MICROORGANISMS ASSOCIATED WITH THE SPOILAGE OF TOMATOES

BY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FUFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.Sc. HONS) IN MICROBIOLOGY

CERTIFICATION

This is to certify that this project work was carried out by **Excellenta OAMEN (Miss)** with the matriculation number **LSC1806777** in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City.

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(PROJECT SUPERVISOR)	
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(HEAD OF DEPARTMENT)	

APPROVAL

This project was carried out by Excellenta Ofure OAMEN (Miss) under the supervision of PROF. (MRS.) E.E AKORTHA in partial fulfillment of the award of a Bachelor of Science (B.Sc.) degree in Microbiology.

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(PROJECT SUPERVISOR)	

DEDICATION

I wish to dedicate this work to GOD Almighty for his endless blessings and mercy and also to my family for their encouragement and support.

ACKNOWLEDGEMENT

I wish to acknowledge God Almighty for his endless protection, love and favor over my life.

To my supervisor, PROF. (MRS.) E.E AKORTHA thank you for your guidance and patience.

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ABSTRACT

The research was carried out to evaluate the spoilage microorganisms of tomatoes sold in Benin City. Standard bacteriological methods were used to enumerate the total bacterial and fungal count of the tomatoes using pour plate methods after serial dilution. The bacterial isolates were characterized and identified using morphological and biochemical methods and sugar fermentation test. The percentage distribution and frequency of the isolates were evaluated using statistical method. The result obtained in this study showed that the highest bacterial, coliform and fungal population was obtained in Adolor sample with values of 6.07554±1.00, 5.09691±0.5 and 4.767155±0.5 $\log_{10}$ cfu/g respectively while the least bacterial, coliform and fungal count was obtained from Uselu and Ekosodin samples with values of 5.35218±2.00, 3.69897±1.00 and 4.32221±1.00 $\log_{10}$ cfu/g respectively. Using the cultural and morphological characteristics, the fungal isolates obtained in this study were *Aspergillus niger, Trichoderma sp, Penicillium* sp and *Rhizopus arrhizus* while the bacterial isolates obtained were *Escherichia coli, Bacillus* sp, *Salmonella* sp, *Shigella* sp, *Klebsiella* sp and *Staphylococcus* sp. From the result, isolates were resistance to many of the antibiotics including, collistin, Erytromycin, metronidazole and clindamycin but were susceptible to ciprofloxacin, augmentin and gentamycin. The result in this study has shed light into the gaining of entrance of food borne pathogens as well as some spoilage microorganisms (mostly fungi) during selling, harvesting and cultivation which may result in food poisoning.

CHAPTER ONE

INTRODUCTION

1.0 BACKGROUND STUDY

Tomato (*Solanum lycopersicum*) is an important crop that belongs to the Solanaceae family, together with potato (*Solanum tuberosum*), hot pepper (*Capsicum frutescens*), pepper (Capsicum annum) and eggplant (*Solanum melongena*) (Shah, *et al*., 2013). It ranks first among plants widely grown in the world, and accounts for over 14% of the world's fruit production (FAO, 2011). According to the Food and Agriculture Organisation Corporate Statistical Database (FAOSTAT), China is the leading tomato producer in the world, followed by India, United States, Turkey, Egypt, Iran and Italy with these countries accounting for more than 80% of global tomato production (FAO, 2011). Egypt is the only African country amongst the top ten world's tomato producers (DAFF, 2015). In South Africa, tomatoes are the second most important and popular crop after potato from the Solanaceae family and also one of the main plants sold on both local and export markets (DAFF, 2015).

Furthermore, the Department of Agriculture Forestry and Fisheries (DAFF) reported that 75% of the tomatoes in South Africa are produced in the northern areas of Limpopo province while the remaining 25% are produced between Onderberg area of Mpumalanga province and the border area of the Eastern Cape province (DAFF, 2015). Tomato is a popular crop choice for smallholder farmers in South Africa because of high demand for the fresh products as well as the opportunities it presents for industrial processing (Tshiala and Olwoch, 2010). There are approximately 695 tomato producers in both the commercial and emerging sectors (DAFF, 2015).

The commercial sector contributes 95% of the total produce while the emerging sector contributes 5% only. Thus, tomato production can serve as a source of income by creating jobs for both rural and peri-urban residents and thereby improve the livelihoods of small-scale producers (DAFF, 2015). After tomato production, the farmers harvest tomatoes and sort them into two classes namely A-grade (marketable) and B-grade (non marketable) tomatoes according to size and quality (Pienaar, 2014). A-grade tomatoes are medium to large in size with a presentable appearance, having no pest affected or deformation marks, while B-grade tomatoes are characterised by small fruits with pest-affected areas (Parfitt *et al*., 2010). Smallholder farming system results in B-grade tomatoes due to certain challenges (Jovanovic *et al*., 2018; Parfitt *et al*., 2010; Pienaar, 2014).

It's possible to eat tomatoes both raw and cooked, making them a popular fruit. Lycopersicum Esculentum is a plant in the Solanaceae family with the botanical name Lycopersicum Esculentum. Vitamin A and vitamin C are abundant, as are carbohydrates, proteins, lipids, fibers, and potassium. It contains a lot of lycopene, which provides a lot of health benefits. It includes a considerable volume of water, making it more prone to deterioration due to bacterial action. Tomatoes have a lower sugar concentration than other fruits, making them less sweet (André, *et al*., 2017). Food deterioration refers to a variety of changes in food that make it less appealing or even harmful to consumers. Changes in fragrance, taste, appearance, or texture may accompany these changes (Akinmusire, 2011). Vitamins B, C, and E are abundant in this fruit. Carbohydrates like fructose and glucose, as well as trace minerals like iron, copper, zinc, and dietary fiber, are all essential nutrients for human health. Due to their high-water content, tomatoes are more susceptible to microbial degradation (Obunkwu *et al*, 2018). Tomatoes are very important for their dietary needs, and they can be consumed in a variety of ways. They can

be cooked as a vegetable, used as an ingredient in a variety of recipes and sauces, used to make stew, fruit juices, and eaten raw in salads (Onuorah and Orji, 2015). Tomato rotting refers to when the quality of tomatoes deteriorates due to a combination of biological and physical reasons. The fruits' taste, smell, look, or texture may alter as a result of these modifications. In 2015, Onuorah and Orji. According to estimates, nearly a third of the produce is wasted before it reaches the consumer (Mbajiuka and Emmanuel, 2014). This loss has been linked to a variety of reasons, including physical (mechanical breakage, bruising) and microbiological (fungi and bacteria) damages (Onuorah and Orji, 2015). Tomatoes are prone to deterioration during storage, shipping, and waiting to be processed. The microbial degradation of tomato fruits lowers the market value and nutritional quality of the product. Contaminations with mycotoxins, which form aflatoxins in humans after inhalation or ingestion, render tomato fruits unfit for consumption, resulting in food poisoning (Bello *et al*., 2016). Some research has been done to discover bacteria and fungi that are linked to tomato rotting. *Bacillus subtilis, Klebsiellaaerogenes, Pseudomonas aeruginosa, Salmonella typhi, Proteus mirabilis*, and *Staphylococcus aureus* were isolated from spoiled tomatoes in Benin City by Wogu and Ofuase in 2014. In Lagos State, Nigeria, a similar study found significant amounts of *Staphylococcus* spp, *Bacillus* spp, and *Escherichia coli* (Ogundipe *et al*., 2012). Rhizopussp have been linked to tomato rotting, according to Akinmusire (2011).

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). 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. The high water content of tomatoes makes it more susceptible to spoilage by the action of microorganisms (Obunkwu *et al*, 2018). 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 (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. (Onuorah and Orji, 2015). 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).

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. Spoilage refers to any change in the condition of food in which the food becomes harmful for human consumption. The susceptibility of tomatoes to microbial spoilage is largely attributed to its high moisture content. In northern Nigeria, freshly harvested tomato fruits are stored, 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).

