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Fungal Pathogens Associated with Post-Harvest Deterioration of Tomato Fruits in Jega Local Government Area, Kebbi State, Nigeria.

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ABSTRACT

A study was conducted to identify fungal pathogens associated with spoilage of tomato fruits sold in jega local government of kebbi state area, Nigeria a total of fifty (50) fresh and physically damage tomato samples were collected from five different locations namely: Jega, Kyarmi, Nasarawa, Danwarai, Gindi of the Study Area. A total of eight (8) Species of fungi: *Aspergillus Niger, Candida Albican, Fusarium Oxynoforum, Penicillium Chrysogenum, Tricoderma Viride, Rhizoctonia Oryzae, And Verticillium Albo-Atrum* were identified from the samples collected using standard methods. From the result obtained, *Aspergillus Niger* has the highest percentage of occurrence of (23.81%) followed by *Cladosporium Iride* (19.5%) while *Penicillium Chrysogenum* had the lowest (4.79%). Base on the locations jega central market has the highest prevalence of fungal load (62.5%) followed by kyarmi (50%) while nasarawa had the lowest (25). The result revealed that tomatoes rot are caused by different species of fungi and their activities, therefore proper handling procedure especially during post – havest should be adopted to avoid the fruits spoilage.

Key words: Tomatoes, fruits, spoilage, Jega, *Aspergillus niger*, occurrence.

1. Introduction

The tomato plant (*Solanum esculentum* Mill; family Solanaceae), is an economically important herbaceous crop cultivated globally for its various health and nutritional benefits (Ramakrishnan and selvakumar, 2012; Abubakar et al. 2019). The family Solanaceae includes other well-known species such as Potato, Tobacco, Pepper and Egg plants (Yadav et al. 2016; Kutawa et al. 2020). Tomato in West Africa is grown in gardens and irrigation schemes and in Nigeria, most tomatoes are grown in the northern parts of the country and there is no record of any systematic or organized traditional storage method for vegetables and fruits. They are usually sold immediately after harvesting as they are packed in baskets, cardboard boxes, or wooden crates ready for transportation to the markets (Kimura and Sinha, 2010; Kabir et al. 2020).

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 (Kimura and Sinha, 2010; Yang and Kim, 2020). Because of the importance of tomato as food, it has been bred to improve productivity, fruit quality, and resistance to biotic and abiotic stresses (Kimura and Sinha, 2010; Yang and Kim, 2020). Tomato has been widely used not only as food, but also 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 Gravani, 2016). They contain high amount of carbohydrates, fats, organic acids, water, minerals, vitamins and pigments. It is estimated that ripe tomato fruits contain approximately 94 % of water, 4.3 % carbohydrates, 1 % protein, 0.1 % fat, 0.6 % fiber and vitamins. (Yoltao, 2011; Yang and Kim, 2020**).** They are good sources of natural antioxidants which include carotenoids, vitamins, phenolic compounds, flavonoids, dietary glutathione, and endogenous metabolites and have been shown to eliminate free radicals, (Sravanthi and Gangadhar, 2015; Ali et al. 2020).

Tomato is the most perishable vegetable during handling, transportation and storage. This is because tomato contains large amount of water which makes them susceptible to spoilage by the action of microorganisms such as fungi, bacteria and protozoans (Ross, 2015).Naturally, Fruits and vegetables carry epiphytic micro flora. During growth, harvest, transportation, further processing and handling, the produce can be further contaminated with non-pathogenic and pathogenic organisms from soil, human or animal sources (Beuchart, 2010). Pathogenic fungi, such as *Altternaria, Aspergillus, Fusarium, Mucor sp., Penicillium, Rhizopus and Trichoderma*have been implicated in some crop spoilage (Beuchat, 2010). Fungi contamination of many agricultural products, including tomatoes starts in the fields (Sellitto et al. 2019). Both the biological and physical damages during the harvest and transportation phases, coupled with large amount of water and soft endocarp makes tomatoes more susceptible to spoilage by fungi (Asan and Ekmeki, 2012; Olayemi et al. 2021).

