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Mycological Assessment of Deteriorated *Lycopersicum esculentum* Fruits Sold in Wukari Nigeria

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Abstract

Lycopersicum esculentum (Tomato) fruits are widely consumed in raw and processed forms owing to their high nutritional value. However, high water content makes them more prone to microbial attack especially spoilage by fungi. In this study, fungi associated with deteriorated tomato fruits sold in Wukari, Nigeria were assessed. A total of thirty (30) deteriorated tomato fruits were collected, ten (10) each from the three markets (Federal University up-gate mini market, New market and Yam market) and assessed for fungi using standard microbiological methods. The result shows that fungal load in all the markets ranged from 1.0×10^5 sfu/g to 3.0×10^5 sfu/g and *Aspergillus niger, Aspergillus flavus, Rhodotorula* species, *Mucor* species, *Saccharomyces cerevisiae* and *Fusarium* species were isolated from the various samples. The percentage occurrences of the various isolates on the samples show that *Aspergillus niger* was the most frequent (63.33%), followed by *Fusarium* species (43.33%), *Aspergillus flavus* (33.33%), *Mucor* species (26.67%) and *Rhodotorula* species (23.33%) while *Saccharomyces cerevisiae* (10.00%) was the least. In each sampling location, the percentage occurrences of all the species were highest at Yam market site (35%), followed by Federal University up-gate mini market (33.33%) while New market site was the least (31.67%). The pathogenicity result showed that the healthy tomato fruits after being inoculated with these fungi isolates deteriorated spontaneously through the period of 5 days and the organisms were recovered after culture on freshly prepared nutrient medium. Some of these organisms are known to be human pathogens, hence care must be taken when handling and using deteriorated tomatoes to avoid infections from these organisms.

Keywords: Fungi; Spoilage; Pathogenicity; Pathogens; Deterioration.

1. Introduction

Tomato (*Lycopersicum esculentum*) fruit is a very nutritious vegetable crop which are consumed globally in processed forms (stews, soups, sauce) or eaten raw with other vegetables as salad. Ripe tomato fruits contain lycopene and good source of vitamin A, B and C as well as other minerals [1, 2]. Tomato fruits have been reported to contain 4.3% carbohydrate, 1% protein, 0.6% fiber, 0.1% fat and high moisture content of 94% and are very rich in vitamins, minerals and other phytochemicals such as carotenoids, lycopene, phenolic compounds and flavonoids which are known antioxidants [3, 4].

The deterioration and contamination of tomato fruits by microorganisms could start from the field during harvesting or transportation, processing and handling of the producers. Moreover, high moisture content in tomatoes contribute to spoilage by fungi [5, 6]. The report of Ghosh [7] on microorganisms responsible for tomato spoilage indicated that fungi were the major spoilage organisms for tomato fruits. Different fungal species have been implicated in the spoilage or deterioration of tomato fruits, including *Aspergillus niger, Aspergillus flavus* [8, 9], *Aspergillus phoenicis, Absidia* species, *Trichodema* species, *Alteneria altenata, Fusarium oxysporum, Fusarium moniliformis, Mucor* species, *Rhizopus* species, *Penicillium* species, *Geotrichum* species and *Phytophthora* species

[7, 10-15]. These organisms could be of public health concerns due to the fact that some of them are associated with toxin production which could result to serious health problems when consumed in food items. For instance, *Aspergillus fluvus* produces aflatoxin which causes hepatocellular carcinoma both in animal and humans [16, 17].

Tomato fruits deteriorate easily after harvest and during storage which results to postharvest loses. Most of these loses are reported to be as a result of spoilage due to microbial attacks, physiological breakdown as well as environmental conditions like high temperature [18, 19]. However, in many parts of Nigeria, especially the Southeast and Southwest, commercial food vendors and restaurants in addition to supposedly poor urban dwellers use physically damaged or deteriorated tomato fruits for their cooking intentionally. This is due to the fact that these type of impaired tomatoes are cheaper than fresh intact ones. Moreover, it is generally believed that the physical damage of the fruits does not affect their nutritional values[9]. Hence, the need to investigate fungi responsible for the spoilage of tomatoes sold in Wukari, a town located in Northeastern part of Nigeria. Therefore, the present study evaluates fungi associated with the deterioration of (tomato) fruits sold in Wukari, Nigeria.

2. Materials and Methods

2.1. Sample Collection

Ten (10) bulbs of spoiled tomato fruits samples each were purchased from Federal University up-gate mini market, New market and Yam market in Wukari, Taraba State, Nigeria. The samples were moved in a sterile polythene bags avoiding further contamination to Biological Sciences laboratory of Federal University Wukari for mycological analysis.