Tomatoes are susceptible to fruit spoilage caused by numerous fungal pathogens (Barkai-Golan and Paster, 2008; Samuel and Orji, 2015; Sanzani *et al*., 2016; Tournas and Katsoudas, 2005). Examples of fungal induced tomato diseases include Alternaria rot caused *by Alternaria solani and Alternaria tenuis, Phytophthora* rot caused by *Phytophthora infestans and Phytophthora nicotianae var. parasitica*, Anthracnose ripe rot caused by *Colletotrichum phomoides, Phoma* rot caused by *Phoma destructiva* and *Fusarium* rot caused by Fusarium spp. (Wani, 2011). Diseases caused by the above-mentioned fungi may be due to large nutrient composition or other factors such as high water content and low pH (Droby *et al*., 1992). These factors may make the produce to become highly susceptible to pathogenic attack. Diseases reduce the yield of tomatoes by up to 25% in industrialized countries and more than 50% in developing countries (Nunes, 2012). However, there are numerous methods for preventing diseases in fruits and one of them is the use of fungicides. The use of synthetic fungicides has been a primary method for managing the postharvest spoilage of tomatoes (Spadaro and Gullino, 2004). However, there are increasing concerns over fungicide use such as environmental pollution risks, inability to control fungal diseases due to fungicide resistance, and persistence of fungicide residues on the tomato (Ippolito and Nigro, 2000). All those challenges have resulted in the search for safe and effective

alternative strategies for the control of plant pathogens (Liu *et al*., 2013). Such strategies include biological control (such as the microbial antagonists) of fungal pathogens in tomatoes using naturally occurring microorganisms (Droby *et al*., 2009). Moreover, this biological control is effective, nontoxic and environmentally friendly alternatives to fungicides (Janisiewicz and Korsten, 2002).

1.1 AIM AND OBJECTIVES

The aim of this study was to evaluate microorganisms associated with spoilage of tomatoes

The objectives are:

1. Enumeration of bacteria and fungi from tomatoes
2. Characterization and identification of bacterial and fungal isolates
3. Antibiotic profiling of bacterial isolates

CHAPTER TWO

LITERATURE REVIEW

2.1. A brief description of the tomato

Tomato (*Lycopersicon esculentum*) is one of the most important vegetables worldwide. It is a self-pollinated fruit that belongs to the Solanaceae family (Arah *et al*., 2015). The family also includes plant drugs such as Tobacco (*Nicotiana tabacum*), deadly nightshade *(Atropa belladonna*), mandrake (*Mandragora officinarum*), jimson weed (*Datura stramonium*) and petunia (Petunia hybrida) (Shah *et al*., 2013). Tomato is widely cultivated in tropical, sub-tropical and temperate climates and is ranked third in the world for vegetable production (FAO, 2011). Tomato is known by different names worldwide, for example, tomate (German, France), tomati (West Africa), tomaatti (Finish), tomat (Indonesia), pomidoro (Italy), kamalis (Malay), jitomate (Spain, Mexico), pomidor (Russia), faan ke'e (China), tomatl (Nahuatl), nyanya (Swahili) and tamatar (Hindi) (Naika *et al*., 2005). Tomato originated in the South America Andes, in the mountains of Peru (Shnain *et al*., 2017). It was taken to other parts of the world by the early travelers where it was planted as an ornamental curiosity but not eaten (Arah *et al*., 2015). By 500 BC it had been moved to Mexico for the purposes of domestication. Tomato was brought to Europe in 1554 by the Spanish conquistadors. It was later cultured in the U.S. in 1710, and introduced from Europe into southern and eastern Asia, Africa and the Middle East. Thereafter, tomato became popular and was exported around the world by 1850 for commercial production (Shnain *et al*., 2017).

2.1.1. Scientific classification of tomato

Kingdom: Plantae

Sub kingdom: Tracheobionia

Class: Magnoliopsoda

Sub class: Asterielae

Order: Sultanates

Family: Solanaceae

Genus: Lycopersicon

Species: esculentum

2.1.2. Different botanical varieties of tomato

There have been numerous changes to the botanical name for tomato. For several years it was known as Solanum lycopersicum, which later changed to Lycopersicon esculentum (Naika *et al*., 2005). Tomato is a true diploid with 2n = 24 (Stack and Anderson, 1986). The plant is annual with a herbaceous prostrate stem having determinate or indeterminate growth habit (Naika *et al*., 2005). Tomato has three vine types, namely, indeterminate (sprawling, staggered ripening and tall type), semi-determinate (intermediate response and semi-bush type) and determinate (compact, uniform ripening and bush type) (Naika *et al*., 2005). There are approximately 7 500 tomato varieties which are grown for various purposes (Berrueto, 2017). Tomato varieties can be divided into several categories, based on shape and size. These categories include slicing or globe (also known as round tomatoes), beefsteak (large tomatoes), plum (bred for higher solids) and also grape (smaller variation of a plum tomato) (Berrueto, 2017).

2.1.3. The economic, health and nutritional values of tomatoes

Tomato has become an important cash and industrial crop in many parts of the world. This is not only because of its economic importance but also its nutritional value in the human diet and subsequent importance for human health as a result of the essential nutrients it provides (Ayandiji and Adeniyi, 2011; Yadav *et al*., 2017). It is also a versatile crop that can be classified according to use into two categories as fresh market tomatoes for direct consumption and processing tomatoes which are cultivated for industrial canning and processed foods, respectively (Osman, 2015). Tomato is rich in vitamins A, B, C and E; carbohydrates such as fructose and glucose; 10 minerals such as phosphorus, sodium, potassium, calcium and magnesium and trace elements such as iron, copper, zinc and dietary fibers (Ayandiji and Adeniyi, 2011; John *et al*., 2016; Yadav *et al*., 2017). It therefore serves as a source of essential nutrients when consumed (Arah *et al*., 2015; Guil-Guerrero and Rebolloso-Fuentes, 2009). An average size (70 - 150 g weight and 50 - 70 mm diameter) tomato fruit contains energy (18 kcal), protein (0.95 g), fat (0.11 g), carbohydrate (4.01 g), total sugar (2.49 g), niacin (0.731 mg), calcium (11.0 mg), iron (0.68 mg), magnesium (9.0 mg), phosphorus (28.0 mg), potassium (218.0 mg), sodium (11.0 mg), zinc (0.14 mg), thiamin (0.036 mg), riboflavin (0.022 mg), carotene (vitamin A) 320 IU, vitamin B (60.079 mg), vitamin C (16.9 mg), and ascorbic acid (31 mg) per 100 g pulp of fruit (Arah *et al*., 2015; Yadav *et al*., 2017). Tomatoes are ready-to-eat food, and are thus minimally processed (John *et al*., 2016). They are consumed in various ways such as raw in salads and sandwiches, cooked or processed in ketchup, sauces, soup, chutney, pickles, paste, puree, juices, dried powder and whole canned fruits, while it also forms an important ingredient in the cocktail known as a Bloody Mary (Ayandiji and Adeniyi, 2011; Chaudhary, 2014; Yadav *et al*., 2017). The deep-red coloration of the ripened tomato is due to

the high amount of lycopene, a form of B-carotenoid pigment and a notable antioxidant that is beneficial in reducing the incidence of certain chronic diseases such as prostate cancer, cardiovascular disease and diabetes (Ram *et al*., 2014; Wu and Tanksley, 2010). Tomato juice promotes gastric secretion, acts as a blood purifier and works as an intestinal antiseptic (Chaudhary, 2014). Tomatoes are good sources of vitamin C and vitamin A which are vital in warding off muscular degeneration and improving eyesight. It is also believed to be a powerful blood purifier and clear up urinary tract infections. Tomatoes are high in fiber which aids easy digestion and may assist in weight loss (Arah *et al*., 2015). 11 Tomatoes have numerous advantages that make them economically important (Naika *et al*., 2005). These advantages include the following: relatively short-duration vegetable crop, short production period, growth as an uncovered field crop and in protected cultivation, easy fitting into different cropping systems, high economic value, and high micronutrient content (Naika *et al*., 2005).

