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DETERMINATION OF FUNGAL PATHOGENS ASSOCIATED WITH THE SPOILAGE OF *Citrus sinensis* (sweet orange) IN GOMBE MAIN MARKET GOMBE STATE, NIGERIA.

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ABSTRACT

Isolation and identification of fungal pathogens associated with the spoilage of *citrus sinensis* was carried out between September to December 2022 in Gombe main market. Total of thirty samples collected for the analyses. Potato dextrose Agar (PDA) was used for isolation of fungi from the spoilt *Citrus sinensis* fruits and for the preparation of pure cultures. The isolates pathogenicity was also carried from the pure cultures. Four fungal pathogens were which includes *Aspergillus Niger* with high frequency number of 47.6%, *Aspergillus fumigatus* with 29.5% frequency followed by *Aspergillus flavus* with 14.2% and lastly *Rhizopus stolonifer* with the lowest frequency of 9.5%. The isolates were identified by comparing their characteristics with those of known taxa and also based on macro-morphological and micro-morphological characteristics.

Keywords: Pathogenic fungi; *Citrus sinensis*; Gombe state; Nigeria

INTRODUCTION

Fruits are very important and have high dietary and nutritional qualities. Consumption of fruits products has dramatically increased by more than 30% during the past few decades (Barth *et al.*, 2009). They are good sources of nutrients for growth, repair and control of body processes as most of them contain sugar, vitamins, mineral elements and small quantities of protein and oil (Zubbair, 2009). *Citrus* fruits widely used as edible fruits all over the world and it belongs to genus *Citrus* and family Rutaceae, containing 130 genera in the seven subfamilies with many important fruits and fruits products. It is cultivated throughout the tropical and temperate regions of the world. *Citrus* fruits include oranges, lemons, limes and grape fruits. Among the *Citrus* fruits, oranges (sweet, mandarin and sour) are the most important as fresh fruits and they contribute to about 80 percent of the world's *Citrus* fruits production (Sidana *et al.*, 2013).*Citrus sinensis*, also known as sweet orange, is the most popular of the *Citrus* fruits. It is widely cultivated in most regions of the world (Muhammad *et al.*, 2013).

According to United Nations Conference on Trade and Development (UNCTAD), in 2004 there were 140 *Citrus* producing countries. 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). In Nigeria, the *Citrus sinensis* producing States include Benue, Taraba, Oyo, Imo, Ebonyi, Kwara, Kogi, Kaduna, Ogun, Ondo, Ekiti, Edo, Delta and Osun State (Oyegun, 2002). Production of *Citrus sinensis* in Nigeria is forecast up from 3,325,000 tons to 3,800,000 in 2013 (Food and Agricultural Organization of the United Nations [FAO], 2015). Nigeria is among the top orange producers in the world. About 30 to 50 percent orange produced is wasted during storage and transportation and is frequently reduce by fungal attack. The occurrence of fungal

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spoilage of orange fruits is also recognized as a source of potential health hazard to man and animals. The research is aimed at determine fungal pathogens associated with the spoilage of *Citrus sinensis* in Gombe main market gombe State, Nigeria.

MATERIALS AND METHODS

Study Area

The research was conducted in Gombe metropolis, Gombe State, Nigeria. It has an Area of 20.265Km2(7.246sqm) and a population of 2,353,000 at the 2006 census, and located at 10o17IN-11o10IE / 10.283oN-11.167oE. Average rainfall is 85mm falling between April and October and dry season last from November to March. The temperature ranges between 29oC -39oC, while the mean annual temperature is 340C(Bukar 2009).

