Bima Journal of Science and Technology, Vol. 7 (4) Dec, 2023 ISSN: 2536-6041

DOI: 10.56892/bima.v7i4.535

Assessment of Fungal Pathogens Linked to *Citrus sinensis* (L.) Osbeck Spoilage in Gombe Metropolis, Gombe State, Nigeria

[1]Umar A. M., [1]Abubakar A., [1]Abubakar B., [1]Aako A. O. and [1]Atayi M.G.

Department of Science Laboratory Technology, Federal Polytechnic Kaltungo, Gombe, Nigeria

Corresponding Author: adamci1027@gmail.com

ABSTRACT

Citrus sinensis (L.) Osbeck (Sweet orange) is one of the most popular commercial fruits that is consumed in large quantities as juice or fresh fruit. This study was carried out to isolate and identify fungal pathogens linked to the rotten *Citrus sinensis* fruits in Gombe State. A total of one hundred samples of rotted fruits were taken from the main market in Gombe. Each sample's border line between healthy and diseased tissue was cut and inoculated in petri dish containing Potato Dextrose Agar, and incubated at room temperature for 10 days. Four fungal pathogens were identified as *Aspergillus niger*, *Rhizopus stolonifer*, *Candida tropicalis* and *Fusarium oxysporum*, with the frequencies of occurrence of 53.4%, 23.3%, 17.8% and 5.5% respectively. The pathogenicity test revealed that all fungal isolates were pathogenic. Consumption of spoilt oranges should be avoided.

Keywords: Fungi, *Citrus sinensis,* Spoilage, Isolation, Identification, Pathogens.

INTRODUCTION

Fruits are highly nutritious, which makes them essential. Muhammad *et al.* (2013) report that over the last few decades, there has been a significant increase in fruit product consumption of over 30%. Since most fruits have sugar, vitamins, minerals, and trace amounts of protein and oil, they are essential sources of nutrients for bodily functions like growth, repair, and regulation (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. Oranges among the *Citrus* fruits, 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 (L.) Osbeck, also known as sweet orange, is widely cultivated in most regions of the world with production of 161.8 million tons in more than 10.2 million hectares in 2021, and Nigeria is among the top 10 orange producers in the world. *Citrus sinensis* producing States in Nigeria, include Taraba, Benue, Ebonyi, Kogi, Kwara, Oyo, Imo, Ogun, Ondo, Ekiti, Edo, Kaduna, Delta and Osun State (Food and Agricultural Organization of the United Nations, 2023).

Citrus sinensis is a major source of vitamins (Di Majo *et al.,* 2005). Several studies have shown that oranges are very important. Etebu and Nwauzoma (2014) stated that orange have health benefits which include: high blood pressure treatment of arteriosclerosis, kidney stones, prevention of cancer, stomach ulcers and reduction in cholesterol level and strengthening of the immune system. These health benefits are as a result of phytochemical compounds like synephrine, liminoids, polyphenols, hesperidin flavonoid, pectin etc. and vitamins, especially vitamin C. A single orange is said to have over 60 flavonoids and about 170 phytonutrients and

DOI: 10.56892/bima.v7i4.535

with blood clot inhibiting, anti-tumor, anti-inflammatory and antioxidant properties.

Any changes in the condition of the fruits in which they become less palatable is termed as Spoilage of *Citrus sinensis*. Modifications in appearance, taste, smell, and/or texture may accompany these disorders (Akinmusire, 2011). Fruits are particularly vulnerable to pathogenic fungal attacks because of their greater moisture content, low pH, and nutrient makeup, and these fungi can cause rots in fruits and even render them unfit for human consumption by creating mycotoxins. (Moss, 2002).

Fungal infection spread principle of fruits supports that one infected orange fruit can infect other orange fruits while they are being stored and transported (Jay, 2003). Plants are usually infected by soil-infesting fungi and bacteria that cause the loss of fleshy tissue at the time of or shortly before harvesting. On the other hand, handling or storage after harvest may result in infection. Common air molds, like Penicillium species, have the potential to penetrate the vulnerable tissue during packaging and result in loss. By these pathogens, the extensive spore production ensures its presence wherever fruit was handled, including packing house, field, equipment, storage rooms, de-greening, market place and transit containers (Ismail and Zhang, 2004).