2.2. Smallholder agriculture leading to tomato contamination

One of the agricultural pathways towards sustainable food and nutrition security is through the local production of food, where smallholder farmers play a crucial role (Dorward *et al*., 2005; Maliwichi *et al*., 2014; Wiggins and Keats, 2013). The value of smallholder agriculture is being recognized in the developing countries and, hence, governments are implementing programs in agricultural development that are leading to the empowerment of the smallholder farmers (Aliber and Hall, 2012). A smallholder farmer is categorized as a farmer that owns small plot of land whereby crops are grown mainly to support the family. Depending on the yield produced smallholder farming can range from subsistence to commercial (Raphela, 2014; Shao *et al*., 2004; Thamaga-Chitja and Morojele, 2014). Smallholder farmers play a significantly positive role in

poverty alleviation and household food security (Shao *et al*., 2004; Thamaga-Chitja and Morojele, 2014; Wiggins and Keats, 2013).

According to Poulton *et al*. (2006), the productivity of smallholders in agriculture contributes to an increase in market profits, encourages a reasonable supply of income and creates both the backward and forward linkages necessary for economic growth (Raphela, 2014; Thamaga-Chitja and Morojele, 2014). According to Van Averbeke and Mohamed (2006), there are three different types of smallholder farmers:

I. Subsistence farmers – These farmers produce for household consumption with very limited sales. They make up the majority of the small-scale farmers.
II. Emerging smallholder farmers – These farmers wish to work increasingly towards commercializing their production.
III. Commercial smallholder farmers – These farmers receive an income from the sale of their produce. They constitute the minority of the small-scale farmers.

2.3 MICROORGANISMS ASSOCIATED WITH SPOILAGE OF TOMATOES

Chandu et.al. (2016) conducted a study on tomatoes, in his research, he used the serial dilution approach to generate different sample solutions in different concentrations. After being injected with several specialized media for the identification of organisms, he conducted research and discovered a specific organism *Bacillus substilis, Aspergillus niger* (Chandu *et.al.,*2016). In his prior research, he produced specialized media potato dextrose agar for the identification organism, and in his investigation, he discovered the unique organism *Aspergillus fiavus, Penicillium Waksmani, Botryodiplodia theobromae, Fusarium Oxysporum, Colletrichum asianum*. (Kator *et.al*., 2018) Previous research was conducted in Kebbi State's Alerio Local

Government Area. He made potato dextrose agar and nutritional agar in his lab. To isolate the fungus and bacteria, they used the spread plate and streak plate methods. During his research, he discovered this unique organism Candida species, *Penicillium digitatum, Aspergillus niger, Alternaria, Mucor species Trichoderma* species *Saccharomyces cerevisiae*. (Aminu *et.al*.,2021) In Benin City, serious research was conducted in four markets: Oba, New Benin, Santana, and Vegetable. He created sample solution in concentration up to 10 in serial dilution for this study. He also inoculated numerous specialized media for the identification of organisms, and through his research, he discovered this particular organism *Bacillus subtiles, B. cereus, B. Aureus, Escherichia coli, Klebsiella aerogenes, Pseudomonas aeruginosa, Salmonella typhi, Proteus mirabilis* and *Staphylococus aureus*. (Wogu *et.al*., 2014) Previous research was conducted in the Nigerian city of Awka, in the state of Anambra. They employed the corplate method, in which a serially dilute sample was dispensed into a conical flask containing strial sabouraud dextrose agar and chloramphenicol was added to suppress bacterial growth. After inoculating several biochemical media in search of an identification organism, he studied and discovered this unique organism *Aspergillus niger, Rhizopus stolonifer, Fusarium oxysporum, Saccharomyces cerevisiae, Alternaria alternate, Penicillum digitatum, Geotrichum candidum* (Samuel and Orji, 2015)

2.3. Tomato production

On a global scale, the annual production of fresh tomatoes amounts to approximately 159 million tons with more than a quarter of these 159 million tons grown for the processing industry, thus making tomatoes the world's leading vegetable for processing (Noonari *et al*., 2015). Tomato is cultivated in both the tropics and subtropics of the world and is also cultivated in kitchen gardens, commercial fields under greenhouse and polyhouse conditions and soil-less

culture or hydroponic systems (Chaudhary, 2014). Although the root structure of a tomato plant is able to penetrate various soil types up to depths of two meters, the highest percentage of the roots will be found in the top 600 mm of the soil. Tomatoes are grown and produced optimally when the mean temperatures are between 20 °C and 24 °C. When average daily temperature is above 32 °C and the night temperature fails below 21 °C the fruit set is poor (Starke, 2014). It takes tomato plants three to four months to bear fruits that are ready for harvesting. Tomato planting involves different techniques and methods for determinant (generally grown under open field condition) and indeterminate (normally grown under poly-house condition) varieties (Yadav *et al*., 2017).

Moreover, tomato can grow well in soil, organic substrates, soilless mixes, perlite, sand or hydroponics (Shamshiri *et al*., 2018). Tomato industry may also increase the foreign export earnings of many African countries, thereby contributing to their gross domestic product (GDP) (Chandio *et al*., 2016). Studies have shown that the full potential of the crop has been under exploited as a result of the many challenges involved in tomato production (Geoffrey *et al*., 2014; Jayne *et al*., 2010). These challenges include physical infrastructure (poor roads, transport and telecommunications), long production and exacerbating risks, lack of land policy (farmers have no rights to the land they farm), social constraints (the role of women farmers in agricultural production tends to be underestimated), and lack of investment (low output prices, high cost of inputs and limited access to credit make it difficult for smallholder farmers to produce sufficient food efficiently). Other challenges include, environmental constraints (climate change and its related impacts on food production), production constraints (very low average production due to the rain-fed crops and cultivation using unsuitable agricultural practices that increase soil erosion, thereby resulting in low yields), lack of post-harvest processing, inadequate storage facilities and

marketing systems (which leads to post-harvest losses of the produce) and pre-harvest losses (Aliber and Hall, 2012b; Arah *et al*., 2015; Dorward *et al*., 2005; Ortmann and King, 2007). To mitigate some of the challenges, quality management practices should be put in place. The quality management starts in the field and continues until the produce reaches the end user (Albrigo, 1978). Understanding and managing the various roles that pre-harvest factors play in 16 qualities are very important in the maximum harvest and post-harvest quality of any crop (Meaza *et al*., 2007). Generally, pre-harvest conditions are known to be important in determining storage performance (Zhao *et al*., 2011).

In some instances, their effects may even be greater than the effects of the adjustment of the storage environment. To date, pre-harvest treatment recommendations for fruits and vegetables have been established primarily in order to enhance productivity, and not as diagnostics for good quality, nutritive value and optimum shelf life (Miglioria *et al*., 2017). As a result, the need for the integration of pre- and post-harvest treatment for the improvement of shelf life remains critical. Post-harvest losses (PHLs) are measured qualitatively and quantitatively along the supply chain, from the beginning of the harvest period until the product is either consumed or used (Hodges *et al*., 2011). The qualitative losses include reduction in nutrient value and change in the color, taste, and texture of food whereas the quantitative losses refer to the decrease in the volume and weight of food (Buzby and Hyman, 2012). Post-harvest losses result primarily from physiological, physical and environmental factors, namely, high crop perishability, mechanical damage, humidity, rain and excessive exposure to high ambient temperature. It is also caused by inappropriate post-harvest handling, poor infrastructure, poor marketing systems, pests (birds, rodents, insects), disease attack (contamination by spoilage fungus and bacteria), insufficient

transport facilities, storage and the processing techniques in relation to the product between the farm and distribution (John *et al*., 2016).