2.2. Enumeration Isolation and Identification of Fungi from the Spoiled Tomatoes

One gram (1.0g) of each sample was gently crushed in a conical flask containing 9 mL of distilled water to form 10^{-1} stock solution and then serially diluted using ten-fold dilution as described by Ugbogu, *et al.* [20]. Exactly 1.0 mL from 10^{-5} dilution was inoculated into petri-dish and mixed with 20 mL aliquot of freshly prepared Sabouraud Dextrose Agar and then incubated at 28^0C for 48 hrs. Thereafter, plates were examined for fungi colonies and then counted. The colonies were calculated and expressed as spore forming unit per gram (SFU/g). The distinct fungal colonies were subcultured on fresh media to obtain pure cultures using spot inoculation for moulds and successive streaking for the yeasts. The pure fungi isolates were identified on the basis of their physiological, morphological and biochemical characteristics and with reference to Larone [21], Barnett, *et al.* [22] and De-Hoog, *et al.* [23].

2.3. Pathogenicity Test of the Isolates

Six healthy tomatoes were properly washed and rinsed with sterile distilled water and the surface was disinfected with 70% ethanol. Sterile cork borers were used to bore holes in each of the tomato fruits. Each of the isolated fungi was thereafter inoculated into the fruits after which the cores of the fruits were replaced. Sterile petroleum jelly was used to seal the holes of the fruits to prevent contamination. Six tomato fruits wounded with the cork borers but were not inoculated with the fungi served as controls. The inoculated tomato fruits and the controls were placed in sterile polythene bags (one fruit per bag). Each of the fruits was moistened with wet balls of absorbent cotton wool to create a humid condition. The fruits were thereafter incubated at room temperature for five days and observed for spoilage. The fungi were re-isolated from the fruits and compared with the original isolates [6].

3. Results

Table 1 presents the fungal load of the various spoiled tomato fruits samples. The result shows that the total count ranged from 1.0×10^5 sfu/g to 3.0×10^5 sfu/g (Federal University mini market), from 1.0×10^5 sfu/g to 2.0×10^5 sfu/g (New market) and from 2.0×10^5 sfu/g to 3.0×10^5 sfu/g (Yam Market).

The fungi isolated from the various spoiled tomato fruit samples were and presence in each sample and location show that *Rhodotorula* species was isolated from FUM3, NM3, NM 6, YM4,YM5, YM8 and YM9, *Aspergillu sniger* was isolated from FUM 1, FUM 2, FUM 4, FUM 5, FUM 6, FUM 7, FUM 8, FUM 10, NM1, NM 4, NM 6, NM 7, NM 8, NM 10, YM2, YM 3, YM 5, YM 6, YM 10, *Aspergillus flavus* was isolated from FUM 3, FUM 5,FUM 8, FUM 9, NM2, NM 7, NM 9, YM 2, YM 6, YM 7, *Mucor* species was isolated from FUM1, NM1, NM5, NM 9, YM1, YM 5, YM 7, YM 8, *Fusarium* species was present on FUM 2, FUM 4, FUM 6, FUM 7, FUM 10, NM 3, NM 5, NM 6, NM 10 while *Saccharomyces cerevisiae* was only present in samples FUM 10, NM 8 and YM3 (Table 2).

The percentage occurrences of the various isolates on the samples show that *Aspergillus niger* was the most common (63.33%), followed by *Fusarium* species (43.33%), *Aspergillus flavus* (33.33%), *Mucor* species (26.67%) and *Rhodotorula* species (23.33%) while *Saccharomyces cerevisiae* was the least with occurrence of 10.00% (Table 3).

Table 4 presented the frequency of occurrence of the isolates from each sampling location. The result shows that the percentage occurrence was highest at Yam market site (35%), followed by Federal University up-gate mini market (33.33%) while New market site was the least (31.67%).