During storage and transit, between 30 and 50 percent of the orange crop is wasted, and fungal attacks often cause this amount to decrease. Orange fruit rotting caused by fungi poses a risk to human and animal health (Oviesogie *et al.,* 2015). Referring to this perspective, it is crucial to isolate and identify the fungal pathogens linked to *Citrus sinensis* spoiling in order to determine the best course of action.

MATERIALS AND METHODS

Study Area

The research was conducted in Gombe metropolis, Gombe State, Nigeria. It has an Area of 20.265Km^2 (7.246sqm) and a population of 2,353,000 at the 2006 census, and located at 10^{o}17^{I}N-11^{o}10^{I}E / 10.283^{o}N-11.167^{o}E. Average rainfall falling between April and October is 85mm and dry season last from November to March with a temperature ranges between 29^{o}C -39^{o}C, while the mean annual temperature is 34^{0}C.

Sample Collection

A total of one hundred (100) samples were randomly obtained from Gombe main market in Gombe metropolis on weekly basis. After the fungi were isolated, ten (10) healthy orange fruits were obtained for the pathogenicity test. Each sample was meticulously carried to the Department of Biological Sciences' Biology Laboratory, Gombe State University, Gombe, for examination

Culture Medium Preparation

In this study, Potato Dextrose Agar (PDA) is used for isolation of fungi and for the preparation of pure cultures. The medium was prepared in accordance with the manufacturer's guidelines. A beaker filled with 1000 milliliters of distilled water was used to dissolve aliquots of thirty-nine (39) grams of potato dextrose agar powder. The medium was put into a sterile conical flask that was wrapped in aluminum foil paper and cotton wool. After that, it was sterilized for 15 minutes at 121°C in an autoclave. Following autoclave sterilization, the medium was cooled to 50 °C before being aseptically dispensed into sterile Petri dishes. The addition of 0.5%w/v chloramphenicol was made to prevent bacterial growth.

DOI: 10.56892/bima.v7i4.535

Isolation of Fungi

The surface fruit's border between healthy and infected tissue was cut into small sections using sterile scissors. Ethanol (75% concentration) was used for two minutes to disinfect the area of the wound that had been cut. After that, these were rinsed three times with distilled water. Each infected cut piece was put in Potato Dextrose Agar plates with 0.5%w/v chloramphenicol to stop the bacteria from growing. These were incubated for ten days at room temperature. To promote fungal growth, 50% lactic acid was added. The resulting fungal colonies were obtained as pure cultures from the subcultures of the primary plates.

Identification of Fungi

The fungal isolates were identified by comparing their features with those of known taxa, as described by Oviesogie *et al.* (2015). Both macro- and micro-morphological traits were used to identify the fungal isolates. Shape, conidia, hyphae color, septation, and pigmentation are examples of microscopic features, whereas colony morphology, color, and appearance are examples of macroscopic characteristics. The colonies were noted after being examined under a compound microscope at 10X and 40X magnification. During slide preparation, a small amount of the mycelium from the fungal cultures was collected and added to a drop of Lactophenol cotton blue stain that had been applied to a clean glass slide using an inoculating needle. After evenly spreading the mycelium across the slide, cover slip was applied.

Pathogenicity Test

The pathogenicity test was performed in accordance with Chukwuka *et al.* (2010) and Baiyewu *et al*. (2007) instructions. Healthy clean mature fruits were washed with distilled water and their surfaces were sterilized with 75% ethanol. A hole was made with a 3mm cork borer on each of the fruit. A colony of fungi isolate (from each pure culture) was inoculated into the hole and the point of inoculation was sealed with petroleum jelly to prevent contamination. Controls of orange fruits were wounded but not inoculated with fungal colony. Both the inoculated fruits and the controls were placed in clean polyethylene bag (one fruit per bag). To create a humid environment for them each fruit was moistened with wet balls of absorbent cotton wool and incubated at ambient temperature for 4 days (Oviesogie *et al.,* 2015).