The extent of these losses often depends on the relative vulnerability of the product to physical damage (Kitinoja and Kader, 2015). Total yield of crops is known to reduced due to postharvest diseases, in fact Naureen *et al*. (2009) stated that post-harvest diseases destroy the total yield of crops by 10 to 30% globally. While in developing countries postharvest diseases destroy more than 30% of the yield perishable crops, and much less is recorded in developed countries (Fatima *et al*., 2009; Rehman *et al*., 2007; World Bank, 2011). Post-harvest losses (PHLs) in tomatoes may be as high as 25 to 42% globally (Rehman *et al*., 2007). Estimations on PHLs for Africa are often between 20 to 40% (World Bank, 2011). In 2011, PHLs were valued at USD1.6 billion per year in the eastern and southern regions of Africa (World Bank, 2011). Mandiriza-Mukwirimba *et al*. (2016) reported that approximately 61.3% of the farmers in South Africa were not using chemicals to control diseases, compared to 38.7% of farmers who were using such chemicals. The increase in food losses due to PHLs has a negative impact such as low returns to farmers, processors, consumers and traders, as well as the country as a whole, which is adversely affected in terms of foreign exchange earnings (FAO, 2011). The post-harvest potential of tomatoes not only depends upon post-harvest handling but may also depend on pre-harvest factors such as cultural practices (nutrient, water supply and harvesting methods), genetic and environmental conditions and also biotic, chemical and hormonal factors (Leonardi *et al*., 2000). Quality management of handling fruits starts in the field and continues until the product reaches the end user (Meaza *et al*., 2007). Numerous microbial defects (signs and symptoms) of tomatoes are characterized by the type of microorganism responsible for the deterioration in the process of infection which, in the case of fungal invasion follows the development of the fungal penetrating

structure (John *et al*., 2016). The susceptibility of tomato to microbial colonization is due to its differential chemical composition such as a high level of sugar, low pH (4.9-6.5) and its high-water activity which favors the growth of microorganisms (John *et al*., 2016). Fungi are the most important and prevalent pathogens, infecting a wide range of fruits and causing destructive and economically important losses in fruits during storage, transportation and marketing (Etebu *et al*., 2013).

2.4. Fungal diseases of tomatoes

In total, there are more than 200 species of fungi that may infect the tomato crop, with diseases often being the limiting factor in tomato production (Agrios, 2004; Suprapta, 2012). The epidemics of a disease depend on complex interactions between host, pathogen and environment as well as cultural practices such as fertilization and irrigation (Osman, 2015; Aust and Hoyningen Huene, 1986). Plant pathogens use different strategies to survive and spread to new hosts (Osman, 2015). Most pathogens have a life cycle that includes both plants and soil, although they usually need to infect a specific host to increase their population (Abdul-baki, 1996; Berlin, 2005). Fresh vegetable fruits are fairly perishable because their high moisture content renders them vulnerable to microbial diseases as well as to physiological deterioration (Deribe *et al*., 2016; Naika *et al*., 2005; Osman, 2015; Peet and Welles, 2005). A lack of adequate pre-harvest and post-harvest handling factors may lead to diseases such as those caused by certain pests, namely, Aculops lycorpersici (causes rusty brown and coarse surface cracking), and Thrips tabaci (causes blossom drop and scarring of the fruit) as well as some virus species such as fruit necrosis caused by the Tomato marchitez virus (ToMarV), fruit marbling caused Pepino mosaic virus (PepMV) (Hanssen, 2010). There are also some bacterial diseases such as

bacterial speck caused by Pseudomonas syringae, bacterial wilt (Rhizopus solanacearum), bacterial Spot (Xanthomonas campestris) and bacterial canker (Clavibacter michiganensis) (Rashid *et al*., 2016). Tomatoes are also affected by the physiological disorders such as blossom end rot which is caused by a shortage in the availability of calcium, and growth cracks caused by the fruit expansion which stretches the epidermis (skin) beyond its capacity, as well as diseases caused by viruses such as the tomato mosaic virus which have been reported on tomato (Arli-Sokmen and Sevik, 2006; Kennelly, 2009).

In addition, many of the smallholder farmers in South Africa encounter attacks of pathogenic fungi because they possess inadequate technical information, in particular relating to crop diseases (Mandiriza-Mukwirimba *et al*., 2016). It has been reported that the highest percentage cause of the PHLs of tomato fruit are associated with different species of soil-borne phytopathogenic fungi (Etebu *et al*., 2013; Fatima *et al*., 2009). These species cause diseases such as early blight (Alternaria solani), anthracnose (Colletotrichum spp.), Sclerotium wilt (Sclerotium rolfsii), damping off (R. solani), tomato wilt (Fusarium oxysporum), Phoma rot (Phoma destructive), Fusarium wilt (Fusarium oxysporum), late blight wilting (Phytophthora capsici), Septoria leaf spot (Septoria lycopersici) and Rhizopus rot (Rhizopus stolonifer) (Fatima *et al*., 2009; Ignjatov *et al*., 2012; Kleemann *et al*., 2008; Kumar *et al*., 2008; Osman, 2015).

These pathogens are severe wound pathogens that may infect the fruit in the packing house, and throughout subsequent handling or storage, thereby limiting production and reducing both crop yield and crop quality (Palou *et al*., 2008). Pathogenic microorganisms in tomato are recognized as a source of potential health hazard to both man and animals following ingestion as a result of their production of mycotoxins, which are capable of causing diseases such as respiratory infection, meningitis, gastroenteritis and diarrhea in man (Beuchat, 2006).

2.5 PREVENTION AND CONTROL OF MICROBIAL SPOILAGE

To control germs on fresh-cut food, a variety of thermal and non-thermal treatments have been developed. Hot water, heat steam, and hot sanitizing solution are all examples of thermal processing used to treat fresh-cut vegetables. In the fresh-cut produce industry, thermal processing is a relatively recent technology. Physical and chemical processing technologies are two types of non-thermal processing. High pressure, irradiation, pulsed electric fields, pulsed white light, ultrasound, and ultraviolet radiation are examples of physical technology. Some of these approaches are not economically viable due to their high cost (high pressure and pulsed electric fields), lack of consumer acceptance (irradiation), or the need for process confirmation of efficacy (UV and pulsed white light) (Ohlsson and Bengtsson 2002). Based on the physical condition of the chemical utilized, chemical methods can be separated into gas-phase sanitation and liquid phase sanitation. Ozone and chlorine dioxide are examples of gas-phase sanitation. One of the challenges in implementing gas phase technologies is that the treatment of produce necessitates the use of a unique in-line closed system. Employee safety may be jeopardized by these applications. Chlorinated water is the most extensively utilized chemical treatment in the fresh-cut produce business. Raw material quality, processing technology, good manufacturing practices (GMP), packing, and temperature management are all essential factors in preventing microbial spoilage in addition to these active control techniques. The use of high-quality raw materials can lower the risk of surface contamination while also enhancing the plant's self-defense mechanism. Current techniques of prevention and treatment make it difficult to sanitize diseased or damaged products, and they can contaminate products with low quantities of microorganisms. Yeast populations were observed to be substantially greater on cut honeydew melons with soft tissue during storage than on firm honeydew melons (Zhuang H *et al*. 2003).

The increase in respiration just prior to full ripening in climacteric fruits is well known to coincide with a significant decline in fruit resistance to pathogens.

2.6. Current methods to control post-harvest losses

The response of tomatoes during storage and the post-harvest qualities depend to a certain extent on pre-harvest factors such as cultural practices, the use of natural plant extracts, fertilizers, manure, and genetic and environmental conditions (Meaza *et al*., 2007; Pretorius *et al*., 2003). The losses of untreated fruit from fungal decay have been estimated to be as high as 90% during 20 post-harvest handling and marketing (Albrigo, 1978). Nevertheless, decay in tomato fruits can be controlled by various methods that are explained below.

2.6.1. Physical control

Controlling the storage temperature is the most well-known physical treatment. Such treatment may be applied in the form of a hot water dip, hot water rinsing and brushing, vapor, hot air and curing (Conway *et al*., 2004; Fallik, 2004). The temperature is calculated using an adaptive management framework and the TOMGRO model (Jones *et al*., 1992; Shamshiri *et al*., 2018). During the entire tomato growing season, optimal air temperatures from 18 to 32.2 °C are considered with 50 to 70% humidity (Peet and Welles, 2005; Shamshiri *et al*., 2018). In the green house, the cultivation of tomato temperature is maintained at 17 to 28°C in coastal areas and 17 to 22 °C in inland areas with 85 to 95% humidity (Puyaubert & Baudouin, 2014). During storage, temperature greatly encourages the rate of respiration of fruits and vegetables, and is certainly one of the most important factors in maintaining the post-harvest quality of tomato fruits (Žnidarčič *et al*., 2010). The chilling injury and ripening rate is minimal at 10 to 15 °C

temperature and 85 to 95% relative humidity which may extend the postharvest life of fruits (Žnidarčič *et al*., 2010) . Ultraviolet light (UV-C, 254 nm) hormesis has been identified as one of the physical methods which may be used to stimulate positive responses in order to induce resistance to storage diseases and extend the shelf-life of fruits and vegetables (Liu *et al*., 1993).

