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Fungi Associated With Spoilage of *Citrus Sinensis* in Fruits and Vegetables Market, Sokoto, Nigeria

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Research on fungal pathogens associated with spoilage of *Citrus sinensis* in vegetables market was conducted to identify the fungal organisms responsible for the various forms of infection. 75 rotten orange fruits were randomly selected from different locations of the market. Rate of occurrence of each fungal pathogen was determined. Diseased portions of the samples were cut and sterilized with sodium hypochlorite, cut into pieces and placed in a Potato Dextrose Ager amended with Streptomycin (PDAs) medium contained in petridish and arranged in the laboratory in a CRD. Pure cultures were obtained, colonial and morphological characteristics of the isolates identified. 10 fungal pathogens were observed to be among the causes of orange fruits spoilage in the market with the highest occurrence by *Aspergillus niger* Effort should be made to develop management practices of the identified pathogens in order to enhance the quality and nutritional value of the orange fruits.

Keywords: Fungi, Spoilage, Orange,

INTRODUCTION

Citrus Sinensis is a member of the family of "Rutaceae" which contains about 150 genera and nearly 2000 species, probably originated in North Eastern India, in Burma and in the adjoin areas (FAO, 2004). The genus citrus contain all the species widely cultivated in West Africa including Nigeria. The sweet orange are evergreen trees of small to medium stature. They often have thorny (prickly) stem 1.6 – 1.9m tall with a rounded symmetrical spreading crown. The leaves are shiny and leathery they are elliptical and up to 10.2cm long, they have wings on their petioles (leaf steams). The approximate composition of edible portion is

water (86%), protein (0.6%), fat (0.1%). Micro-nutrients per 100g; calcium (24mg), vitamin A (12mg), thiamine (0.06mg), riboflavin (0.02mg) niacin (0.1mg), also, one medium orange supplies about 66mg of vitamin C, a 100 percent of the daily dietary requirement for adults (Alfred and Patrick, 1985). Diseases that harm or destroy the Citrus fruits are through the impairment of beneficial physiological or biochemical processes caused by continuous irritation initiated by primary causal agent/pathogens resulting in the reduction of nutritional and market values of the fruits in a given environment (Nnadi and Madabuike, 2000).

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Table 1 Summary of Pathogens identified to be responsible for the spoilage of oranges in the study area.

Pathogens	Frequency (Infected oranges)	Percentage (%)
<i>Aspergillus niger</i>	43	57.3
<i>Aspergillus flavus</i>	09	12.0
<i>Aspergillus fumigatus</i>	13	17.0
<i>Aspergillus terreus</i>	11	14.7
<i>Absidia corybifera</i>	09	12.0
<i>Fusarium oxysporum</i>	10	20.0
<i>Mucor racemosus</i>	10	13.3
<i>Mucor hiemalis</i>	11	14.7
<i>Rhizopus stolonifera</i>	05	6.7
<i>Troloopsis candida</i>	22	29.3

MATERIALS AND METHODS

The research was conducted in Sokoto North Local Government Area of Sokoto State, Nigeria. It has an Area of 51km² (19.7sqm) and a population of 232,846 at the 2006 census, and located 13⁰ 03N-50⁰ 14E. (The Wikipedia, free encyclopedia)

Annual rainfall ranges from 500 to 750mm falling between May and September and dry season last from October to April and extend to May or June in some parts of the State. Average temperature is about 32⁰c during west season and up to 40⁰c in the dry season. (S.S.D, 2000)

Seventy five rotten fruits of *Citrus sinensis* were randomly collected from different locations in the fruits and vegetable market (Ramen kura) in the study area. The diseased oranges were put in a sterile polythene bags and taken to Mycology laboratory for identification. Diseased samples were cut washed with distilled water, cut into pieces and sterilized for five minutes using 0.5% sodium hypochlorite and rinsed thrice with sterile distilled water and blotted dry with a sterile filter paper (Rizvi and Yang, 1996). The pieces were then placed onto a 90 mm (diameter) petridish containing freshly prepared Potato Dextrose Ager with streptomycin (PDAs) and labeled. The plates were incubated at 27⁰C and observed daily (Ndiaye, 2007). Fungal mycelia of the isolated organisms were sub-cultured on fresh PDAs to obtain pure cultures. The colonial characteristics of the isolates were observed and detailed morphological features determined. The fungal species were identified using an Atlas of Robert and Ellen (1988).

RESULTS

Results of fungal pathogens responsible for the spoilage of orange fruits in the study area are presented in Table 1. Among the 75 spoiled orange fruits sampled, *Aspergillus niger* had occurred in 45 of them (57.3%), followed by *Troloopsis candida* with 29.3% of occurrence in 22 fruits while *Rhizopus stolonifera* had the least frequency of occurrence (6.7%) in only 5 fruits.

DISCUSSION

Fungal pathogens responsible for the spoilage of oranges include *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigates*, *Aspergillus terrus*, *Mucor heimalis*, *Mucor racemosus*, *Absidia*, *Carmbifera*, *Torulopsis candida*, *Rhizophus stolonifera* and *Fusarium oxysporum* are associated with rotten citrus species. The presence of these pathogens could be as a result of sufficient sugary flavor in the orange which favours their growth. The spoilage of the fruit is influenced by combination of intrinsic and extrinsic factors, such as: acid, temperature, nutrient and anti-microbial compound (Anonymous, 2010). Acid is a major factor in spoilage, acid fruit are generally spoiled by fungi organism.

