microorganisms were identified as the major causal agents of the tomato fruits spoilage in the study area, proper handling procedure especially during post-harvest and pre harvest as well as during selling and buying of the tomato fruits should be employed to avoid damage that usually results to the spoilage of these vital vegetable.

Keywords—Tomatoes, microorganisms, mean, market and Aliero

I. INTRODUCTION

Tomato (*Lycopersicum esculentum* M.), is an annual plant, belong to the family of solanaceae, having a weak woody stem covered with glistering reddish yellow glandular hairs that grows up to 10 feet tall, but most species are less than three feet's tall on average which is usually referred to as "Tumatori*"* in Hausa [1]. The tomato fruits have smooth skin with diverse in size and shape, ranging from small and round to large and variable shapes depending on the species and environmental conditions [2]. Similarly, it is green when immature but becomes bright red or yellow as it ripens. This ripens fruit are used in Kebbi state and worldwide to preparing delicacy soups, stews or can be eaten raw with salads. According to [3], matured tomato fruits are good source of both micronutrients as well as macro elements. Due to its higher value and importance as food, tomato fruit has been bred to improve on its productivity, fruit quality, and resistance to biotic and a biotic stresses [4].

Fruit spoilage may be defined as any change that renders fruit unfit for human consumption. Every change in tomatoes that causes it to lose its desired quality, damaged and eventually become unfits for human consumptions could be referred tomatoes spoilage or rotting [5]. Food scarcity is one of the challenges faced worldwide due to the implication of microbes after poverty. Many developing and developed countries faced food scarcity which is one of the important major problems especially in Nigeria. According to a report, about 1 billion people are being faced by severe hunger in these nations of which 10% actually die from hunger-related complications. This problem arises due to inadequate agricultural storage, packaging, handling, transportation and preservation from microbes-induced spoilages [1]. The microbial contamination of tomato fruits has been a thing of concern in Aliero Local government and Nigeria at large, as it's used on daily bases. As these microbes come in contact with tomatoes during growth, harvesting, transportation, selling at market, processing, preservation and handling, the produce are contaminated with nonpathogenic and pathogenic organisms from soil, environments, human or animal sources [6]. Consumption of spoiled tomato fruit that has been contaminated with these microbes could be health hazard to human and also may enhance economic loss to farmers by reducing yield.

II. RELATIVED WORK

Many studies about Tomato fruits fungi were done in Turkey [7] Ebonyi [8] and Jega L.G.A, Kebbi State [9]. So

far, to the best of our knowledge studies on fungi and bacteria species associated with Tomato fruits were not done in Aliero central market. This research work focused on identifying and characterizing fungal and bacterial species associated with the deterioration of tomato fruits in Kebbi state more especially in Aliero communities.

III. MATERIALS AND METHODS

Study Area

The study was conducted in Aleiro Local Government Area of Kebbi State. It is located in the South east of Kebbi State. The town shares common borders with Gwandu local Government from the South West, to the East is bordered to Tambuwal in Sokoto State. It covers a total mass of 412 square kilometers, with a total population of 125,785 inhabitants [10]. The area is located at longitude of 11º3S, 12º44N, latitude 3º6W and 4º2E. The topography of the Area is flat and slightly undulating with compact, stony brown soil. Aliero Local Government falls in the Sudan Savanna region of the State with major seasons; dry and rainy season [11]. Most of the people in Aliero communities are agrarian, with emphasis on vegetation, especially onion, tomatoes and pepper. The relative humidity of the area ranges from 17 to 80% respectively.

Sample Collection

Survey was conducted to selected tomatoes fruits in Aliero market, Kebbi state, Nigeria. Three (3) categories of tomatoes fruit samples were collected in a sterilized poly-then bags, varying degree of spoilage level as; highly rotten, slightly rotten and fresh harvest tomatoes. A total of sixty (60) tomatoes were collected, well labelled for easier identification and taken to the Department of Plant Science and Biotechnology, Aliero for further analysis.

Figure 1: Tomato fruits at Market

Sterilization of the Materials

All glass wares were soaked overnight in 70% ethanol, washed with detergent, rinsed with distilled water and then air-dried. Petri dishes, glass slides and bottles to be used were sterilized by dried heat oven at 160^{oC} for one hour [12].

Media Preparation

The media Potato Dextrose Agar (PDA) and Nutrient Agar (N.A) used in this research work were prepared in accordance to the manufacturer's specific instructions using standard aseptically technique.

Sample Processing and Serial Dilution

Three (3) categories of tomatoes fruits collected were grinded using blender separately and 20.0g of each sample was weight and used for serial dilutions as described by Keta *et al*. [1]**.**

Isolation of bacterial and Fungal Species

Isolation technique methods such as spread plate and streak plate were employed to determine bacterial and fungal growth as described by Keta *et al*. [1].

Identification of Bacterial and Fungal Species

The bacterial species isolates were identified using colony formation characters and biochemical tests as described by [12]. While fungal isolates were identified using spore formation and cellular characteristics with the help of microscopic and mycology atlas as method adopted by Keta *et al*. [1].