Tomatoes are treated with UV-C doses from 1.3 to 40 KJ/m2 in order to induce resistance to the various fungal pathogens that lead to spoilage (Buzby and Hyman, 2012; Tang *et al*., 2015; Vaklounakis, 1991). However, it must be noted that the use of temperature and UV-C lights during storage changes the aroma profile and the taste of fruits after six days of storage (Baloch and Bibi, 2012; de LeónSánchez *et al*., 2009; Ponce-Valadez *et al*., 2016).

2.6.2. Chemical control

Strategies such as synthetic fungicides and pesticides applications, resistant-variety cultivation and crop rotation are used to control fungal diseases in crops with pesticide application remaining as the most common control strategy (Gao *et al*., 2017). These strategies are fairly inexpensive, easy to apply and demonstrate both curative and preventive actions against various infections. The azoxystrobin, fludioxonil, and pyrimethanil fungicides were introduced for the post-harvest management of citrus mold (Kanetis *et al*., 2007). These are also chemicals such as spore kill, vinclozolin, copper oxychloride, benomyl and kitazin that are being used against various fungal pathogens that cause spoilage in fruits and vegetables (Amini and Sidovich, 2010; Lee *et al*., 2012; Leroux, 2007; Nel *et al*., 2007; Sahu *et al*., 2013; Stansly *et al*., 2004). However, the intensive use of synthetic pesticides and fungicides may cause pathogen resistance and pesticide residues and release fungicides in the environment (Ma *et al*., 2015; Yang *et al*., 2015). Their use is becoming more restricted because of the concerns of the consumers and the administration about human health (De Curtis *et al*., 2010; Usall *et al*., 2016). Moreover,

effective chemical treatments cannot inhibit the growth of some plant diseases and consumers are increasingly demanding pesticide-free food Wang *et al*., 2009).

2.6.3. Biological control

The non-biodegradable nature and the environmental pollution caused by chemical control applications have led to the alternative production of naturally derived substances (Migliori *et al*., 2017). Among these alternatives, biological control using microorganisms with a strong fungal activity such as growth and ecological fitness has been identified (Pal and Gardener, 2006; Shafiq, 2015; Zong *et al*., 2010). There are mechanisms that have been suggested as being liable to the antagonistic activities of biocontrol agents, including competition for nutrients and space, mycoparasitism of the pathogen, emission of antifungal compounds, antibiotics, volatile 22 metabolites, induction of host resistance, biofilm development and the participation of the reactive oxygen species (ROS) in the defense response (Dukare *et al*., 2018; Liu *et al*., 2013).

These biocontrol agents are safe for the environment, they improve crop production and they limit pesticide resistance (Khonglah and Kayang, 2018; Shafiq, 2015). The successful application of these agents, by either spraying, dipping or drenching, occurs during the postharvest period (Di Francesco *et al*., 2016; Liu *et al*., 2013). The antagonists used to manage postharvest diseases include bacteria and yeast and it is only recently that fungi have been reviewed as well (Liu *et al*., 2013; Lledó *et al*., 2016; Nunes, 2012). Antagonism is a phenomenon whereby a microorganism inhibits the growth or interferes with the development of another microorganism (Liu, *et al*., 2013; Rodrigo *et al*., 2017). Fungal antagonists such as *Debaryomyces hanseniis, Candida guilliermondii, Byssochlamys spectabilis, Trichoderma harzianum, Trochoderma viride, Phythium debaryanum, Gliocladium roseum, Aureobasidium pullulans, Phythophthora cryptogea* and *Cryptococcus laurentii* are among the effective

antagonists that have been identified as the best alternatives to monitor postharvest diseases on citrus fruits (Agrios, 2004; Castoria *et al*., 2001; De Curtis *et al*., 2010; Gomathi and Ambikapathy, 2011; Naglot *et al*., 2015; Zong *et al*., 2010).

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CHAPTER THREE

MATERIALS AND METHODS

3.1 SAMPLE COLLECTION

Samples were obtained from five markets in Benin city, Edo state and were transported aseptically to the Microbiology laboratory in University of Benin for microbial analysis.

3.2 Culture Preparation and Sterilization

Manufacturers' specification was strictly adhered to when culture media used for this research were being prepared. The media were also sterilized following specification at 121ºC at 15 psi for 15 minutes unless otherwise stated by manufacturer.

3.3.1 POTATO DEXTROSE AGAR (PDA)

PDA medium (39g) was dissolved in 1000ml of distilled water in a conical flask then closed with a cork stopper. The suspension was first dissolved completely by shaking and then sterilized by auto cleaving at 121°c for 15 minutes. The medium was allowed to cool then dispensed aseptically into sterile petri dishes. The petri dishes were covered and allowed to solidify.

3.3.2 NUTRIENT AGAR (NA)

Nutrient (28g) was dissolved in 1000ml of distilled water in a conical flask then closed with a cork stopper. The suspension was first dissolved completely by shaking and then sterilized by auto cleaving at 121°c for 15 minutes. The medium was allowed to cool then dispensed aseptically into sterile petri dishes. The petri dishes were covered and allowed to solidify.

3.4 Enumeration and isolation of total heterotrophic bacterial and fungal count

Ten-fold serial dilution of the samples were prepared aseptically in sterile physiological saline. Samples were collected in sterile bottles and a stock (10-fold) was prepared by dissolving 10g of tomatoes in 90 ml sterile saline water (SSW) or normal saline. The samples were serially diluted with a factor of 10 until a thousand dilution fold (1000) was attained. An aliquot of 0.1 ml was inoculated using the pour plating technique. Appropriate media were used for fungal and bacterial enumeration. Nutrient agar (supplemented with fluconazole) for bacteria and potato dextrose agar (supplemented with chloramphenicol) for fungi. Plates were cultured at 37±2ºC for 24 hours. The number of colony forming unit per milliliter (cfu/ml) was calculated using the formula below:

$$\frac{cfu}{ml} = \frac{number\ of\ colonies\ x\ dilution\ fold/series}{volume\ of\ inoculum}$$

(Willey *et al.,* 2008)

3.5 Phenotypic Identification of Bacteria from Samples

Following successful pour plate technique, isolation and culture was made from a single colony and characterized using cultural, morphological and biochemical methods using the Bergey's manual. Several tests such as Gram reaction, catalase, urease, indole, oxidase, sugar fermentation, citrate utilization, respective reaction on triple sugar iron agar tests were carried out to presumptively identify bacterial isolates (Holt *et al*., 1994).

3.6 Morphology identification

The morphological identity of each bacteria isolate was obtained by Gram staining so as to know the gram reaction, cell morphology and arrangement by viewing under the microscope. The Gram stain procedure is as follows:

A smear of the bacteria isolate was made on grease free slide and heat fix by passing over flame. The smear was flooded with crystal violet which is the primary stain for 1min then washed with distilled water.

Subsequently the slides were flooded with Lugol's iodine solution for 30sec and then washed off with distilled water.

95% alcohol was used for decolorization for 10sec and immediately washed off with distilled water.

Finally, the smear was counter stained with safranin for 1min and washed off.

The slides were allowed to air dry before observing under the microscope using an oil immersion objective lens of ×100 magnifications to view the slides.

3.7 Biochemical identification

Biochemical test was carried out so as to help in the identification of the bacteria isolates as phenotypic (cultural) characteristics is not sufficient. The various biochemical test carried out are shown below;

3.7.1 Oxidase test

This is mainly used to differentiate between *pseudomonas* from other gram-negative rod bacteria. Oxidase test was carried out to identify bacteria species that will produce cytochrome oxidase enzyme. *Staphylococcus aureus* and *Escherichia coli* which are gram positive and gram negative respectively were employed as control. A piece of filter paper using sterilizes wire loop 2-3

drops of freshly prepared oxidase reagent (1% aqueous tetramethyl-3-phenyl nediamine dichloride) was added. A positive oxidase test is indicated by purple coloration within 10 seconds.