RESULTS

Out of the 100 samples collected, four fungal species were isolated and identified based on both macro- and micro-morphological traits. These are: *Aspergillus niger*, *Rhizopus stolonifer*, *Candida tropicalis* and *Fusarium oxysporum*, as shown in Plate I-IV.

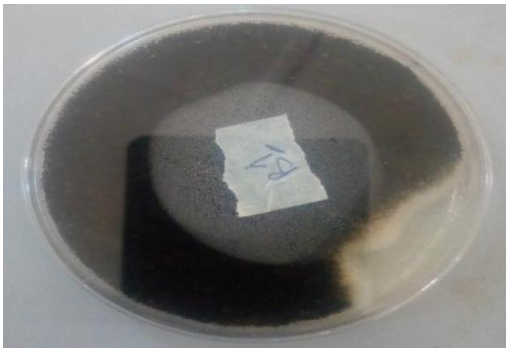

Plate I: *Aspergillus niger*

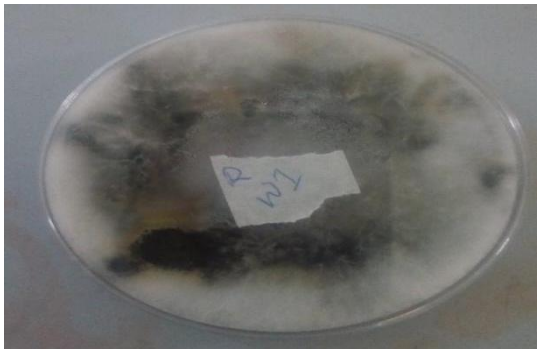

Plate II: *Rhizopus stolonifer*

Bima Journal of Science and Technology, Vol. 7 (4) Dec, 2023 ISSN: 2536-6041

DOI: 10.56892/bima.v7i4.535

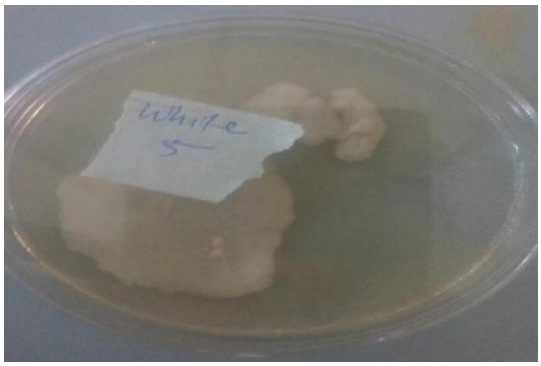

Plate III: *Candida tropicalis*

Plate IV*: Fusarium oxysporum*

Figure 1 displays the frequency of occurrence for each fungal isolate. With a frequency of 53.4%, *Aspergillus niger* was most common, followed by *Rhizopus stolonifer* (23.3%) and *Candida tropicalis* (17.8%). *Fusarium oxysporum* was least common, occurring in 5.5% of cases.

