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Full Length Research Article

Isolation and Identification of Fungi Associated with the Spoilage of Sweet Orange (*Citrus Sinensis*) Fruits In Sokoto State

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ABSTRACT: This study was carried out in Sokoto Metropolis to isolate and identify fungi associated with the deterioration of sweet orange fruits. A total of one hundred samples of fresh sweet Oranges (*Citrus sinensis* L) were used. First, a total of seventy samples were obtained from the three selected marketing centres in Sokoto metropolis (Central market, Kasuwar daji and Old market respectively), and transported immediately to mycology laboratory Usmanu Danfodio University, Sokoto for analysis. The oranges were kept under room temperature and observed after two weeks for spoilage. Each of the orange was cut and the liquid content inoculated on potato Dextrose Agar and incubated at 25^{0}c and observed for 3-28 days after which the different colonies obtained were identified using the slide culture technique. The fungal analysis shows that *Apergillus fumigatus, Apergillus niger, Aspergillus flavus and Rhizopus stolonifer* are associated with the spoilt sweet orange fruit (*Citrus sinensis*) with frequencies of occurrence of 22%, 17 %, 25 % 36% respectively. Fresh sweet orange fruits (thirty samples: ten each from the three markets respectively) were obtained, and the isolated culture for each of the identified fungi specie inoculated on each of the freshly purchased oranges and incubated to check for their spoilage ability. The result shows that *R. stolonifer* and *A. flavus* were the most active with rots diameter of 45 mm and 35 mm, respectively. And the least active fungus was *A. niger* having a rots diameter of 25mm.
Keywords: Fungal analysis, pathogenic, frequency of occurrence, *Citrus sinensis.*

INTRODUCTION

Citrus sinensis (L.), family Rutaceae, is one of the major commercial fruit crops that are widely consumed both as fresh fruit or juice due to its high vitamin C content and antioxidant potential (Gorinstein *et'al.,* 2001). The crop is mainly cultivated in the tropical and subtropical regions of the world in over 137 countries on six continents (Ismail and Zhang, 2004). Brazil is the largest producer followed by the United State of America (USA), China and Mexico. Spain, USA and South Africa are the largest exporting countries followed by Turkey and Morocco (Citrus Commodity Notes, 2005). Sweet orange is an important fruit crop in international trade next to grapes requiring excellent quality and shelf life attributes. Unfortunately, it is known to be attacked by several pathogens that affect the fruit quality. In developing countries, where protection and proper handling of fresh fruit is an adequate, losses during transit and storage can represent an excess of 50% of the harvested crop (Eckert and Ogawa, 1985).

Spoilage microorganisms can be introduced into the crop on the seed itself, during crop growth in the field, during harvesting and postharvest handling, or during storage and distribution (Barth *et al.,* 2009). Most citrus fruits are, in association with a variety of bacteria, and fungi, but due to a particular environmental condition, only a small proportion of the kind of microorganism(s) present will be able to grow rapidly and cause its deterioration (Alfred and Patrick, 1985). Postharvest losses and decay of Citrus fruits can be traced to infections that occur either between flowering and fruit maturity or during harvesting and subsequent handling and storage activities. Pre-harvest infections are mainly caused by fungal pathogens such as *Phytophthora spp, Colletotrichum glocosporiodes* (Browning *et al*., 1995: El Ghaouth *et al.,* 2002). Isolation and identification of the pathogens are desirable in order to strategize the control measures with a view to reducing losses due to spoilage or infections (Singleton *et al.,* 1992). Occasionally, a disease is caused by new, previously unknown pathogens that must be isolated and studied. If the identity of the pathogen is suspected or determined and a specific nutrient medium that allows only the growth of that pathogen is available, then the isolation of the particular pathogen is achieved by growing a small section of infected tissue on such media (Tsao, 1970). Ifeanyi (1995), states that fungi such as yeast and mould are mainly associated with the diseased and deteriorated citrus fruits.

Apart from mycotoxin contamination of orange fruits, the presence of fungi eventually leads to disease development in the field when the infected seeds in the fruits are planted. The objective of this study was

to isolate and identify fungi associated with post-harvest deterioration of sweet orange fruits in Sokoto metropolis, Nigeria.

MATERIAL AND METHODS

Samples Collection

A total of seventy (70) sweet orange fruits (*Citrus sinensis*) were obtained from three different markets in Sokoto metropolis and thirty healthy orange fruits were later obtained for the pathogenecity test after the isolation of the fungi. All the samples collected were placed in a sterile polythene bags separately and labelled appropriately and transported to Mycology laboratory, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto for the fungal analysis.

Isolation of Fungi

The infected citrus fruits were surface sterilized with cotton wool soaked in 70% alcohol. The fruits were then cut out into small segments (3mm diameter) using sterilized scalpel, the segments of the infected fruits were then plated on solidified Potato Dextrose Agar plates(90mm diameter) aseptically. Inoculated plates were incubated at 28±3^{0}c for 7days.

From the incubated plates the different fungal isolates with different colorations observed includes; (i) Brown (ii) Black (iii) Green and (iv) White 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.