3.7.2 Urease test

This is used to test organisms that have the abilities to produce the enzyme urease which catalyzes the breakdown of urea to produce ammonia. The test is usually used to differentiate organisms like *Proteus mirabilis* from other non-urease positive organisms. A sterilized medium was dispensed into test tubes aseptically and the test bacteria isolated were inoculated into the medium and incubated at 37 degrees centigrade for 24 hours. A change in color from yellow to red-pink confirmed the presence of urease.

3.7.3 Indole production test

This test was used to determine which of the isolates has the ability to split indole from tryptophan present in peptone water. The best is usually used in differentiating gram-negative bacilli especially those of Enterobacteriaceae. Five grams of commercially available peptone broth was dissolved in 1litre of distilled water. The medium was then sterilized by autoclaving at 121 degrees centigrade for 15 minutes. The 4 ml of the medium was dispensed into sterile test tube and each of the bacteria isolates was inoculated into the peptone broth. The inoculated media was incubated 37 degrees centigrade for 24 hours after which few drops of KOVAC reagent was added. KOVAC reagents consist of 150ml of amyl alcohol, 10g dimethylamino benzaldehyde and 150ml of concentrated hydrochloric acid. Positive test was indicated by the red coloration that occurs immediately at the upper part of the test tube.

3.7.4 Citrate utilization test

This test is used to identify which of the isolate can utilize citrate as the sole source of carbon for metabolism. The medium used for this test is Simon's citrate agar. In the preparation, 22 grams of commercially available Simon's citrate agar was dissolved in liter of distilled water and sterilized by autoclaving at 121 degrees centigrade for 15 minutes. The medium is dispensed into test tubes and the test organism was inoculated by stabling the medium on the tubes using sterile straight inoculation wire containing culture. The tubes were incubated at 37 degrees centigrade for about 24 hours. Positive result is indicated by a change in color from green to bright blue coloration.

3.7.5 Catalase test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyzes the breakdowns of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme positive.

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

3.7.6 Sugar fermentation and production of gases using Triple sugar iron agar (TSI)

TSI was prepared following manufacturer's instruction and the prepared media was placed in a test tube and kept in a slant position for it to solidify. The slant and butt of the medium was inoculated with the test bacterium using a sterile loop and it was incubated for 18- 24 hr. The results were read on the basis of acid or alkaline production in the slant or butt region of the tube and gas production was confirmed by the presentence of crack or air bubbles in the slant or but region. More so, production of hydrogen sulphide was confirmed by the blackening of the

medium. A prepared laboratory chat was used for result interpretation in line with microbiological standard protocol as well as other biochemical tests carried out on the isolates to confirm or ascertain their identity.

3.8 Antibiotic susceptibility test

The bacterial isolates were subjected to commonly used antibiotics in Nigeria using Kirby Bauer agar disc diffusion technique as described by Aromolaran and Badejo (2014) and Akinyemi *et al*. (2005). Pure colonies of bacterial isolates were streaked on sterile Muller Hinton agar (MHA) plates and incubated at 37 ºC for 24 h. The bacterial cells were harvested into sterile normal saline solution and standardized using 0.5 McFarland standards. The cultured cells were introduced on the surface of sterile MHA using sterile swab sticks and multi-disc antibiotics were placed on the culture media aseptically and incubated at 37 ºC for 24 h. The antibiotic discs used were ceftazidime (30 µg), cefuroxime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), nitrofurantoin (300 µg), ampicillin (10 µg), and augmentin (30 µg). The diameter of the zones of inhibition around each disc was measured after the incubation period and recorded.

3.9 Enumeration of Fungal Isolates

Samples were collected in sterile bottles and a stock (10-fold) was prepared by dissolving 10g of tomatoes in 90 ml sterile saline water (SSW) or normal saline. The samples were serially diluted with a factor of 10 until a thousand dilution fold (1000) was attained. Thereafter, an inoculum volume of 0.1 ml from the 1000 dilution was transferred unto sterile petri dishes to which was added potato dextrose agar (supplemented with 1% chloramphenicol). Replicates of samples

were prepared for fungal plates cultured using pour plate method and with the formula employed given below in equation (1)

$$Dilution\ factor\ =\ \frac{final\ volume}{aliquot\ volume} \qquad (1)$$

$where: final\ volume = aliquot\ volume\ (sample\ volume)\ + diluent\ volume$

(Ogofure and Igbinosa, 2021)

Enumeration of the bacterial/fungal isolates was carried out using the formula delineated by Willey *et al*. (2008) and it is shown in the equation (2) below.

$$\frac{cfu}{g} = \frac{number\ of\ colonies\ x\ dilution\ factor}{volume\ of\ inoculum} \qquad (2)$$

3.10 Microscopic Identification of fungal isolates

After successful enumeration, the fungal isolates were subcultured on PDA and the molds and yeasts were morphologically characterized after being stained with Lactophenol cotton blue. The results obtained were then compared with standard references for proper identification of the isolates. A drop of lactophenol blue stain was placed on a clean grease free sterilized glass slide and after this a sterile inoculating wire loop was used to pick the mycelium unto the glass slide from the mold culture. The mycelium was spread evenly on the slide and then covered with cover slips gently and then allowed to stay for some seconds before observing under x40 under the microscope. The colonial and morphological characteristics of each isolate were determined, appearance of special structures including the nature of spore/ascospores if produced. The growth, the appearance of the colony from initial to the time of maturity was also taken into consideration as well as the presence or absence of septate hyphae.

3.11 Statistical analysis

The data was analyzed using the SPSS package version 21.0. All data are mean of three replicates. The mean, range and standard deviation of each parameter was determined.

CHAPTER FOUR

RESULTS

This study was aimed at evaluating microbial spoilage microorganisms of tomatoes.

Figure 1: Shows a chart of the total heterotrophic bacterial count of tomatoes samples

Figure 2: Indicates a chart of the total coliform bacterial counts of the sample

Figure 3: Shows a chart of the total fungal count of the tomato samples

Table 1: Shows total heterotrophic bacterial count of tomatoes samples

Table 2: Indicates the total coliform bacterial counts of the samples

Table 3: Shows the total fungal count of the tomato samples

Table 4: Indicates the cultural, morphological and sugar fermentation of bacteria

Table 5: Shows the cultural and morphological characteristics of fungal isolates

Table 6: Shows the antibiotic sensitivity test of bacterial isolates

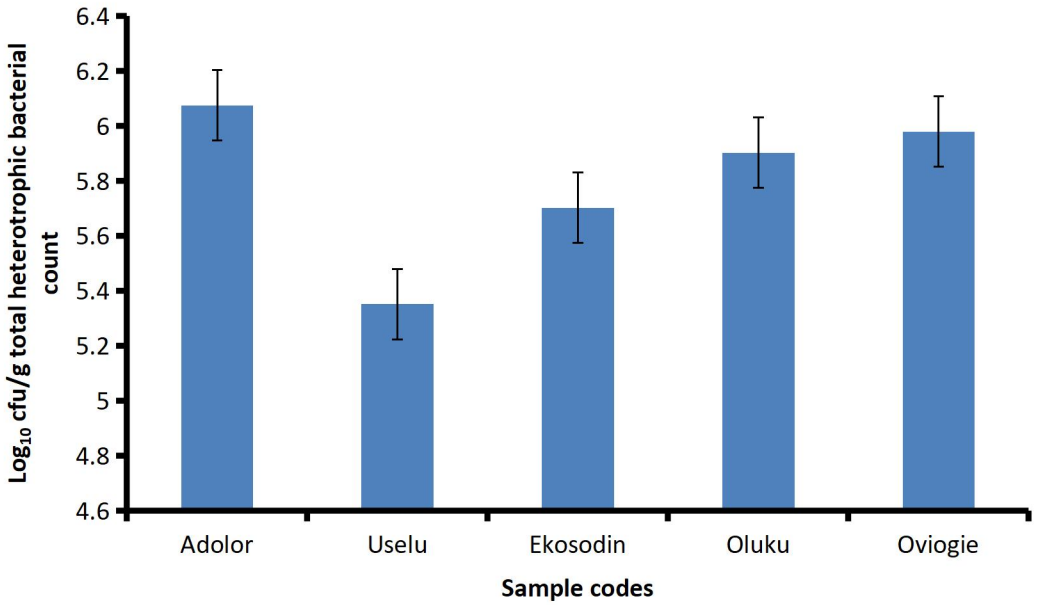

Figure 1: Total heterotrophic bacterial count (log10 cfu/g)