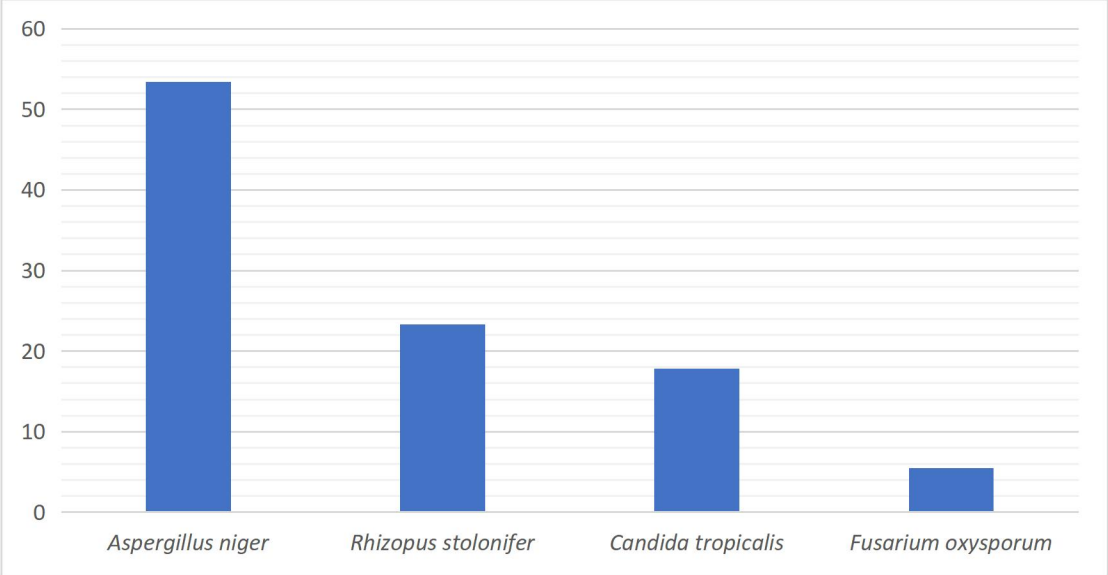

Figure 1: Frequency (%) of occurrence for each fungal isolate

The pathogenicity test results on fresh, healthy Citrus sinensis fruit samples are displayed in Table 1. The results of the test showed that fresh, healthy fruits could be infected by any fungal isolate. After four days of incubation, it also disclosed the diameter of the rot that each isolated fungal species produced.

Table 1: Pathogenicity test on fresh healthy fruits after four (4) days of re-infection

Fungal isolates	**Diameter of rot (mm)**
Aspergillus niger	13
Rhizopus stolonifer	29
Candida tropicalis	10
Fusarium oxysporum	13

The patterns of spoilage on *Citrus sinensis* produced by isolated fungal species were displayed in Plate V-VIII.

Bima Journal of Science and Technology, Vol. 7 (4) Dec, 2023 ISSN: 2536-6041

DOI: 10.56892/bima.v7i4.535

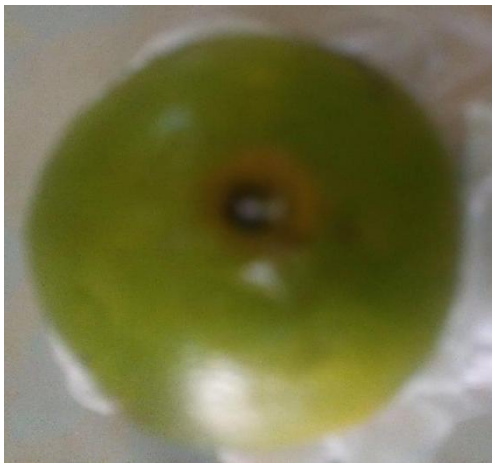

Plate V: Spoilage pattern by *A. niger*

Plate VI: *Spoilage pattern by R. stolonifer*

Plate VII: Spoilage pattern by *C. tropicalis*

Plate VIII*: Spoilage pattern by F. oxysporum*

DISCUSSION

Citrus sinensis fruits are particularly attractive to fungi that cause decay because they have low pH levels and high sugar and nutrient content (Singh and Sharma, 2007). The evaluation of fungal pathogens linked to *Citrus sinensis* spoiling in the Gombe metropolis was the main objective of this study. Out of the 100 samples analysed, 73% were found to be infected with one or two fungal infections. Four fungal species: *Aspergillus niger, Rhizopus stolonifer, Candida tropicalis,* and *Fusarium oxysporum* were isolated and identified. This is comparable to the findings of Bukar *et al*. (2009), who stated that *Aspergillus* sp., *Rhizopus* sp., and *Fusarium* sp. were discovered to be infected in diseased oranges obtained from vendors at the Na'ibawa Yan Lemu market in Kano metropolis.