Sample collection

A total of thirty(30) samples were randomly obtained from Gombe main market in Gombe metropolis on weekly basis, and five (5) healthy orange fruits were later obtained for the pathogenicity test after the isolation of the fungi. All the samples collected were transported to Biology laboratory, Department of Biological Sciences, Gombe State University, Gombe for the fungal analysis.Preparation of culture medium

Potato dextrose Agar (PDA) was used for isolation of fungi from the spoilt *Citrus sinensis* fruits and for the preparation of pure cultures. The medium was prepared following the manufacturer's instruction. Thirty-nine (39) grams of Potato Dextrose Agar powder was dissolved in 1000 ml of distilled water in a beaker. The medium was placed in sterile conical flask covered with cotton wool and aluminium foil paper. It was then sterilized in autoclave at 121oC for 15 minutes. The medium was cooled after autoclaving to 50 degree Celsius and then dispense aseptically into sterile Petri dishes. Chloramphenicol (0.5%w/v) was added to the medium to inhibit growth of bacteria.

Isolation of fungi

The borderline between healthy and infected tissue of surface fruits was cut with sterile scissors into small segments. The cut portion of the wound was disinfected with ethanol of 75% concentration for 2 min. These were then be rinse in three different changes of distilled water. Each cut portion of the infected part showing infection was placed in Potato Dextrose Agar plates containing Chloramphenicol (0.5%w/v) to inhibit the growth of bacteria. These were incubated at ambient temperature for 10 days. 50% lactic acid was added to enhance fungal growth. Pure cultures of the resulting fungal colonies were obtained from the subcultures of the primary plates as described by Akinmuse (2011).

Identification of fungal Isolates

The isolates were identified by comparing their characteristics with those of known taxa, as described by (Oviesogie 2015). The fungal isolates were identified based on macro-morphological and micro-morphological characteristics. Macroscopic characteristics include colony morphology, colour, shape and appearance, while microscopic characteristics are conidia shape, hyphae colour, septation and pigmentation. The colonies were observed under compound microscope at magnification of 10X and 40X and recorded. During slide preparation, a drop of Lactophenol cotton blue stain was placed on a clean glass slide and with the aid of inoculating needle, a small portion of the mycelium from the fungal cultures was collected and placed in the drop of the stain. The mycelium was spread very well

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on the slide and then covered with cover slip.

RESULTS

From the research, Four fungal species were isolated and identified on the basis of macro-morphological and micro-morphological characteristics. These are: *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus and Rhizopus stolonifer,* as shown in Table 1. The frequency of occurrence of each fungal isolate is shown in Table 2. *Aspergillus niger* had the highest occurrence of (44.4%), *Aspergillus fumigatus (*26.6%) and Aspergillus flavus (13.3%), while *Rhizopus stolonifer* had the least occurrence of (8.8%).

Macroscopic examination of *Aspergillus Niger, Aspergillus fumigatus, Aspergillus flavus and Rhizopus stolonif*er are black colors with white edges, growth recognized within three days and consist of felt green and white colouration, a powdery masses of yellowish green spores on the upper surface and reddish gold on the lower surface, the colonies where white and cotory later become dark brown respectively.

Microscopic examination of *Aspergillus Niger Aspergillus fumigatus Aspergillus flavus and Rhizopus stolonifer* are conidiophore were broad, long, and unbranched conidial leads were large, conidial lead are typically columner and uniseriate, uniseriate as pergilla columnar conidial heads, flasher-shaped vesisles, non septate any celia with branches rhizoids present.

The frequency of occurance of *Aspergillus Niger* is 20 with frequency of 47.6 *Aspergillus fumigatus* 12 with frequency of 28.5 *Aspergillus flavus* 6 with frequency of 14.2 then *Rhizopus stolonifer* 4 with 9.5 frequency.

Table 1: Frequency of occurrences of various fungal isolate.