Statistical Analysis

Statistical Package for Social Science was used for the data analysis. The fungi isolated were recorded as colony formed per unit (cfu/g) for bacteria and spore formed per unit (sfu/ml) in fungi using tables as a descriptive statistical tool.

IV. RESULTS AND DISCUSSION

Table 1 showed the morphological features of fungal species isolates from fresh, rotten and slightly Tomato fruits namely; *Candida* species, *Penicillium digitatum*, *Aspergillus niger*, *Alternaria alternate*, *Mucor* species, *Trichoderma* species and *Saccharomyces cerevisiae* as seen in Table 1. Biochemical characterization of bacterial species isolates from three collected sample tomato were presented in Table 2. The results of spore formed shown that fresh tomatoes fruits had the lowest fungal spore formed $3.0 \times 10^3 - 4.2 \times 10^2$, slightly rotten tomatoes $3.6 \times 10^3 - 4.4 \times 10^2$ and $4.6 \times 10^3 - 5.6 \times 10^2$ in rotten tomato fruits were obtained highest as showed in Table 3. Bacterial variable count of tomato fruits ranged from $2.9 \times 10^3 - 6.0 \times 10^2$ as presented in Table 4 respectively.

Table 1: Morphological features of fungal isolates from tomatoes fruits in Aliero market

Isolates Fungal Species	Culture Characteristics	Microscopic Features
Candida species	Creamy mucous colonies with a formative odour	Oval budding cells pseudo hyhae formation
Penicillium digitatum	Creamy cotton like with dirty white pigments producing a formative odor	Bluish oval shape cells with bud formation
Aspergillus niger	Whitish yellow with blue green pigments	Double branching septate hyphae with short conidiospores
Alternaria alternate	Dark brown conidiosphores, flexuous in	Pigment (brown, tan or black) hypae often

	appearance, smooth surface	constructed at the point of septation
Trichoderma species	Found white to crème deep red, yellow to yellow brown to red wine	Slender clavete to pyriform
Mucor species	Grey spore becoming brownish	Non-septate mycelia with branches sporangiosphores
Saccharomyces cerevisiae	Growth is observed within few days. Appear creamy- yellowish green. Appear in green flat circular colony. Spherical spores often in group of fours.	Appear spherical or ovoid in shape. Have simple pseudo hyphae Flat, smooth, and cream color.

Table 2: Biochemical characterization of three collected sample tomato test.

SAMPLE CODE	SPORE FORMATION	MURTALITY	CATALASE	CITRATE	INDOLE	COAGULATE	URASE	METHYL RED	VOGES PROST	GRAM REACTION	COLONY CLISCIRPTION	PROBABLE ORGANISM	
FRT_1	+	+	+	-	-	-	-	+	-	+ rods chains	Gray white Swaming innature	*Bacillus* species	FRT_1
FRT_2	+	+	-	-	-	+	+	+	-	- with short rods	Creamy white non viscous flat	*E. coli*	2
FRT_3	+	+	+	+	-	+	+	+	-	+ rods In chains	Cream smooth with cicular colonies	*Staphylococcus* species	3
SRF_1	+	+	+	-	-	-	+	+	-	+ rod chains	Swaming gray white colonies	*Proteus* species	SRT_1
SRF_2	+	+	+	-	-	-	+	+	+	+ rod chains	Creamy smooth colonies	*Staphylococcus* species	2
SRF_3	+	+	+	-	-	+	-	+	-	+ rod chains	Whitish rough Edge flat colonies	*Bacillus* species	3
HRF_1	+	-	-	-	-	-	+	+	-	- short rods	Creamy smooth flat colonies	*E. coli*	HRT_1
HRF_2	+	+	+	+	-	+	+	+	-	+ cocci	Creamy smooth colonies	*Staphylococcus* species	2
HRF_3	+	+	+	-	-	-	+	+	+	+ rods in chains	Gray white in color rougedge nature	*Bacillus* Species	3

HRT = highly rotten tomatoes
SRT = slightly rotten tomatoes
FRT = fresh harvest tomatoes

Table 3: Fungal spore formed per unit (sfu/g) of the different tomato fruits sample in Aliero market

Sample Code	**Fungal spore count (Sfu/g)**	**Average**
FRT1	3.0×10^3	1.5×10^3
FRT2	4.2×10^2	2.1×10^2
SRT1	3.6×10^3	3.3×10^3
SRT2	4.4×10^2	2.2×10^3
HRT_1	4.6×10^3	2.3×10^3
HRT2	5.6×10^2	5.3×10^2

Key
HRT = highly rotten tomatoes
SRT = slightly rotten tomatoes
FRT = fresh harvest tomatoes

Table 4: Bacterial variable count of tomatoes fruits

Sample	**Bacterial variable count**	**Average**
FRT1	2.9×10^3	1.4×10^3
FRT2	4.4×10^2	2.2×10^2
SRT1	3.0×10^3	1.5×10^3
SRT2	5.8×10^2	2.4×10^2
HRT_1	4.0×10^3	2.3×10^3
HRT2	6.0×10^2	3.0×10^2