Fungal spoilage of tomatoes has been recognized as a source of potential health hazard to humans and animals due to the fact that they produce mycotoxins which are capable of causing mycotoxicoses in man following ingestion or inhalation (Yoltao, 2011; Wu et al. 2022). The mycotoxins are not limited to their areas of infections since tomatoes contain large amount of fluid. So, also it has been reported that 20% of all fruits and vegetables harvested for human consumption are lost through microbial spoilage causing one or more of 250 market diseases. This spoilage of fresh tomato usually occurs during storage and transit as well as while waiting to be processed. Losses in tomato fruits yields due to fungal attacked can be as high as 60% (Shehu, et al, 2014; Naciri et al. 2021). Despite these damages, there is little work has been done to this effect in the study area. There is therefore a need to evaluate the fungi associated with the spoilage of tomato fruits as proper isolation and identification of these organisms in tomatoes will aid greatly in mitigating the management strategies thereby reducing the rate of spoilage of this perishable fruits as this will enhance tomatoes production and improve the economy in the area. The research aimed at determining the pathogenic effect of the fungal isolates associated with the spoilage of tomato fruits in Jega Local Government Area, Kebbi State Nigeria.

2.0. Materials and Methods

2.1. Study Area

The study was conducted in Jega Local Government Area of Kebbi State, Nigeria. It has an estimated land mass of 1043 km, located on latitude 11°30'N and 11°50'N, ,longitude 4°00'E and 4°00'E and has a population of 73, 495 during the 2006 census. The area is characterized with two seasons -wet and dry seasons. The rainy season lasts between May and October with a unimodal peak of rainfall in August. The mean annual temperature is 31°C and annual rainfall is 500mm. The people of Jega are mostly farmers and traders (Suleiman, 2008).

2.2. Field Survey and Sample Collection

A total of forty (500) spoilt tomato fruits were obtained from five different locations viz: Jega central market, Nassarawa, kyarmi, Gindi, and Danwarai markets. The samples were transported to the department of Plant Science and Biotechnology Laboratory, Kebbi State University of Science and Technology, Aliero for the mycological analysis.

2.3. Sterilization of Glass Wares

All glass wares were initially washed with detergent and rinsed with water. They were then allowed to air dry and later sterilized in an oven at a room temperature of 160^0c for one hour. They were then allowed to cool for another one hour. The wire loop used was sterilized by holding it on a spirit lamp until it turned red and then allowed to cool down before using it for inoculation.

2.4. Preparation of Culture media

The media used was potato Dextrose Agar (PDA) which was prepared according to the manufacturer's instructions. Thirty nine grams (39g) of the dehydrated PDA was weighed using weighing balance and suspended into conical flask containing 1000ml of distilled water. One gram (1g) of antibiotic (Streptomycin) was added to inhibit the growth of the bacteria. The suspension was heated to dissolve completely on a hot plate which was then autoclaved at a temperature of 121^0c for 15 minutes. It was allowed to cool down to room temperature. The media was taken to incubation room and 25ml of the media was poured into ten sterilized petri dishes, it was then allowed to solidify for the purpose of the experiment.

2.5. Mycological Analysis

Each of the infected sample was washed and surface sterilized in 1% commercial bleach for one minute. These were then rinsed in three successive changes of sterile distilled water and blotted dry with sterile filter paper. From each sample, five pieces of segments measuring $3mm^3$ from the advancing margins of rotted lesions were cut out with flame sterile scalpel and forceps, and plated on acidified potato dextrose agar (PDA) in 90 mm Petri dishes. The plates were incubated at room temperature (28 ± 2°C) for seven days. When fungal growth from the tissue was visible, fungi were sub cultured onto freshly prepared sterile PDA plates to obtain a pure cultures for identification. Where there is a mixed culture, fungi were continuously sub cultured until pure isolates were obtained. Stock cultures of the pure isolates were prepared and preserved at 4^0C in the refrigerator (Oyeleke and Manga, 2008).

2.6. Identification of Fungal Isolates

The fungal isolates were subjected to comparative morphological studies by an image and analysis system using published descriptions in a mycological atlas obtained at the Microbiology Laboratory of Kebbi State University of Science and Technology, Aliero. This was followed by a slide mount of each isolate. The characteristics observed were matched with those available in the aforementioned mycological atlas and were identified accordingly (Akintobi and Okonko, 2011).

2.7. Determination of Frequency of Occurrence of the Isolates

This was done to determine the percentage occurrence of the different fungal isolates. The number of occurrence for each of the isolates were recorded and calculated using the formula as described by Carlson (2014). % Frequency = Number of identified fungi/Total number of fungi x100

2.8. Data Analysis

The data generated were processed and subjected to descriptive statistics using means and percentages so as to provide summary description of the subject using descriptive statistical tools by means of tables.