Identification of the Fungal Isolates

The one to four weeks pure cultures of the fungal isolates were identified using cultural and morphological features such as colony growth pattern, conidial morphology and pigmentation (Sparrow, 1976), by slide culture techniques (Oyeleke and Manga, 2008). A small portion of the aerial mycelia from the representative culture was picked using a sterile inoculating needle and inoculated on a slide containing a fraction of a prepared solidified Potato Dextrose agar and incubated for 24-48hours, after which it was viewed under the light microscope first with (x10) and then with (x40) objective lens to detect spore, hyphae and other special structures.

The Morphological characteristics and appearance of the fungal isolated from the rotten *Citrus sinensis* fruits used in this study were confirmed and authenticated with the help of Mycological Atlas of Robert and Ellen (1988).

Pathogenecity Test

Pathogenecity or decay test was carried out in order to know if the isolated fungi were really responsible for the spoilage of citrus fruits. Healthy fruits were surface sterilized with 75% alcohol. Cylindrical plug tissues were cut out from the fruits using a sterilized 2mm sized cork borer. Agar disc containing one-week old fungal culture were aseptically placed in these holes, then covered and sealed off by means of petroleum jelly. The procedure was repeated separately across each of the fungal isolates. The inoculated samples and the control were placed in sterile polythene bags and incubated at 28 ± 3^{o}C for 14 days. The point of inoculation of each type of fungus was examined and recorded. The diameter of the rotten portion of the orange fruits was measured. The fungi were later re-isolated from the inoculated fruits and compared with the initial isolates.

RESULTS AND DISCUSSION

The isolated fungi from the rotten of the *Citrus sinensis* fruit and their frequencies of occurrence are shown in Table 1. The pathogenicity of the isolated fungi from the rotten *Citrus sinensis* fruit after fourteen days of incubation shown in Table 2.

Table 1: Fungi isolated from rotten of *Citrus sinensis* Fruits in Sokoto, Nigeria.

Fungal Isolates	Frequency (%)
Rhizopus stolonifer	36.0
Aspergillus flavus	25.0
Aspergillus fumigatus	22.0
Aspergillus niger	17.0

Table 2: Decay rate of Fungi isolated from rotten *Citrus sinensis* fruits after 14 days of incubation

Fungal Isolates	Diameter of Rot (mm)
Rhizopus stolonifer	45.0
Aspergillus flavus	35.0
Aspergillus fumigatus	30.0
Aspergillus niger	25.0

This study shows that *A. fumigatus, A. niger, A. flavus* and *R. stolonifer* and some yeasts were found in the spoilt sweet orange fruits sold in Sokoto State, Nigeria. Some of these pathogens have been reportedly isolated from Pawpaw fruits in Nigeria (Baiyewu *et al.,* 2007; Chukwuka *et al*., 2010). Out of the fungi isolated, *R. stolonifer* has the highest frequency of occurrence (36 %) followed by *A. flavus* (25 %) then *A. fumigatus* (22%) and *A. niger* with 17 % frequency of occurrence. This is however in agreement with Ifeanyi, (1995) and Bello (2010)

whom both isolated about seven different fungal genera from different fruits including sweet orange fruits and When these isolates were aseptically inoculated into healthy susceptible fruits, the characteristic symptoms originally observed were also noticed. All the four organisms were successfully taking part in the decay and are thus confirmed as the causal organism of fruit decay (Baiyewu *et al*., 2007; Chukwuka *et al*., 2010).Thus These fungi were also found to be associated with the deterioration of orange fruits, All the four organisms isolated were confirmed to cause spoilage on the sweet orange fruits but in varying degrees. Of all the isolated fungi, *R. stolonifer* and *A. flavus* were the most pathogenic with rapid disintegration of the treated fruits in 14days having a rots diameter of 45mm and 35mm, respectively. And the least pathogenic fungus was *A. niger* having a rots diameter of 25mm.

Generally, fungi that cause spoilage are considered toxigenic or pathogenic (Al-Hindi *et al.,* 2011). Some moulds may produce mycotoxins (Tournas and Stack, 2001). The fungi isolated in this study have been reported to produce secondary metabolites in plants tissues. These secondary metabolites are potentially harmful to humans and animals (Eaton and Groopman, 1994; Baiyewu *et al.,* 2007). A good example is Aflatoxin which has been implicated in cancer of the liver (heplatoma), aflatoxicosis and also acute hepatitis in humans, especially in the developing world (Krogh, 1992; Prasad, 1992; Eaton and Groopman, 1994; Muhammad *et al.,* 2004; Baiyewu *et'al.,* 2007). Pathogenic fungi, on the other hand, could cause infections or allergies (Monso, 2004).

CONCLUSION

In this study, it was found that *A. fumigatus, A. niger, A. flavus* and *R. stolonifer* are detected in spoilt sweet oranges. Therefore, sweet orange fruits should be properly refrigerated and should be discarded of there are any changes notice in the colour or taste of the fruit as will be hazardous to human health.

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