Sample codes	**Log_{10} cfu/g total heterotrophic bacterial count**
Adolor	6.075546961
Uselu	5.352182518
Ekosodin	5.703291378
Oluku	5.903089987
Oviogie	5.980003372

Table 1: Total heterotrophic bacterial count (log10 cfu/g

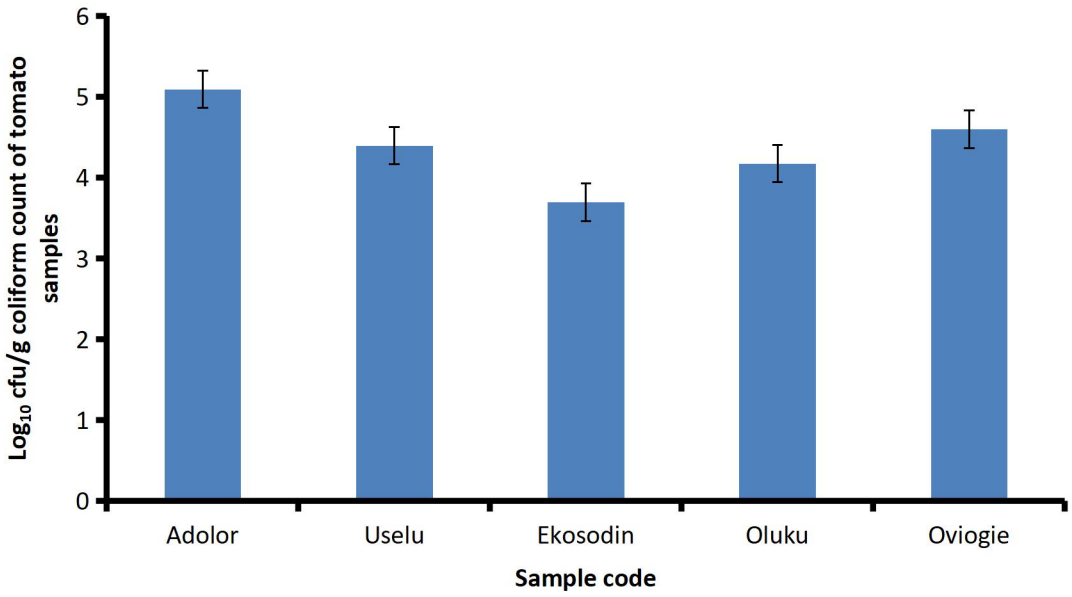

Figure 2: Log_{10} cfu/g total coliform count

Sample codes	**Log_{10} cfu/g coliform count of tomato samples**
Adolor	5.096910013
Uselu	4.397940009
Ekosodin	3.698970004
Oluku	4.176091259
Oviogie	4.602059991

Table 2: Log_{10} cfu/g total coliform coun

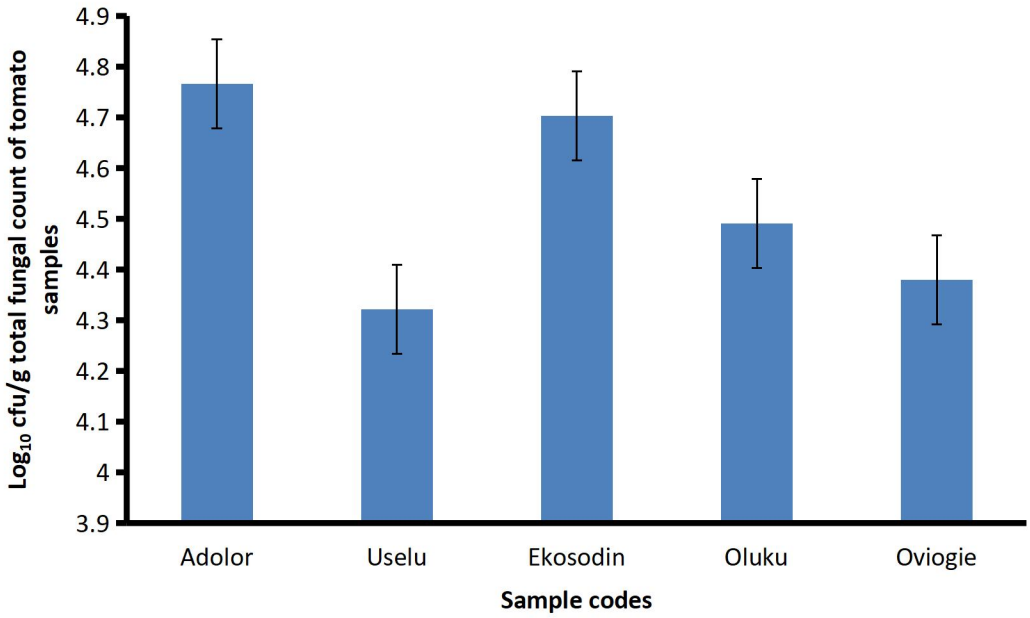

Figure 3: Total fungal count (log_{10} cfu/g)

Samples codes	**Log_{10} cfu/g total fungal count of tomatoes samples**
Adolor	4.767155866
Uselu	4.322219295
Ekosodin	4.703291378
Oluku	4.497361694
Oviogie	4.380211242

Table 3: Total fungal count (log_{10} cfu/g)

Table 4: Cultural, Morphological and Biochemical Characteristics of Isolates

Shape	Circular	Circular	Circular	Circular	Circular	Circular
Elevation	Convex	Convex	Convex	Convex	Convex	Convex
Margin	Entire	Entire	Entire	Entire	Entire	Entire
Morphological characteristics						
KOH	+	+	-	-	+	+
Gram stain	-	-	+	+	-	-
Cell morphology	Rod	Rod	Cocci	Rod	Rod	Rod
Cell arrangement	Single	Single	Clusters	Single	Chains	Clusters
Biochemical characteristics						
Catalase	+	+	+	+	+	+
Coagulase	-	-	+	-	-	-
Indole	+	-	-	-	-	-
Oxidase	-	-	-	-	-	-
Citrate	-	+	+	+	+	+
H_2S	-	-	-	-	+	-
Glucose	+	+	+	+	-	-
Lactose	+	+	+	-	+	-
Sucrose	+	+	+	+	+	+
Gr. Diff.	Green metallic Sheen (EMB)		Yellow (MSA)	Straw (BCA)		Cream
Identity	*E. coli*	*Klebsiella* sp	S. *aureus*	*Bacillus* sp.	*Salmonella* sp	*Shigella* sp

Table 5: Cultural and Morphological Characteristics of Fungal Isolates

Color of mycelium on agar plate	Dark brown colored growth	Green mycelium	Army green and entire, non-luxuriant with concentric ring	Initially white, with age turning gray and developing black dots
color of plate culture reverse	Dark	Pale yellow	Orange	light gray
Microscopic characteristics				
Nature of hyphae	Septate	Septate	Septate	Non- septate
Type of Spore	Conidiospore	Conidiospore	Conidiospore	Sporangiophores
Spore structure/Attachment	consists of a smooth and colorless conidiophores and spores.	Conidia size and shape are similar to Penicillium and Aspergillus but Trichoderma forms sticky clumps of conidia with a distinctive green pigment rather than in chains. Typical green spore clumps are identified as Trichoderma.	clear (not pigmented) hyphae with smooth-walled conidiophores, stipes are rather long and is biverticillate	Single and unbranched sporangiophore
Rhizoids	Absent	Absent	Absent	Present
Appearance of special structure	Conidial heads radiate, becoming columnar when mature; conidiophores are long and smooth-walled; biseriate; two rows of phialides cover the entire vesicle.	Conidiophores hyaline and loosely branched at right angles. Phialides flask-shaped and inflated at the base, with very short collarettes	Conidiophore stipes smooth-walled; phialides mono- or biverticillate, flask-shaped. Phialides do not show long pointed extensions at the tips	Rhizoids occur at the junction of stolon and sporangiophore
Class of fungi	Ascomycetes	Ascomycetes	Ascomycetes	Zygomycetes
Possible Identity	*Aspergillus niger*	*Trichoderma* sp.	*Penicillium* sp.	*Rhizopus arrhizus*