3.0 Results

The fungi isolates associated with the spoilage of tomato fruits were identified based on colony and morphology characteristic as presented in **table 1**. They include *Aspergillus niger , Candida albican, fusarim oxynoforum, Penicillum chrysogenum, Tricoderma viride, Rhizoctonia oryzae, Verticillium albo-atrum* and *Cladosporium iridie.* The prevalence of fungi isolates with respect to the location of sample collection shows that Jega central market has the highest prevalence of fungal load (62.5%) followed by Kyarmi (50%) while Nassarwa had the lowest (25%) **(Table 2)**.The frequency of occurrence of fungi on the fruits was revealed that *Aspergillus niger* has the highest percentage (23.81%) followed by *Cladosporium iride* (19.5%) while *penicillium chrysogenum* had the lowest (4.79%) **Fig 1.**

Table 1: Cultural and Morphological Characteristics of Fungi Isolated from Fresh Tomato Fruits

Colony Appearance	**Morphology and Cellular Characteristics**	**Fungi Isolated**
Colonies with loose white mycillium rapidly becoming dark brown to black on the development of conidia	The conidiospore are large with septate hyphae	*Aspergilus niger*
White floccose colonies with the aerial mycelia becoming tinged in purple	Sickle shape macro conidia.	*Fusarium oxynoporum*
Yellow fluffy myecilia and some black sporongiospores	Septate hyphae with filamentous structure	*Culvularia geniculate*
White at early stage, purple when colonies get older	Sickle shape micro conidia	*Penicillium chrysogemum*
White at early stage but brown when colonies get older	Hyphae and septate are lacking	*Trichoderma viride*
Black, yellow to pale in medium	Absence of white mycelia at the margin	*Cladosporium iridie*
Black, brown to black in media	Greenish black, white mycelia at the margin about 1-2 mm, white foam in the center.	*Rhizoctonia oryzae*

Table 2: Prevalence of fungi isolates with Respect to locations of the Study Area.

Location	**Fungal Isolates**	**Prevalence (%)**
Jega	*Aspergillus, Candida albican iridie, Fusarium oxynoforum Culvularia geniculate*	62.5
Danwarai	*Penicillium chrysogenum, Rhizoctonia oryzae, Candida albican*	37.5
Gindi	*Fusarium oxynoforum, Candida albican, Rhizoctonia oryzae*	37.5
Kyarmi	*Aspergillus niger, Verticillium albo-atrum, Culvularia geniculata, Candida albica*	50
Nasarawa	*Tricoderma viride, Rhizoctonia oryzae*	25