Table-1. The fungal load of the various spoiled tomato fruit samples

S/N	Samples codes	Fungal count (Sfu/g)
1	FUM 1	2.0×10^5
2	FUM 2	2.0×10^5
3	FUM 3	2.0×10^5
4	FUM 4	2.0×10^5
5	FUM 5	2.0×10^5
6	FUM 6	2.0×10^5
7	FUM 7	2.0×10^5
8	FUM 8	2.0×10^5
9	FUM 9	1.0×10^5
10	FUM 10	3.0×10^5
11	NM 1	2.0×10^5
12	NM 2	1.0×10^5
13	NM 3	2.0×10^5
14	NM 4	1.0×10^5
15	NM 5	2.0×10^5
16	NM 6	3.0×10^5
17	NM 7	2.0×10^5
18	NM 8	2.0×10^5
19	NM 9	2.0×10^5
20	NM 10	2.0×10^5
21	YM 1	2.0×10^5
22	YM 2	2.0×10^5
23	YM 3	2.0×10^5
24	YM 4	2.0×10^5
25	YM 5	3.0×10^5
26	YM 6	2.0×10^5
27	YM 7	2.0×10^5
28	YM 8	2.0×10^5
29	YM 9	2.0×10^5
30	YM 10	2.0×10^5

Key: SFU/mL = Spore forming unit per mililitre, FUM 1-10 = samples from Federal University Up-gate mini Market; NM 1-10 = Samples from New market; YM 1-10 = Samples from Yam market

Table-2. Occurrence of fungi isolates in various spoiled tomato fruit samples

Samples	*Rhodotorula* species	*Aspergillusniger*	*Aspergillusflavus*	*Mucor* species.	*Fusarium* species	*Saccharomyces cerevisiae*
FUM 1	-	+	-	+	-	-
FUM 2	-	+	-	-	+	-
FUM 3	+	-	+	-	-	-
FUM 4	-	+	-	-	+	-
FUM 5	-	+	+	-	-	-
FUM 6	-	+	-	-	+	-
FUM 7	-	+	-	-	+	-
FUM 8	-	+	+	-	-	-
FUM 9	-	-	+	-	-	-
FUM10	-	+	-	-	+	+
NM 1	-	+	-	+	-	-
NM 2	-	-	+	-	-	-
NM 3	+	-	-	-	+	-
NM 4	-	+	-	-	-	-
NM 5	-	-	-	+	+	-
NM 6	+	+	-	-	+	-
NM 7	-	+	+	-	-	-
NM 8	-	+	-	-	-	+
NM 9	-	-	+	+	-	-
NM 10	-	+	-	-	+	-
YM 1	-	-	-	+	+	-
YM 2	-	+	+	-	-	-
YM 3	-	+	-	-	-	+
YM 4	+	-	-	-	+	-
YM 5	+	+	-	+	-	-

YM 6	-	+	+	-	-	-
YM 7	-	-	-	+	+	-
YM 8	+	-	-	+	-	-
YM 9	+	-	+	-	-	-
YM 10	-	+	-	-	+	-

Key: FUM 1-10 = samples from Federal University Up-gate mini Market; NM 1-10 = Samples from New market; YM 1-10 = Samples from Yam market; + = Positive; - = Negative

Table-3. Percentage occurrence of each Isolate from the Samples

S/N	Isolates	Number examined	Number positive	Percentage
1	*Rhodotorula* species	30	7	23.33%
2	*Aspergillus niger*	30	19	63.33%
3	*Aspergillus flavus*	30	10	33.33%
4	*Mucor* species	30	8	26.67%
5	*Fusarium* species	30	13	43.33%
6	*Saccharomyces cerevisiae*	30	3	10.00%

Table-4. The percentage occurrence of the fungi isolates in respect to sampling locations

Markets	*Aspergillus niger*	*Aspergillus flavus*	*Fusarium* species	*Rhodotorula* species	*S. cerevisiae*	Mucor species	Total	% occurrence
FUM	8	4	5	1	1	1	20	33.33%
New market	6	3	4	2	1	3	19	31.67%
Yam market	5	3	4	4	1	4	21	35.00%
Total	19	10	13	7	3	8	60	100%
% (Isolates)	31.67%	16.67%	21.67%	11.67%	5.00%	13.33%	100%	

4. Discussion

Tomato fruits have high nutritional and dietary values which made them highly demanded by the increasing population. They contain high amount of water that enhanced their susceptibility to fungal spoilage. Spoiled tomato fruit due to fungi can lead to foodborne infection and intoxication [6]. In the present study, fungi associated with the deterioration of tomato fruits sold in Wukari, Nigeria were assessed. The fungal count ranged from 1.0×10^5 sfu/g to 3.0×10^5 sfu/g, this count is higher than that reported by Onuorah and Orji [6] who found fungal count in the range of 1.3×10^3 to 2.0×10^3 cfu/ml during their study on "fungi associated with the spoilage of Post-harvest tomato fruits sold in Awka, Nigeria". This could be due to the fact that prevailing conditions leading to the spoilage of tomato fruits in Wukari encouraged the multiplication of the organisms more rapidly.