Muhammad *et al*. (2013) also mentioned that in Niger state, sweet orange spoiling is linked to *Aspergillus niger, Aspergillus flavus, Candida tropicalis, Penicillium digitatum, Penicillium chrysogenum,* and *Fusarium oxysporum*. This might be the result of the area's poor storage practices or the handlers' unhygienic handling practices. This is also consistent with the findings of Tafinta *et al*. (2013), who discovered that Sokoto State's spoilt sweet orange fruits were linked to *Aspergillus fumigatus, Aspergillus niger,*

DOI: 10.56892/bima.v7i4.535

Aspergillus flavus, and *Rhizopus stolonifera*. *Aspergillus niger* and *Candida tropicalis* were also identified by Akinro *et al*. (2015) as the pathogens causing the spoilage of sweet oranges sampled from a chosen market in Iree Town of Boripe Local Government, Osun State.

This finding is also comparable to that of Oviesogie *et al*. (2015), who reported that the pathogens responsible for the spoilage of sweet orange in Benin City, Edo State, were identified as *Penicillium* species, *Aspergillus* species, *Rhizopus* species, *Mucor* species, *Candida tropicalis*, *Alternaria* species, and *Saccharomyces cerevisiae*. Additionally, he proposed that the presence of the resistant spores and the fungi most likely came from the farms where the fruits were harvested, with some of them finding their way into the stores as a result of contamination from the already-spoiled fruits. With this work, similar species were also reported by Akhtar (2013), Samuel *et al*. (2015), Nasiru *et al*. (2015), and Akinmusire (2011). They stated that among the pathogens linked to orange spoiling were *Aspergillus niger, Candida tropicalis, Rhizopus stolonifer,* and *Fusarium oxysporum*, among others.

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 in locally woven baskets and sacks are transported from distant villages which encourages microorganism survival. Most of these fruits were obtained from Ekiti, Delta, Enugu, Anambra, Ebonyi, Imo,Oyo, Kogi, Benue, Edo, Ogun, Ondo, Taraba, Kwara, Kaduna, 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 quicker the food deteriorates. 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 packaging, storage, handling, and transportation may result in growth and decay 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.

Fungi that cause spoilage generally are considered pathogenic and some of them may produce mycotoxins. Therefore, it is advisably to avoid consumption of such spoilt oranges and it should be discarded properly as that will be hazardous to human health.

CONCLUSION

Conclusively, *Aspergillus niger, Candida tropicalis, Rhizopus stolonifer* and *Fusarium oxysporum* were found to be associated with *Citrus sinensis* spoilage in Gombe metropolis. These fungi are known to be pathogenic to health and were observed to be able to have short time negative effect to the healthy oranges, which pose a serious threat economically to oranges sellers in Gombe metropolis. Further research should be done on on the best ways to manage the fungi that cause the orange fruits in the study area to spoil. Well-vented plastic bags and polyethylene should be used to market oranges, and if possible, oranges should be singly sealed. Consumption of such spoilt oranges should be avoided and should be discarded properly and hygienically.

REFERENCES

Akhtar, N., Anjum, T. and Jabeen, R. (2013). Isolation and Identification of Storage Fungi from Citrus Sampled from Major Growing areas of Punjab, Pakistan.

DOI: 10.56892/bima.v7i4.535

International journal of agriculture and biology, 1814–9596. Retrieved from http://www.fspublishers.org.

Akinmusire, O.O. (2011). Fungal species associated with the spoilage of some edible fruits in Maiduguri, North Eastern, Nigeria. *Advances in Environmental Biology*, *5*(1), 157-161. Retrieved from http://www.aensiweb. com/old/aeb/2017/157-161.

Akinro, E.B., Adetuberu, I.A., Efunwole, O.O. and Olakunle, T. P. (2015). Isolation and Identification of Fungal Species Associated with the Spoilage of Some Selected Edible Fruits in Iree Town Of Boripe Local Government, Osun State, Nigeria. *Journal of Research in Pharmaceutical Science*, *2(7)*, 7-10. Retrieved from http://www.questjournals.org.

Baiyewu, R.A., Amusa, N.A., Ayoola, O.A. and Babalola, O.O. (2007). Survey of the postharvest diseases and aflatoxin contamination of marketed Pawpaw fruit (*Carica papaya* L.) in South Western Nigeria. *African Journal of Agriculture*, 2(4), 178-181.