Key
HRT = highly rotten tomatoes
SRT = slightly rotten tomatoes
FRT = fresh harvest tomatoes

Discussions

A total of six (7) fungal and four (4) bacterial species were isolates from three different deterioration stages of tomato fruits namely; highly rotten, slightly rotten and fresh

tomatoes fruits obtained at Aliero local government Centre market and the results of this study revealed the presence of *Candida* species, *Alternaria alternata*, *Trichoderma*, *Penicillium digitatum*, *Mucor* species and *Aspergillus* species and *Bacillus* species, *E. coli*, *Staphylococcus* species and *Proteus* species (Table 1 & 2). All identified microbes in tomato fruits varied with colony formed per unit and spore form per unit. In a similar research carried out by Abubakar *et al*. [9], on their study reported some similar fungal species such as *Aspergillus niger*, *Penicillium digitatum*, *Alternaria alternate* and *Saccharomyces cerevisiae*. These species may be present due to similar environmental factors such as temperature and humidity that favor the growth and sporulation of these fungal species in the study area. Furthermore, this studied disagreed with the findings of Ugwu *et al.* [13], that observed the absence of both fungi and bacteria species in fresh healthy tomatoes but in line with their study in spoilt tomato fruits, hence they recorded species of fungi; *Candida tropicalis*, *Penicillium notatum*, *Aspergillus niger*, *Fusarium oxysporum*, *Absidia corynbifera*, *Rhizopus stonolifer* and 4 species of bacteria; *Escherichia coli*, *Klebsiella* spp., *Salmonella* spp. and *Pseudomonas aeruginosa*. Some of these microorganisms were not obtained in our research, this could be due to methods used, location, temperature differences, cultivars, storage methods/facilities and transportation.

The presences of these fungal species (Table 1) on deteriorated of tomato fruits obtained in this study collaborated with the study of [8], on his finding research isolation and identification of fungi associated with post-harvest decay of *Lycopersicum esculentum* M. sold in Abakaliki, Nigeria. However, in our present research, *Aspergillus* species were the most frequent isolates. Also, Bashir *et al*. [5], identified two bacteria species; *Staphylococcus aureus* and *Bacillus* and two fungal species; *Aspergillus flavus* and *Rhizopus stolonifer* on decay tomatoes. Also, [14] isolated and identified *Bacillus* species in *Rhizosphere* bacteria from *Capsicum chinense*. Many researchers such as; [7,15], isolated and identified *Aspergillus niger* as most prevalence fungi on decay and rotten tomato fruits sold in the market. However, the *Bacillus* species, *E. coli*, *Staphylococcus aureus* and *Proteus* species obtained could be due to the activities of sellers and buyers during sales in the markets. Microbes are the most agent causes of fruits and food spoilage that appeared everywhere at the same time. According to Hammond *et al*. [16], microorganisms are too small to be seen with the naked eye, except for molds, colonization of exposed foods by bacteria and yeasts may remain unnoticed. The main source of the fungal contamination in tomato fruits are soil, air, moisture content, nutrient, water activity that allowed the development of these fungal species by colonization.

According to [1], reported that, some *Aspergillus* species such as *Aspergillus niger*, *Aspergillus flavus* are responsible for production aflatoxins that generated mycotoxins and contaminated tomato fruits are mostly preferred by food sellers than fresh tomato fruits in Kebbi state as its less cost. These rotten tomatoes are contaminated with microorganisms such as *Aspergillus* and *Penicillium* species that could be hazardous to human health. According to [7], aflatoxin produce by species of *Aspergillus* are very resistances to heat and UV light. However, *Aspergillus flavus* is the main producer of carcinogenic aflatoxins and its presence in food is of great concern in terms of food safety and food security [17]. Therefore, the buyers, sellers and farmers that obtained this produce should take appropriate precautions during buying, harvesting, transportation, storage and sales of tomato fruits to reduce the risk of these toxins and other health implications. Environments in which tomatoes are displayed such as market places are not hygienic and this could be another avenue for fungal contamination. However, retailers are seen displaying in open baskets, beside the gutter or refuse heaps, these also encourages fungal attack and subsequent production of toxins [18]. Poor environmental sanitation and high human traffic and inability to separated deteriorated and fresh tomato fruits are also implicated.

V. CONCLUSION

This study revealed that many bacterial as; *Bacillus* species, *Escherichia coli*, *Staphylococcus aureus* and *Proteus* species and fungal species were; *Saccharomyces cerevisiae*, *Candida* species, *Alternaria alternate*, *Trichoderma* species, *Penicillium digitatum*, *Mucor* species and *Aspergillus* were found responsible for deterioration of tomato fruits in the study area. These organisms are a source of potential health hazard to human and animals following ingestion. This is due to their production of toxins which are capable of causing diseases and economic loss as well as food poisoning. Proper handling methods should be adopted by both farmers and consumers to minimize the microbial contamination of fruits in general more especially tomato fruits in the study area.

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