ASSESSMENT OF FUNGAL SPECIES ASSOCIATED WITH TOMATO SPOILAGE SOLD IN DUTSIN-MA METROPOLIS, KATSINA STATE, NIGERIA

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ABSTRACT

Tomato is an annual plant having a weak woody stem covered with glistering reddish yellow glandular hairs, it is widely cultivated in many part of world. This study was under taken to find out the fungal species responsible for the spoilage of tomatoes in Dutsin-ma metropolis, Katsina State, Nigeria. This research revealed that fungal species are responsible for the spoilage of the tomato fruit in the study area. Samples with fungi were grown on potato dextrose agar and sabouraud dextrose agar. The fungi count was found to be 3.8x105, 3.5x104 and 4.5x104 for Aspergillus niger at Yara dole market, Wednesday Market and Tsohuwar kasuwa, respectively. While fungal count of Fusarium oxysporum was 3.9x103, 4.8x103 and 4.2x105 for Yara dole market, Wednesday Market and Tsohuwar kasuwa, respectively. The fungal count of Rhizopus stolonifer was 3.5x103, 4.4x102 and 5.1x104 for Yara dole market, Wednesday Market and Tsohuwar kasuwa, respectively. The storage of tomato should be done at the temperature and relative humidity that does not favour the growth of fungi.

Key words: Assessment, fungal species, tomato, spoilage, Dutsin-ma.

INTRODUCTION

Food, microorganism and human have had a long interesting association that develop long before the beginning of recorded history. Foods are not only of nutritional value to those who consume them but often are ideal culture media for microbial growth. Food also serves as vehicle of disease transmission, and the detection and control of pathogens and food spoilage microorganism are important part of food microbiology (Prescott *et al.,* 2005). Microbial growth in food involves successful changes with intrinsic or food related factors such as redox potential and extrinsic or environmental factors interacting with the microbial community over time (Jay, 2000).

Tomato is an annual plant having a weak woody stem covered with glistering reddish yellow glandular hairs, it is widely cultivated in many part of world. It is green when immature but becomes bright red or yellow as it ripens (Bashir *et al.,* 2014). Microbial action is one of the factors that influence spoilage of fruit and vegetables. In fruits, there is generally the absence of bacteria except the lactic acid bacteria in the incipient spoilage of the fruit due to low pH 3.5-4.5. Most of the bacteria are saprophyte such as *Coliforms, Penicillium, Rhizopus, Altenaria* and *Mucor* spp. are often present but in low numbers compared to bacteria (Ezeama, 2007).

Over the last century, tomato (*Lycopersicon esculentum* Mill.) as an important vegetable crop has attained a tremendous popularity. It can be grown in most places all over the world, like growing in the field, greenhouses and net houses. The tomato crop is grown and used for both fresh market and processing and it is an adaptable crop. Over the past 25 years, the demand on tomato recorded highly data in both producing and consuming and it has grown quite rapidly (FAO, 1980).

Nowadays, tomato occupied an area of about 3.9 million hectares all over the world for growing and annually about 108 million metric tonnes of tomato will be produce for both fresh market and processing (Bhatia *et al*., 2004).

In general terms, food can be said to be spoilt if they have suffered decay or decomposition of undesirable nature. Spoilage of food may be due to one of the following factors which include: The growth and activities of microorganism, Infestation of insects, the action of food enzymes, chemical reaction other than those mediated by food enzymes and Physical changes taking place during food treatment. All foods are protected from attack of microorganism by some sort of covering and as soon as these protective

coverings are removed, damage or bruised microorganisms set in for their activity. Fruits contain high levels of sugars and nutrient elements and their low pH values make them particularly desirable to fungal decay (Singh and Sharma, 2007). These fruits are usually displayed on benches and in baskets for prospective customers in the open markets until sold, thereby exposing them to further microbial infection beside those associated with these whole fruit surface and those from adjacent infected fruits (Butter, 2014; Ghosh and Ishagel, 2009).

Food spoilage refers to various changes in which the food becomes less palatable or even toxic to consumers these changes may be accompanied by alterations in taste, smell, appearance or texture. Numerous microbial defects of agricultural crops are characterized by the types of microorganisms responsible for their deterioration (Akinmusire, 2011).

Tomato fruits are very rich in mineral, vitamins, and carbohydrate. Udoh *et al.* (2005) reported that the tomato fruit has 94% water and 4.3% carbohydrate. In view of these, the fruit is often attacked by microorganisms especially after harvest, thus a fast and high rate of spoilage is often observed in storage. Barth *et al.* (2009) stated that fungal species destroys fruits more than bacteria, and isolated the following fungal species from soft rot infected tomatoes: *Fusarium oxyporum, Fusarium muniliforme, Aspergillus niger and Rhizopus stolonifer*.