Bukar, A., Mukhtar, M.D. and Adamu, S. (2009). Isolation and identification of postharvest spoilage fungi Associated with sweet oranges (*citrus sinensis*) traded in Kano metropolis. *Bayero Journal of Pure and Applied Sciences, 2*(1), 122 – 124.

Chukwuka, K.S., Okonko, I.O. and Adekunle, A.A. (2010). Microbial ecology of organisms causing Pawpaw (*Carica papaya* L.) fruit decay in Oyo state, Nigeria. *Amrican European Journal of Toxicological sciences*, *2*(1), 43-50.

Di Majo, D., Giammanco, M., Guardia, M.L., Tripoli, E., Giammanco, S., and Finotti, E. (2005). Flavones in citrus fruit: structure antioxidant activity relationship. *Food Research International*, *38*(10), 1161-1166.

Etebu, E. and Nwauzoma, A. B. (2014). A review on sweet orange (*Citrus Sinensis* Osbeck): health, diseases, and management. *American Journal of Research Communication*, *2*(2), 33-70.

Food and Agriculture Organization of the United Nations (2023). *FAO Statistics 2023*. Retrieved June 2, 2023.

Ismail, M. and Zhang, J. (2004). Post-Harvest Citrus diseases and their control, outlooks on pest management. *Peouen*, *15*, 29-35.

Jay, J.M. (2003). *Microbial Spoilage of Food. Modern Food Microbiology* (4th ed.). New York: Chapman and Hall Incorporated. 187-195.

Moss, M.O. (2002). Mycotoxin review. *Aspergillus* and *Penicillium*. *Mycology, 16*, 116-119.

Muhammad, S.S.D., Kumar, N., Damisa, D., Bala, E., Muhammad, I.L., Muhammad, R.G. and Yunusa, A. (2013). Fungi associated with decayed sweet oranges (*Citrus sinensis*) collected from Lapai, Niger state, Nigeria. *Indian Journal of life Sciences, 2*(2), 123-131.

Nasiru, A.M., Salau, I.A. and Yakubu, M. (2015). Fungi Associated with Spoilage of *Citrus Sinensis* in Fruits and Vegetables Market, Sokoto, Nigeria. *Global Advanced Research Journal of Agricultural Science, 4*(12), 919-922. Retrieved from http://garj.org/garjas/home

Oviasogie, F.E., Ogofure, A.G., Beshiru, A., Ode, J.N. and Omeje, F.I. (2015). Assessment of fungal pathogen associated with orange spoilage. *African Journal of Microbiology Research, 2(*14*),* 1758-1759.

Samuel, O., Ifeanyi, O. and Ugochukwu, O. (2015). Filamentous Fungi Associated with the Spoilage of Post-Harvest Sweet

DOI: 10.56892/bima.v7i4.535

Orange Fruits (*Citrus Sinensis*) Sold in Awka Major Markets, Nigeria. *Bioengineering and Bioscience, 3*(3), 44-49.

Sidana, J., Saini, V., Dahiya, S., Nain, P. and Bala, S. (2013). A Review on Citrus – "The Boon of Nature": *International Journal of Pharmaceutical Sciences, 18*(2), 20-27.

Singh, D., and Sharma, R.R. (2007). Post-harvest diseases of fruit and vegetables and their management. In: Prasad, D. (Ed.), *Sustainable Pest Management*. New Delhi, India: Daya Publishing House.

Tafinta, I.Y., Shehu, K., Abdulganiyyu, H., Rabe, A.M. and Usman, A. (2013). Isolation and Identification of Fungi Associated with the Spoilage of Sweet Orange (*Citrus Sinensis*) Fruits in Sokoto State. *Nigerian Journal of Basic and Applied Science, 21*(3), 193-196.

Zubbair, N.A. (2009). Determination of microbial characteristics of selected fruits sold in major markets in Ilorin metropolis. *African Sciences*, *10*(2), 1595-688.