Fungal isolate	Number of isolate	% Frequency
Aspergillus niger	20	47.6
Aspergillus fumigatus	12	28.5
Aspergillus flavus	6	14.2
Rhizopus stolonifera	4	9.5

DISCUSSION

*Citrus sinensi*s fruits contain high levels of sugars and nutrients and their low pH values make them particularly desirable to fungal decay (Singh and Sharma, 2007). This study focused on assessment of fungal pathogens associated with *Citrus sinensis* spoilage in Gombe metropolis. Out of the 30 samples analysed, 47% were found to be infected with one or two fungal infections. Four fungal species were isolated and identified as: *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus* and *Rhizopus stolonifer*. This is similar to the work of (Bukar 2009) who reported that diseased oranges sampled from retailers at Na'ibawa Yan Lemu market in Kano metropolis were found to be infected with Aspergillus sp., Rhizopus sp. and Fusarium sp. (Muhammad *et al*. 2013;) also reported that *Fusarium oxysporum, Fusarium solani, Aspergillus niger, Aspergillus flavus, Candida tropicalis, Rhizopus stolonifer, Penicillium digitatum* and *Penicillium chrysogenum* are associated with Sweet orange spoilage in Niger state. This may be due to the poor hygiene of the handlers or poor storage method adopted in the area.

Tafinta *et al*., (2013), revealed that *Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus* and *Rhizopus stolonifer* were found to be associated with the spoilt Sweet orange fruits in Sokoto State. Also, (Akinro *et al.* 2015) identified *Aspergillus niger* and *candida tropicalis* as the pathogens responsible for the spoilage of

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Sweet oranges sampled from selected market in Iree Town of Boripe Local Government, Osun State, Nigeria. This is similar to the finding of (Oviesogie *et al*. 2015) who reported that *Aspergillus* species, Penicillium species, Mucor species, *Rhizopus s*pecies, Candida tropicalis, Saccharomyces cerevisiae and Alternaria species were identified as the pathogens responsible for the spoilage of Sweet orange in Benin City, Edo State. He also suggested that the presence of the fungi or their resistant spores is most likely to have originated from the farms where the fruits were harvested and some from the stores due to horizontal contamination by the already spoilt fruits.

(Akhtar 2013), (Samuel *et al*. 2015), (Nasiru *et al.* 2015) and (Akinmusire 2011) also reported similar species with this work. They reported that *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus* and *Rhizopus stolonifer* etc., were among the pathogens associated with orange spoilage. All these similarities arose possibly as a result of some certain factors, among them is the source of the fruits. The fruits used in this study and those used in the other studies were not cultivated in the study areas but are transported to from distant villages in locally woven baskets and sacks under weather conditions that encourage the incubation of these contaminating microorganisms. Most of these fruits were obtained from Benue, Enugu, Kogi, Edo, Anambra, Ogun, Oyo, Ondo, Taraba, Imo, Ebonyi, Kwara, Kaduna, Ekiti, Delta and Osun State, which are the main sources of Oranges in Nigeria. Temperature is also a major factor that plays an important role in the growth of fungi, the higher the temperature, the faster spoilage it become. The temperature of this study area (29oC-39oC) is nearly the same with the temperatures of the previous researches' areas. Finally, the mode of infection is also nearly the same because in most of the cited literatures, it was revealed that the improper handling, packaging, storage and transportation may result in decay and growth of the microorganisms. This is also clear in Gombe metropolis. Store houses may also have residues of the pathogens which will re-infect the new products and the circle continuous.

Generally, fungi that cause spoilage are considered toxigenic or pathogenic and some of these moulds may produce mycotoxins. 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 (Baiyewu *et al.*, 2007). *Aspergillus* species are known to produce several toxic metabolites, such as malformins, naphthopyrones and they can produce Ochratoxins (OTA), a mycotoxin which is a very important toxin worldwide because of the hazard it poses to human and animal health (Baiyewu *et al.,* 2007). Therefore it is advisably to avoid consumption of such spoilt oranges and it should be discarded properly instead of giving it to poor people more especially "ALMAJIRAI" as that will be hazardous to human health.

CONCLUSION

Conclusively, *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus* and *Rhizopus stolonifer* were found to be associated with Citrus sinensis spoilage in Gombe metropolis. These fungi are known to be toxigenic or pathogenic to health and were observed to be able to re-infect healthy oranges within short time, which poses a serious economic threat to sellers of oranges in Gombe metropolis. Therefore, effective awareness on control measure is needed to avoid high economic losses. As well, further research should be carry out to find effective methods of controlling the fungal pathogens.

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