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Disparity of fungi responsible for the spoilage of fruits sold in Sokoto Central Senatorial District, Sokoto State, Nigeria

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Abstract

This study was carried out in Sokoto central Senatorial District to identify the disparity of the fungal frequency responsible for spoilage of fruits sold at various markets of the district. A total of 240 spoilage fruits consisting of twelve (12) fruits *each of Mangifera indica, Citrulus lanatus, Citrus sinnensis* and *Cucubita maxima* were collected from five (5) different markets. Each of the fruits was cut into small pieces and inoculated on Potato Dextrose Agar then incubated at 25^0C and observed for 5 days. The isolates were identified using cultural and morphological features. Analysis of variance (ANOVA) was used to determine the significant difference for the mean frequency of these fungal species at *P<0.05* level of significance. The results obtained showed the presence of *Aspergillus niger Rhizopus stolonifera, R. oryzae, Alternaria altinata, Mucor,* and *Fusarium.* Significant disparities were reported from Tangaza (P=0.004), Kware (P=0.000), Binji (P=0.000), and Wamakko (P=0.002) markets. However, there was no significant disparity for the mean frequency of the fungi responsible for the spoilage of fruits in Kasuwar Daji Market (P=0.129). It was recommended that proper storage method be adopted in the area to mitigate the adverse effect of the pathogens on the fruits and human health.

Keywords: Fungi; Spoilage; Disparity; Districts

1. Introduction

Fungi are universal in distribution and are known to be found everywhere on earth where organic materials living or dead are present. Many of fungi are terrestrial and occur in soil rich in dead decaying organic matters. Some fungi are present in organisms as they reside in tissues. Some are aquatic and are considered as primitive. Such aquatic fungi live on decaying organic matter and living organisms found in fresh water. These fungi usually produce flagellate (motile) reproductive cells that swim to near locations (Balali *et al.,* 2015). Many fungi are found in drinking water and some grow in food stuffs such as bread, jams, piglets, fruits and vegetables. They are also present in the air of the surrounding (Chukwuka *et al.,* 2019).

Fungal pathogen responsible for the spoilage of fruits include *Aspergillum flavus, Aspergillums terrus, Aspergillums fumigates, Aspergillums niger, Mucor heimalis, Mucor racemosis, Absidia, Carmbifera, Torlopis candida, Rhizopus stolonifera and Fusarium oxysporum* (Jay, 2018). According to the Food Agriculture Organization Corporate Statistical Database 20% of fruit and vegetables produced are lost to spoilage (Droby, 2009). The improper handling, packaging, storage and transportation of fruits may result in decay and growth of micro-organisms which becomes activated because of the changing physiological state of fruit and vegetables (Ifeanyi *et al.,* 2015).

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Not much information have been scientifically documented on the fungal pathogens associated with fruit spoilage sold in Sokoto Central Senatorial District markets

Currently, Sokoto State has faced a huge shortfall in the food supply, especially fruits. The few fruits that find their way into the markets are sometimes untimely destroyed as a result of rot due to exposure to harmful microorganisms such as pathogenic fungi. Spoilage of fruits has been observed to differ from one region to another due to physiological factors (Honow *et al.,* 2003). There is, therefore, a need to study the disparity of the pathogenic fungi that are responsible for these losses to strategize control measures to reduce the negative effect to farmers, dealers, and consumers of fruits (Ismail and Zhang, 2004).

2. Material and methods

2.1. Sample Collection

A total of 240 spoilage fruits consist of twelve (12) fruits of each *Mangifera indica, Citrulus lanatus, Citrus sinnensis* and *Cucubita maxima* respectively fruits were collected from five (5) different markets namely: Kasuwar Daji, Kware, Tangaza, Binji and Wamakko Markets of Sokoto Central Senatorial Districts, the samples collected were placed in a sterile polythene bags and labeled appropriately then transported to Herbarium Laboratory, Botany Unit, Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, Sokoto State for identification and authentication.

2.2. Identification and Authentication

The samples were identified and authenticated by Taxonomist and voucher number was assigned to each sample.

2.3. Preparation of Media

Potato dextrose agar (PDA) was the medium used for the isolation of fungi. The medium was prepared by dissolving 39 gram of the media and antibiotic in 1000 mil distilled/purified water. The prepared medium was transferred into a conical flask then covered with cotton wool and then caped with aluminum foil. The suspension was heated to dissolve completely. It was then autoclaving for sterilization at 121 ^{0}C for 15 minutes. The medium was then allowed to cool down to 47 ^{0}C, then brought out and allowed to cool further then poured into sterilized Petri dishes and allowed to solidified for a period of 24 hours at room temperature (Jay, 2003).

2.4. Fungal Isolation

2.4.1. Incubation

Each of the infected fruit were surface sterilized with cotton wool soaked in 99% alcohol, the fruits were then cut out into small segments (2 mm diameter) using sterilized needle, the segment of the infected fruits were then placed on solidified PDA aseptically, substrate/plates were incubated at 25 ^{0}C for 5 days (Breidt, 2009).