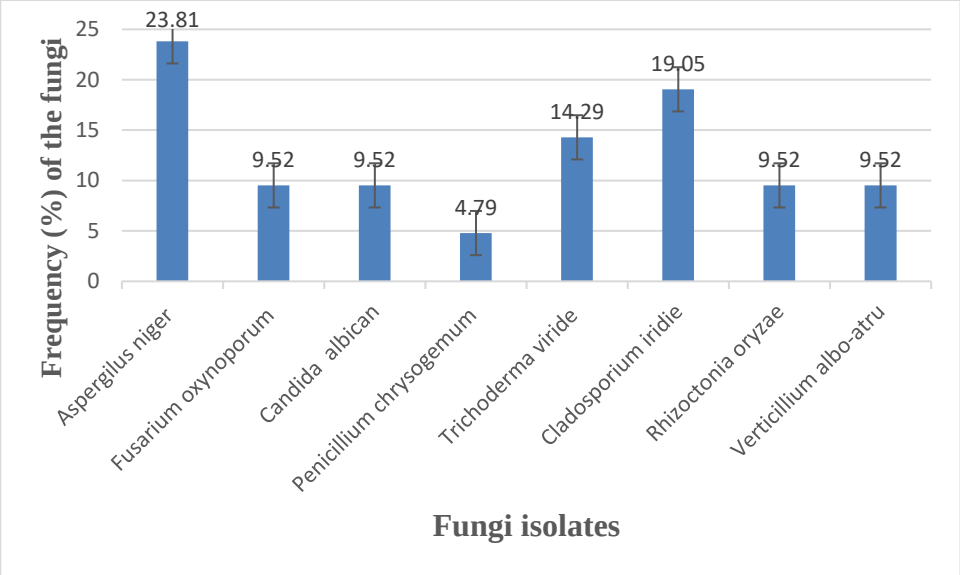

Fig 1: Frequency of occurrence (%) of the Isolated Fungi in the Area

4.0. Discussion

The study was conducted to determine the fungal species associated with spoilage of tomatoes in jega Local Government Area of Kebbi State. A total of eight fungal species were identified in this study of which *Aspergillus niger* had the highest frequency of occurrence (23.81%) while *penicillum chyrogenum* had the lowest (4.79%). The higher number of fungi species identified may be due to climatic conditions such as high temperature and air humidity which favor the growth of microorganism particularly fungal pathogens leading to deterioration of the fruits (Abubakar et al. 2019). Fruits contents of high levels of sugars, nutrient element and their low pH values make them particularly desirable to fungi (Sharma et al. 2007). The highest frequency of occurrence recorded for *Aspergillu sniger* (23.81%) could be related to its high speculating capacity and production of toxins which inhibit the growth of other fungal pathogens (Rakesh and Rajas, 2013). Similar studies (Muhammad et al. 2004) reported that *Aspergillus niger, Cladosporium iridie, Candidia albican* and *penicillium crysogenum* were responsible for market disease and aflatoxin contamination of tomato fruits in Nigeria. The work done by Abubakar et al. (2019) also presented *Aspergillus niger, Rhizopus stolonifer, Fusarium oxynoporum, Saccharomyces cerevisiae, Alternaria alternata* and *Penicillium digitatum* as the major fungal pathothens related to the tomato fruits deterioriation. Lower number of fungal pathogens (*Aspergillus niger, Rhizopus stolonifer* and *Fusarium oxynoporum*) was reported by Kutawa et al. (2020) in Dutsinma metropolis of katsina State, Nigeria. YAa et al (2022) also reported similar results recording few fungal species which include *Aspergillus, Saccharomyces, Mucor and Alternaria species* identified from tomato fruits from Kaduna state Nigeria. The work of Olayemi et al. (2021) described the spatial distribution of micro biota relevant to spoilage of tomato fruits. They revealed that the bacterial isolates identified from the study include *Micrococcus varians, Lactobacillus fermenti, Escherichia coli, Salmonella sp* and *Klebsiella sp* while the isolated fungi were *Rhizopus stolonifer, Fusarium oxysporium, Aspergillus flavus, Geotrichum candidum, Mucor mucedo,* and *Candida tropicalis*. They further demonstrated that all the pathogens were positive for the pathogenicity test and were heterogeneously distributed with remarkable levels of severity across the study area. This is in compliance with the current study reporting large number of pathogens on the tomato fruits. Danaski et al. (2022) reported that 19 microbes with 5 bacteria and 14 fungal pathogens were responsible for tomato fruits spoilage in Maiduguri, Nigeria. Their results showed that *Rhizopus stolonifer, Aspergillus fumigatus* and *Candida tropicalis* were the most frequent fungal microbes associated with the spoilt tomato samples with occurrences of 65.7%, 80.1% and 91.4% respectively while *Aspergillus austus, Penicillium spp* and *Aspergillus oryzae* had the lowest percentage occurrence of 5.71% each. *Rhizopus stolonifer, Aspergillus fumigatus* and *Candida tropicalis* were found to be the most pathogenic in all the three tomato varieties. The high susceptibility of tomato fruits to microbial attack may be related to their low pH, high moisture and nutrient contents which may also lead to production of mycotoxins that are hazardous to both human and animal (Danaski et al. 2022). The occurrence of the isolated fungi with respect to location of sample collection revealed that jega had the highest percentage of the fungal loads (62.5%) followed by Kyarmi with (50%) while Nassarawa had the lowest (25%). The variations in the fungal load may be due to the handling methods of the fruits during packaging transportation or storage. The presence of these fungal pathogens on fresh tomato fruits suggest that they used compromised surfaces of the fruits such as wounds to cause rots. Moreover, most tomato fruit sellers use polythene sheets with poor ventilation to cover the fruits at the end of their sales period and before resuming sales or distributions and condition that favors the growth of rot pathogens especially, the fungi (Abubakar et al. 2019). The fruits must therefore, be properly checked for deep and even light scratches prior to shelving on fruit stalls or packing in storage as these rot pathogens can cause considerable fruit loss if they remain on wound sites.

5. Conclusion

This study has implicated eight fungi species to be responsible for contamination of tomato fruits in Jega Local Government Area, Kebbi State. Fungi are found in the spoiled tomatoes and thus serves as source of inoculants for infection of tomato fruits. Care should be taken during the selection of tomatoes to be used for human consumption while proper handling methods should be adopted by both farmers and consumers to minimize the fungal deterioration of the fruits in the study area. Developing fungicides that will target the major pathogenic fungal species may greatly reduce post-harvest losses associated with tomatoes spoilage in the study area.

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