Table 6: Antibiotic Susceptibility Test

ISOLATES	CS	CIP	GEN	E	TE	M	CD	AG
E. coli	0(R)	17(S)	12(I)	0(R)	0(R)	0(R)	9(R)	12(I)
Staphylococcus sp	0(R)	14(S)	15(S)	8(R)	0(R)	7(R)	11(I)	14(S)
Bacillus sp	0(R)	18(S)	15(S)	10(I)	14(S)	7(R)	0(R)	9(R)
Klebsiella sp	7(R)	22(S)	17(S)	10(I)	8(R)	0(R)	10(R)	15(S)
Shigella sp	0(R)	16(S)	14(S)	0(R)	0(R)	0(R)	0(R)	14(S)
Salmonella sp	7(R)	24(S)	19(S)	10(I)	15(S)	0(R)	10(R)	15(S)

KEY

- R: RESISTANCE
- S: SUSCEPTIBLE
- CS: COLISTIN
- CIP: CIPROFLOXACIN
- GEN: GENTAMICIN
- E: ERYTHROMYCIN
- TE: TETRACYCLIN
- M: METRONIDAZOLE
- CD: CLINDAMYCIN
- AG: AUGMENTIN

CHAPTER FIVE

DISCUSSION

Tomato fruit as well as other fruits and fresh vegetables have epidermal layer which serve as a natural protective cover that effectively guide against most pathogenic microbes and plant spoilage. Tomatoes are prone to deterioration during storage, shipping, and waiting to be processed. The microbial degradation of tomato fruits lowers the market value and nutritional quality of the product. Contaminations with mycotoxins, which form aflatoxins in humans after inhalation or ingestion, render tomato fruits unfit for consumption, resulting in food poisoning (Bello *et al*., 2016). Fungi are the most important and prevalent pathogens that infect a wide range of host plants, causing destruction and economic loss in tomato either in the field, storage or transportation (Kutama *et al*., 2007).

This research was aimed on isolation of microbial pathogens associated with spoilt tomatoes sold in markets. However, the result obtained in this study showed that the highest bacterial, coliform and fungal population was obtained in Adolor sample with values of 6.07554±1.00, 5.09691±0.5 and 4.767155±0.5 $\log_{10}$ cfu/g respectively while the least bacterial, coliform and fungal count was obtained from Uselu and Ekosodin samples with values of 5.35218±2.00, 3.69897±1.00 and 4.32221±1.00 $\log_{10}$ cfu/g respectively. These figures were in the range as the study conducted by Onuorah and Orji, (2015) on microbial pathogens of spoilt tomatoes. Using the cultural and morphological characteristics, the fungal isolates obtained in this study were *Aspergillus niger, Trichoderma sp, Penicillium* sp and *Rhizopus arrhizus* while the bacterial isolates obtained were *Escherichia coli, Bacillus* sp, *Salmonella* sp, *Shigella* sp, *Klebsiella* sp and *Staphylococcus* sp. These isolates were similar to that those obtained by Wogu and Ofuase (2014) while working on

rotten tomatoes. The fruits of tomatoes are popular throughout the world and are used in all kind of stews, soups and also eaten raw in salads. Ripe tomato fruits have high nutritive values, being a good source of vitamin A, B, C and minerals (Kimaru and Sinha, 2010). Because of the importance of tomato as food, it has been bred to improve productivity, fruit quality, and resistance to biotic and abiotic stresses (Haydar *et al*., 2011). The tomato has been extensively used as both food and as research material. It is a major vegetable crop that has achieved tremendous popularity over the last century and it is grown in outdoor fields, greenhouses and net houses (Bihn and Gravini, 2016).

The presence of coliforms such as *Escherichia coli* and *Klebseilla* sp in the tomato samples indicate that other pathogenic organisms of fecal origin may be present. These pathogenic organisms may have been opportunistic pathogens via the use of manure as fertilizer for growing of tomato. *Escherichia coli* is a normal flora of the human gut as well as the intestines of humans and animals. Although most types of *E. coli* are harmless, some types can cause diseases. The worst type of *E. coli*, known as *E. coli* O157:H7, causes bloody diarrhea and can sometimes cause kidney failure and even death. *E. coli* O157:H7 makes a toxin called Shiga toxin and is known as a Shiga toxin producing *E. coli* (STEC) (CDC, 2012). There are many other types of STEC, and some can make you just as sick as *E. coli* O157:H7 (CDC, 2012). One severe complication associated with *E. coli* infection is hemolytic uremic syndrome (HUS) (CDC, 2012). The infection produces toxic substances that destroy red blood cells, causing kidney injury. The application of these fertilizers to tomato plant can infect the fruits with *Escherichia coli* which causes severe diarrhea and can also cause pneumonia, and other respiratory illnesses and urinary tract infections (CDC, 2012). *Salmonella typhi* and *Shigella* sp isolated from these tomato samples can also be as result of manure used as fertilizers or through irrigation with water

contaminated with feces or poor hygiene habits of farmers and traders. The health implications of these organisms are Typhoid fever and Shigellosis respectively. The *Shigella* germ is a family of bacteria that can cause diarrhea in humans (CDC, 2012). People with shigellosis shed the bacteria in their feces (CDC, 2012). The soil is most intensively inhabited by *Aspergillus* and *Penicillium*. The presence of these organisms in the tomato samples is attributed to the cultivation process where the tomato plant in contact with the soil and obtains its nutrients from the soil through its roots.

Fungal spoilage of tomatoes is attributable to the high-water content, environmental conditions, state of handling, state of storage facilities, the fungal load of the handlers and the quality of the tomatoes. These fungi isolated in this study are sources of potent mycotoxins which are detrimental to health. *Aspergillus niger* is a source of Ochratoxin which is considered to be a potent Carcinogen, therefore spoilt tomatoes must not be consumed but disposed of, since such consumption could be detrimental to health. Farmers and marketers of the produce are also advised to take appropriate precautions during the harvesting, transportation, storage and sale of tomatoes to reduce the risk of these toxins and other metabolites that are deleterious to health.

Studies have shown that *Aspergillus* produce aflatoxins. Aflatoxins are associated with some diseases in livestock and humans throughout the world. *Aspergillus* is the main producer of the well-known carcinogenic aflatoxins and its presence in food is of huge concern in terms of food safety they are toxic at low concentrations (Rodriques *et al*., 2007). The dominance of *Aspegillus* in rotten tomatoes could pose a serious health risk especially when the tomatoes are not well cooked. Healthy tomatoes fruit should be preferred as they seldom contain microbes (Ugwu *et al*., 2014). Mbajiuka and Enya (2014) found abundant presence of *Penicillium nalgiovense, Penicillium notatum* and *Penicillim expansium* among other fungi species involved in

deterioration of tomatoes fruit. *Penicillium* and *Fusarium* are among the most important genera of mycotoxigenic fungi (Zain, 2011). The mycotoxins are of greatest agro-economic importance, some molds are capable of producing more than one mycotoxin and some mycotoxins are produced by more than one fungal species (Zain, 2011). From the result, isolates were resistance to many of the antibiotics including, colistin, Erythromycin, metronidazole and clindamycin but were susceptible to ciprofloxacin, Augmentin and gentamycin. There is need to properly handle food items to prevent contaminations which are detrimental to human health.

CONCLUSION

Tomato fruits have high dietary and nutritional qualities. The importance of these fruits with its nutritional and other importance cannot be over emphasized, as its spoilage often result to wastage of economic resources as well as food poisoning. Their spoilage by fungi results in loss of economic resources as well as food poisoning. The result in this study has shed light into the gaining of entrance of food borne pathogens as well as some spoilage microorganisms (mostly fungi) during selling, harvesting and cultivation which may result in food poisoning. In this study, it was revealed that tomatoes are of great benefits to human health. The only constraint in the consumption of tomato is the presence of spoilage and pathogenic organisms. The general public should also be made aware of the potential health concerns linked with the ingestion of relatively less expensive but spoiled ripe tomato fruits, as these could be agents in food-borne bacterial and fungal infections.

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