2.4.2. Inoculation of the Pathogenic Fungi

From the incubated plate the different fungal isolated with difference coloration observed include; whitish, blackish and creamy which signified the occurrence of different fungal colonies. The fungal colonies that emerged were continuously sub-cultured in order to obtain a pure culture of the fungal isolates (Baiyewu *et al.,* 2007).

2.4.3. Subculture

After 5 days, other petri-dishes containing fresh medium (PDA) were used in the incubating room for subculture, the inoculating needle was burnt until red hot and allowed to cool for a while and used to transfer fungal mycelia and spores to the petri-dish containing fresh medium, and different fungal mycelia were sub culture on the fresh (PDA) medium (Droby *et al.,* 2006).

The isolates were then observed and the cultural characteristics of the colonies as well as that of the reserve, presence of absence of aerial growth, rate of growth were used (Alfred and Patrick, 2014).

2.5. Fungal Identification

The fungal isolates were identified using cultural and morphological features such as colony growth pattern, conidial morphology and pigmentation, the identification was achieved by placing a drop of the stain on clean slide with the aid of a mounting needle where a small portion of the aerial mycelia, the inoculating needle was burnt until red hot; A drop

of lacto phenol cotton blue was placed on the center of the clean slide, the inoculating needle was used to transfer a portion of the fungal colonies and spread the 2mm of mycelia growth to prepare a smear on the glass slide and then covered with cover slip; the smear was mounted under the light microscope and examined using X 10 objective lens, the nature of the mycelia, the type of fruit bodies, and spores structure serve as criteria for identification of isolates, The isolate were identified using standard mycological atlas (El-Ghaouth *et al.,* 2009).

2.6. Data Analysis

The fungi isolated was analyzed and recorded as mean frequency using descriptive statistics, analysis of variance (ANOVA) was applied to determine the disparity among the fungal species at P<0.05 level of significant using SPSS version 20.

3. Results

3.1. Disparity of Fungi Responsible for the Spoilage of Fruits in the Study Areas

Disparity of fungal species responsible for the spoilage of fruits sold in Sokoto Central Senatorial Districts was illustrated in table 1. Out of the fruits collected from Kasuwar Daji market to observe the association of fungal species, it was observed that, *A. niger* had the highest mean frequency of 3.75+0.63, followed by *R. stolonifera* (2.25+1.11), sequentially, *R. oryzae* (2.00+0.41) then *Mucor* and *A. altinata* (1.50+0.65) respectively, while *Fusarium* had the least mean frequency of 1.00+0.82. Statistical Significant difference was not reported (P=0.129)

From Tangaza Market, it was reported that, *A. niger* had the highest mean frequency of 4.50+0.41, followed by *R. stolonifera* (2,25+0.50) then *R. oryzae* (1.75+0.63) and *Mucor* (1.75+0.48) while *Fusarium* and *A. altinata* had mean frequency of 1.25+0.49 and 0.75+0.48 respectively, statistically, there was a significant difference for the fungal frequency in Tangaza market (P=0.004)

Frequency of the fungal species among the fruits sold at Kware market indicated that, *A. niger* also had the highest mean frequency (4.5+0.29), followed by *R. stolonifera* (3.25+0.63), then *R. oryzae* (2.00+0.41) while *Mucor*, *Fusarium* and *A. altinata* had the least frequency of 0.75+0.75, 0.75+0.48 and 0.75+0.48 respectively, there was statistical significant difference (P=0.000)

Isolates from Binji market showed that, *A. niger* has the highest mean frequency of 3.75+0.58, followed by *R. stolonifera* (3.25+0.25), *R. oryzae* (2.25+0.25), *Mucor* (1.25+0.25) and then *A. altinata* (1.00+0.58) while *Fusarium* had the lowest mean frequency of 0.50+0.29. Statistical significant difference was observed (P=0.000)

Significant association of fungal species responsible with spoilage of fruits was observed from Wamakko Market (P=0.002), where *A. niger* had the highest mean frequency of 3.75+0.75, followed by *R. stolonifera* (3.25+0.49), then *R. oryzae* (2.00+0.41), and then *Fusarium* (1.25+0.48), subsequently, *A. altinata* (1.00+0.41), while lowest frequency of 0.75+0.48 was reported for *Mucor*.

Table 1 Disparity of fungal species responsible for the spoilage of the fruits

Kasuwar Daji	**Mean + SEM (%)**	***P-value***
A. niger	3.75+0.63	0.129
R. stolonifera	2.25+1.11	
R. oryzae	2.00+0.41	
Mucor	1.50+0.65	
Fusarium	1.00+0.82	
A. altinata	1.50+0.65	
Tangaza Market		
A. niger	4.50+0.41[a]	0.004
R. stolonifera	2.25+0.50 [ab]	