The fungi isolated from the tomato fruits sold in Wukari markets were *Aspergillus niger*, *Aspergillus flavus*, *Rhodotorula* species, *Saccharomyces cerevisiae*, *Mucor* species and *Fusarium* species. *Aspergillus niger* has the highest frequency of occurrence among the isolates. This result agrees with a previous study conducted by Ibrahim, *et al.* [8] who reported that *Aspergillus niger* is one of the major fungi responsible for the production of volatile compounds in spoiled tomato fruits. Similarly, Baker [24] isolated *Aspergillus niger* from rotten tomato fruits and reported that they are pathogenic on tomato fruits hence responsible for the spoilage. Wogu and Ofuase [4], isolated *Aspergillus* and *Fusarium* species from spoiled tomato fruits. Similarly, Dimphna [1] reported the presence of *Penicillium, Aspergillus, Fusarium, Cladosporium and Rhizopus. Aspergillus, Penicillium and Fusarium* in spoiled tomato fruits during their research on "fungi associated with postharvest decay of *Lycopersicum esculentum* M. in Abakiliki, Nigeria". The present study agrees with the findings of Sani, *et al.* [14] who isolated *Aspergillus niger*, *Rhizopus stolonifera*, *Aspergillus flavus*, *Mucor* spp. and *Penicillium* spp. from tomato fruits as organisms responsible for their spoilage which is also consistent with the reports of Ghosh [7], Onuorah and Orji [6] and Mailafia, *et al.* [25] respectively.

In the present study, *Rhodotorula* species and *Mucor* species were isolated as part of the fungi responsible for the deterioration of tomato fruits. These organisms have not been reported by to be responsible for the spoilage of tomato fruits by other researchers. However, *Rhodotorula* species are common environmental yeast that can be found in the juices of fruits which may include tomato fruits. Some strains of *Rhodotorula* are known to cause disease in humans [21]. *Mucor* species on the other hand is commonly found in rotten fruits and as saprophytes. Most species of *Mucor* are unable to infect humans and endothermic animals due to their inability to grow in warm environments close to 37^0C. Sometimes, some of the species are opportunistic and often spread rapidly causing necrotizing infections known as zygomycosis [21]. Therefore, the occurrence and identification of *mucor* species from New market could be attributed to the storage condition of the fruits whereas that of *Rhodotorula* species could be as a result of contaminants from the environment where these fruits are kept or stored, the handling process by the marketers and the transportation means by the producers.

The percentage occurrence of the fungi in relation to the markets showed that there is highest percentage occurrence (35%) in the samples from Yam market while the lowest percentage occurrence (31.67%) was in the samples from New market. The occurrence of more fungi in the tomato fruits from Yam market could be as a result of poor sanitation, overcrowding, poor storage facilities and unhygienic practices by the fruits sellers and handlers. *Aspergillus niger* had the highest percentage of occurrence (63.33%) while *Saccharomyces cerevisiae* had the lowest

percentage of occurrence (10.00 %) in the fruits studied. This result is consistent with some previous studies conducted by Ibrahim, *et al.* [8] and Onuorah and Orji [6], who reported that *Aspergillus niger* had the highest rate of occurrence in the tomato fruits they studied and concluded that the fungus may be a major contaminant responsible for the spoilage of tomato fruits.

From the pathogenicity test, the result showed that the six healthy tomato fruits inoculated with the fungal isolates deteriorated spontaneously after 5 days of observation and the fungi re-isolated were compared with the initial isolates and found to have the same characteristics. This shows that the isolated fungi were really responsible for the spoilage of the tomato fruits. This proves Koch's postulates [26] of disease infection.

5. Significance of the Study

This study discovers that potential pathogenic fungi, which could contribute to outbreak of fungal infections and mycotoxicosis, are present in the deteriorated tomato fruits in Wukari markets. This study will help the researchers to explore the possible environmental factors that favours the growth and multiplication of these microbes. Thus ways of mitigating these factors and reduce possible outbreak of infections resulting from the consumption of deteriorated tomatoes as cheap vegetable could be achieved.

6. Conclusion

Conclusively, this study identified *Aspergillus niger*, *Aspergillus flavus*, *Mucor* spp., *Rhodotorula* species, *Fusarium* spp. and *Saccharomyces cerevisiae* as fungi associated with the spoilage of tomato fruits sold in Wukari, Nigeria. Some of these fungi are associated with toxin production which can lead to serious problems in human and animals when consumed. Hence, the need for adequate quality control measures to be put in place during harvest, transportation, storage and sales of tomato fruits to reduce the risk of contamination by these fungi and as well reduce the public health issues that will arise from consumption of deteriorated tomato fruits.

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