About four hundred thousand people are living in Dutsin-ma Metropolis. Almost all the people in the area uses tomato to cook, and many other things. However, the tomato in the study area is often contaminated, which may cause many diseases when consumed. Spoilage of microorganisms can be introduced to the tomato on the seed itself, during crop growth in the field, during harvesting and post-harvest handling or during storage and distribution (loading and offloading). So far, there is little information in respect to microbiological qualities of spoilt tomatoes sold in Dutsin-ma market. This research work was aimed to isolate and characterise different fungal species associated with tomatoes spoilage in Dutsin-ma metropolis.

MATERIALS AND METHODS

STUDY AREA

The study area (Dutsin-ma metropolis) is located between latitudes 12° 27'17" N to 7°29' N and longitudes 7°20'48" E to 7°37'18" E. It is bounded by Kurfi, Safana, Batagarawa, Danmusa, and Charanchi. (Ministry of Land and Survey Office, 2008). Dutsin-ma is mainly made up of Hausa and Fulani as the main dominant ethnic groups. The 2006 census puts the population of Dutsin-ma at about 469522 (NPC, 2006).

ECONOMIC ACTIVITY

Agriculture is the main stay economic activities in Dutsin-ma and its environs. Fertile land of the region has played a great role in the growth and development of the region, this is because the region serves as one of the centres of cash crops production especially cotton and groundnut and this attracted many farmers, animal rearers and merchants to settle in the region to involve in agricultural activities.

Agricultural land uses are pieces of land kept purposely for agricultural practices including various farming system in the region. The area is blessed with fertile land, sandy-loamy in nature, derived from the local topography, light-brown in cover and easily worked, as such various farming system are practiced ranging from wet season farming, irrigation system, postural farming to the plantation agriculture. In addition to these, some areas were kept as forest reserve to take care of livestock rearing and checking out some ecological problems such as desertification and erosion. In Dutsin-ma and its environs, the land uses for agriculture is very vast, majority of the inhabitants are farmers in one form or another. Crops grown include cereals and vegetables. Another important occupation in the area is fishing as well as livestock rearing. About 62% of the farmers in the area practice mixed farming. Potteries, tailoring local crafts, butchering and marketing business are also practiced in the area.

SAMPLE COLLECTION

A total of 10 samples of tomatoes fruits were collected from three different markets: Wednesday market, Yara dole market and Tsohuwar kasuwa. The samples were taken to the laboratory, washed and drained with water. The fruit samples were kept free from dust and insects at room temperature for up to 7-14 days to undergo a natural process of spoilage before being used in this study.

MEDIA PREPARATION

The media used include Saboraud dextrose agar (SDA) and Potato dextrose agar (PDA), the media were prepared according to the manufacturer's instructions, and it was sterilized in an autoclave prior to use to avoid contamination.

ISOLATION OF FUNGAL SPECIES FROM SPOILT TOMATOES

The tomatoes were cut open, the fleshy and watery content was squeezed out. It was chopped into smaller pieces using mortar and pestle. About 10g of pounded tomato was dissolved in 90ml of sterile peptone water. The stock was shaken for about 10minute. Serial dilution was carried out by transferring 1ml from the stock solution into 9ml sterile peptone water in the universal bottle making 10^{-1} to 10^{-3} dilution (Bashir *et al.*, 2017; Bashir *et al.*, 2018).

A sterile pipette was used to transfer 0.1ml of the 10^{-1}, 10^{-3} and 10^{-5} onto each of the sabouraud dextrose agar (PSA) and Potato dextrose agar PDA. A bent glass rod was flamed and used to spread the inoculums evenly over the PDA and SDA surfaces. The PDA and SDA plates were incubated at room temperature for 4-6 days. After the incubation period, the growth was observed for fungal growth. The isolated colonies were counted and the colony count per ml was obtained using the formula.

$$\frac{\text{No of Colonies/ml X Dilution factor}}{\text{Volume of inoculum}}$$

MORPHOLOGICAL CHARACTERIZATION OF FUNGAL ISOLATES

The fungi isolates were picked from the media and maintained on potato dextrose agar and sabouraud dextrose agar for further identification (Tijjani *et al*., 2018).

IDENTIFICATION OF THE FUNGI

The isolated fungi were identified by observing their cultural and conidial characteristics using wet mount preparation in lactophenal cotton blue. This will be carried out by teasing scrapings made from Sabouraud dextrose agar on a clean grease free glass slide stained with 0.5% aqueous solution of lactophenol cotton blue. The slides were covered with a cover slip and examined under magnification of x100 and x400 (Tijjani *et al* 2018).

RESULTS

The result showed fungal count of $3.8x10^5$, $3.5x10^4$ and $4.5x10^4$ for *Aspergillus niger* at Yara dole market, Wednesday Market and Tsohuwar kasuwa, respectively. While fungal count of *Fusarium oxysporum* was $3.9x10^3$, $4.8x10^3$ and $4.2x10^5$ for Yara dole market, Wednesday Market and Tsohuwar kasuwa, respectively. The fungal count of *Rhizopus stolonifer* was $3.5x10^3$, $4.4x10^2$ and $5.1x10^4$ for Yara dole market, Wednesday Market and Tsohuwar kasuwa, respectively as presented in Table 1 and Figure 1.