R. oryzae	1.75+0.63 [c]	
Mucor	1.75+0.48 [c]	
Fusarium	1.25+0.49 [c]	
A. altinata	0.75+0.48 [c]	
Kware Market		
A. niger	4.50+0.29 [a]	0.000
R. stolonifera	3.25+0.63 [a]	
R. oryzae	2.00+0.41 [ba]	
Mucor	0.75+0.75 [c]	
Fusarium	0.75+0.48 [c]	
A. altinata	0.75+0.48 [c]	
Binji Market		
A. niger	3.75+058 [a]	0.000
R. stolonifera	3.25+0.25 [ba]	
R. oryzae	2.25+0.25 [b]	
Mucor	1.25+0.25 [bc]	
Fusarium	0.50+0.29 [c]	
A. altinata	1.00+0.58 [a]	
Wamakko Market		
A. niger	3.75+0.75 [a]	0.002
R. stolonifera	3.25+0.49 [a]	
R. oryzae	2.00+0.41 [a]	
Mucor	0.75+0.48 [ba]	
Fusarium	1.25+0.48 [b]	
A. altinata	1.00+0.41 [b]	

The results are shown as Mean + SEM of four replicates. At the $P<0.05$ level, values in rows with different superscripts differ significantly (One Way ANOVA followed by Duncan Multiple Range Test)

4. Discussion

Fruits production often suffers from losses on the farm, during transportation, storage, in the market, or even at the consumer end (Chukwuka *et al.,* 2010; Barth *et al.,* 2009). The presence of sugars, minerals, vitamins, amino acids, and low pH in fruits all promote the growth of the saprophytic and parasitic fungi (Hasan and Zanuddin, 2020; Bhale, 2011). The action of these pathogens has led to food shortages with economic consequences. Various saprophytic and parasitic fungi have been reported in Apple, Corn, Grapes, Guava, Mango, Orange, Papaya, and Pomegranate (Chukwuka *et al.,* 2010; Barth *et al.,* 2013; Alhaji *et al.,* 2020).

From the results obtained in this research, it was observed that of the six fungal species isolated (*A. niger, R. stolonifer, R. oryzae, A. altinata, Mucor,* and *Fusarium*), The mean frequencies of these fungal species were significantly different in all selected markets of Sokoto Central Senatorial districts as observed at $p< 0.05$ level of significance with exception of Kasuwar Daji only. The reason for the presence of the fungi in all the markets could be a result of the fact that all the fruits are mainly sourced from the main central market in Sokoto. The differences in the mean frequencies are probably as a result of transportation to the local markets, handling at the different markets, and duration of storage. *A. niger* is the most frequent of the total. This finding is consistent with those of Baiyewu *et al*. (2007), and Chukwuka *et al*., 2010; Mailafiya *et al.* (2017), *A. niger* fungi specie is majorly responsible for the spoilage of the fruits under study. The filthy

condition of most of our markets and warehouses encourages the growth of these microorganisms (Mailafiya *et al.,* 2017). They thrive most in high temperatures and high relative humidity areas (Donnell *et al.,* 2015) like Sokoto. *A. niger* though, very useful in the food and drug industry (Toma *et al.,* 2021) is said to be one of the commonest in the genus *Aspergillus* that is normally responsible for the appearance of black molds on fruits. In fact, most spoilt fruits in Sokoto Market have black molds on them. *A. niger* can also produce ochratoxin and iso-flavone orobol a common contaminant of food. These microorganisms could be dangerous to human health as *A. niger* could cause diseases such as otomycosis (Toma *et al.,*2021). Al-Najada and Al-Suabey (2014) have shown that *A niger* is one of the commonest fungi species affecting fruits.

The second most frequent Fungi, *R. stolonifera* (black bread mold) is classified under Zygomycota. It is mostly found in the soil or air and can be found in all parts of the world and more commonly in the tropics. It is a common contaminant of storage foods, especially moldy ones. Because it is known to grow rapidly, especially in closed environments it is most likely to infest the fruit during storage and transportation while *R. oryzae,* is the third most frequent fungi specie. It is said to be the most common agent of spoilage, especially on ripe fruits. Most *Rhizopus* species are saprobes and help in decomposing dead matter, some of them are parasitic or pathogenic, *Rhizopus* is troublesome in that it spreads and infects neighboring fruit thereby accelerating spoilage of fruits (Marasas *et al*., 1984).

Fusarium fungi specie mostly showed least mean frequency among the species isolated, the fungi are however a very destructive type as it has been reported to pose a real threat to food safety and human health. It is known to produce trichothecene, zearalenone, and fumonisins (Yao, 2018). These are secondary toxic metabolites that are very destructive and have been estimated to cause the global destruction of plants worth billions of dollars yearly (Marasas *et al*., 1984; Aoki *et al.,* 2014). They are also known to cause infections of the cornea and nails (Donnell *et al.,* 2015; Marasas *et al*., 1984).

5. Conclusion

This study isolated and identified the common fungi associated with fruit spoilage in Sokoto Central Senatorial District of Sokoto State. These pathogens affect the yield, quality, taste and shelf life of the fruits. It was found that the common pathogens causing fruit spoilage in some selected markets in the area are *A. niger, R. stolonifera, R. oryzae, A. altinata, Mucor,* and *Fusarium*. The fugal species association was observed to be significantly difference in all selected markets excepts Kasuwar Daji. It was recommended that appropriate measures, especially through education, be adopted in the area to reduce these pathogens

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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