Similarly, temperature of the study area might have influenced the activities of the fungal pathogens; this agrees with the findings of Frazier and Westchoff (1978) that temperature is also a major factor that plays an important role in the growth of fungi, the higher the

Table 2 Colonial Characteristics of Fungal Isolates Associated with Spoilage of *Citrus sinensis*

S/N	Pathogens (Fungi)	Growth	Colonial Characteristics
1.	<i>Aspergillus niger</i>	Fast growth	Thinner but speculating densely, the colonies consist of a compact white or yellow basal felt with a dense layer of dark brown to black.
2.	<i>Aspergillus flavus</i>	Fast growth	Colonies considering dark green conidiophores.
3.	<i>Aspergillus fumigatus</i>	Fast growth	Colonies dark green and speculating heavier conidiophores inter mixed with aerial hyphae bearing conidiophores.
4.	<i>Aspergillus terreus</i>	Fast growth	Similar to <i>Aspergillus niger</i> , but speculating more dense the colonies consist of a white or yellow with a dense layer of brown to black conidiophores.
5.	<i>Absidia corybifera</i>	Superficial	Colony grey white in colour, semi glossy, smooth to ripple.
6.	<i>Fusarium oxysporum</i>	Ovoid growth	Aerial mycelium sparse or floccose, becoming felt, whitish with a purple tinge more incense near the medium surface.
7.	<i>Mucor racemosus</i>	Rapid growth	Colony white and brownish grey consisting of tall and short sporran giosphores.
8.	<i>Mucor hiemalis</i>	Rapid growth	Colony greenish-yellow in day light more grayish in darkness.
9.	<i>Rhizopus stolonifera</i>	Sow growth	Colony whitish and dark brown to black – brown.
10.	<i>Troloopsis candida</i>	Rapidly growing	The colonies are light grayish, snow white and cottony covering the whole Petri –dish within one week.

Table 3 Morphological Features of Fungal Isolates Associated with Spoilage of *Citrus sinensis*

S/N	Fungal Pathogens	Morphological Characteristics
1.	<i>Aspergillus niger</i>	Have a conidial head; radiate tending to split into loose columns with age, conidiophores smooth walled, hyaline but often in brown colour.
2.	<i>Aspergillus flavus</i>	Conidial head typically radiate later splitting into several loose columns, yellow green becoming dark yellow green, conidiophores hyaline coarsely rough ended.
3.	<i>Aspergillus fumigatus</i>	Conidial heads typically columnar, conidiophores short smooth walled, green particularly in the upper part.
4.	<i>Aspergillus terreus</i>	Conidial head radiate tending and split in the loose columns with age. Conidiophores smooth – walled hyaline but often in brown colour.
5.	<i>Absidia corybifera</i>	Hyaline to brownish, smooth walled with an occasional septum, often terminating in a larger sporangium.
6.	<i>Fusarium oxysporum</i>	Septet, borne on lateral, simple often reduce phial ides or on phial ides on short branched conidiophores generally abundant in false heads.
7.	<i>Mucor racemosus</i>	<i>Sporoglyphores</i> branched with short branching, some time recovered with encrusted wall.
8.	<i>Mucor hiemalis</i>	<i>Sporoglyphores</i> ellipsoidal or somewhat kidney shapes, oldie present in substrate hyphae.
9.	<i>Rhizopus stolonifer</i>	Stolons smooth walled and branched opposite the sporoglyphores arising directly with rhizoids.
10.	<i>Troloopsis candida</i>	Blastosphores only round – oval, short armed ramified chains.

temperature, the faster spoilage will occur. John (1969) added that the spoilage of orange in this part of the country could be attributed to high temperature as fungi which brings about decay of fruit response to temperature range for most species is 22-23°C.

Aspergillus niger was observed to have higher rate of occurrence among the sample collected from the study area at (57.23%), this could be due to its faster growth. It also attacked the fruit by entrance into the inner part of the fruit when wound during processes which appear brown colour in the surface of the orange as observed by John (1969).

Black *Aspergillus* species such as: *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus terreus* can cause *Aspergillus* bunch rot in the oranges particularly in warmer climates (Hocking, 2007).

Rhizopus stolonifer attack vegetable and fruits because of their sugary content and low p^H cause soft rot in the edible portion of the fruits. This species has the lowest occurrence within the study area, which attained 6.7% present in the experiment because of its slow growth rate. It makes the fruits soft and mushy, a second major rot in all kind of orange fruits transit rot, so named because it usually develops in boxes during transportation (Ifeanyi, 1995; Carlin et al., 1989).

Troloopsis candida cause some fermentation which changes the appearance of the infected orange designated as grey rot, black rot soft and others.

Mucor spp also causes post harvest rot in orange fruit when it attacks the peel or the fruit that appear brown in cottony and influence the olfactory on orange fruits as claimed by Boike and Damoglus (1987).

Several *Fusarium* species particularly, *F. oxysporum* can invade orange specially when storage temperature are higher or storage period becomes excessive has been reported to cause rot in oranges (Anon. 2011).

CONCLUSION

Several fungal pathogens attacked the internal and external feature of citrus fruits which causes spoilage that could be observed by tactile, olfactory or visual. Hence the need to conduct a research aimed at evolving methods of controlling the fungal pathogens identified to be responsible for the spoilage of the orange fruits in the study area also, management of other non pathogenic factors that could lead to the same menace.

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