Table 1: Fungal count of spoilt tomato samples from three different markets

Fungal Species	YDM CFU/Ml	WM CFU/Ml	TKM CFU/Ml
Aspergillus Niger	$3.8x10^5$	$3.5x10^4$	$4.5x10^4$
Fusarium oxysporum	$3.9x10^3$	$4.8x10^3$	$4.2x10^5$
Rhizopus stolonifer	$3.5x10^3$	$4.4x10^2$	$5.1x10^4$
Average	$3.7x10^4$	$4.2x10^3$	$4.6x10^4$

KEY: - CFU: Colony forming unit, YDM: Yara Dole market, WM: Wednesday market, TKM: Tsohuwar Kasuwa market

Figure 1: (A) showing spoilt tomatoes and (B) showing number of fungal colonies

Based on morphological characteristics, the presence of white cottony colonies with dirty white pigmentation confirmed the isolate to be *Aspergillus niger*, while creamy cottony colonies with whitish orange pigmentation is a unique characteristic of fungus (*Fusarium oxysporum*). *Rhizopus stolonifer* culture showed greenish to dark green cottony colonies with black pigmentation as indicated in Table 2.

Table 1: Morphological characteristics of the fungal isolates

Isolate Codes	Cultural Morphology	Microscopic Examination	Probable Organism
S001	White cottony colonies with dirty white pigmentation	Bluish green cornidiophore develop from septate chain of conidia	*Aspergillus niger.*
S002	Creamy cottony colonies with whitish orange pigmentation	Bluish green cornidiophore develop from aseptate chain of conidia	*Fusarium oxysporum* .
S003	Greenish to dark green cottony colonies with black pigmentation	Bluish green cornidiophore develop from septate chain of conidia	*Rhizopus stolonifer*

DISCUSSION

From the result obtained in Table 1 and 2, it shows that the isolates of fungi that are associated with tomato spoilage were *Aspergillus niger, Rhizopus stolonifer and Fusarium oxysporum*. The findings of this study are in line with the work of Ezeama (2007) by reporting *Fusarium oxysporum* to be one of the causative agent of tomato fruit spoilage. However, it goes contrary to the findings of this work by reporting other fungi like mould and yeast to be associated with tomato spoilage (Nwanekezi, *et al.,* 2002). The findings of this study are also contrary to the work of Barth *et al.* (2009) who stated that the fungi associated with tomato spoilage were yeast cells and *Penicillium* spp.

The high acid content (low pH) and the high percentage of moisture content make tomatoes a suitable medium for microbial growth. However, in fruits like tomatoes there is generally absence of bacteria except the lactic acid bacteria. Also present are most saprophytic bacteria such as coliforms, spore formers. But fungi including Yeast and mould like *Alternaria alternata* etc. are often present (Barth *et al.*, 2009). The presence of *saccharomyces cerevisiae* is an indication of food contamination which may arise through mishandling, exposure, transporting, distribution, under processing and even improper cooking and storage under unhygienic condition. Presence of *saccharomyces cerevisiae,* is an indication of faecal contamination through water (Barth *et al.,* 2009). Practice of basic sanitary rules in handling, storage, cooking with and sales of fresh tomatoes should be employed to improve hygienic condition of these fresh tomatoes consumed to avoid food- borne outbreak.

Fruits such as tomato contain high level of sugars and nutrients element and their low pH value makes them particularly desirable to fungal pathogens (Singh and Sharma, 2007). Fungi have been found to be the most important disease inciting organisms that cause destruction and economic loss of tomato in field, storage, and markets. It has been reported that 21% of tomato produced in Nigeria was lost to rots in the field and 20% in storage, and market (Opadokun, 2003). Spoilage in fresh tomatoes is however higher compared to the sun-dried or canned tomatoes. This is because the sun-dried tomatoes have undergone a considerable degree of treatment so as to reduce the moisture content to a level unfavourable for fungal growth, although improper storage or exposure could attract fungal growth. In the case of the canned tomatoes, the fungal growth is not expected in proper canned tomato, except in under processed canned tomatoes and in canned tomatoes with leakages and those that have been stored for a longer period of time (Akinyele and Akinyumi, 2012).

CONCLUSION

Based on the findings of this research work, there are different fungal species like *Fusarium oxysporum, Rhizopus stolonifer* and *Aspergillus niger* that were found to be associated with tomatoes spoilage in Dutsin-ma metropolis. These fungi cause a significant loss of tomatoes fruits not only to the farmers and fruits vendors, but also to the of people of Dutsin-ma metropolis who uses tomatoes for consumption and other household uses. There is a need in the future to come up with special preservative methods like canning, sun drying and chemical preservatives to minimise tomatoes spoilage. Moreover, the storage of tomato should be done at a temperature and relative humidity that does not favour the growth of fungi. Future researches should focus more on studying several types of microbes that are associated with spoilage of tomatoes fruits.

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