ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VI June 2024

Fungi Associated with Spoilage of Post-Harvest Orange and Tomato Fruits Sold in Selected Markets of Port Harcourt Nigeria

Ezeonuegbu B.A., Nwankwo C.C. and Agunwa C.C.

University of Port Harcourt, Nigeria

DOI : https://doi.org/10.51244/IJRSI.2024.1106019

Received: 13 May 2024; Revised: 31 May 2024; Accepted: 04 June 2024; Published: 05 July 2024

ABSTRACT

High water content, ambient conditions, handling techniques, storage facilities, handler fungal load, and fruit quality all contribute to the fungal deterioration of orange and tomato fruits. This study assessed fungi that cause spoilage of tomato and orange fruits, as well as their frequency of occurrence in selected markets of Port Harcourt metropolis. We purchased the fruit samples from five markets: Choba, Rumuokoro, Mile One, D-Line, and Creek Road Markets. The proximate composition of the fruits was determined, a pathogenicity test was conducted, and the frequency of occurrences of each isolate was calculated as follows; *Alternaria* spp. (72.51%) and *Rhizopus* spp. (27.49%) were isolated from tomato fruits, while isolates from orange fruits were *Alternaria* spp. (55.05%), *Aspergillus* spp. (21.1%), *Penicilium* spp. (20.33%), and *Fusarium* spp. (3.52%). The result obtained from this study showed that only two fungal pathogens (*Alternaria* and *Rhizopus* spp.) were associated with tomato spoilage in Port Harcourt, whereas four fungal pathogens (*Aspergillus*, *Penicillium*, *Fusarrium*, and *Alternaria* spp.) were associated with orange spoilage. *Alternaria* spp. appeared to be the most active of all the pathogens that result in the economic loss of tomato and orange fruits in Port Harcourt. Therefore, to extend the shelf life of orange and tomato fruits, proper handling and sufficient storage facilities must be used.

Keywords: Fungi, Post-Harvest Spoilage, Fruit, Tomato, Orange

INTRODUCTION

Fruits provide essential nutrients for human nutrition, including sugar, vitamins, minerals, a tiny amount of protein, and oil. Everyday bodily operations, growth, repair, and maintenance of normal health and proper body metabolism require these nutrients [1, 2]. Food spoilage refers to several changes that make the food toxic and less palatable to consumers, and these could be associated with alterations in appearance, texture, taste, or smell [3]. The comparatively short shelf-life duration brought on by pathogenic attacks is one of the limiting variables that affects fruit's commercial value. Fungi can infect a fruit during its growing season, harvesting, handling, transporting, storing, and marketing, or even after the consumer has made a purchase [4].

Fruits are prone to spoilage and typically exhibit high metabolic activity during the storage phase. The presence of abundant carbohydrates, minerals, vitamins, amino acids, and low pH levels promotes the thriving and survival of different parasitic and saprophytic fungi [14]. Studies have indicated that a significant portion, approximately 20-25%, of fruits and vegetables go to waste due to spoilage, particularly in the post-harvest phases [4, 5]. There is a connection between this and spoilage fungi, which have the potential to be toxigenic or pathogenic. Previous researchers have successfully identified and isolated toxin-producing fungi from spoiled fruits. There have been reports of pathogenic fungi causing infections or allergies [15, 16].

Microorganisms, particularly fungi, are known to destroy fruits, reducing their suitability for consumption and the profit from fruit sales. Identification of these microorganisms, particularly those that are harmful to humans, is necessary in order to lower the possibility of contamination and illness from handling and eating fruits [14].

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VI June 2024

In a developing country like Nigeria, postharvest losses are often more severe due to inadequate storage and transportation facilities. Orange and tomato fruits are majorly consumed by residents of Port Harcourt Metropolis, resulting in their wide sales across the city in markets, kiosks and sometimes hawked. However, as a city in a developing country, these fruits are plagued with a shorter shelf-life occasioned from attacks by spoilage microorganisms causing losses to traders.

Orange and tomato fruits are popular worldwide. There are numerous reports on the spoilage of these fruits by fungi in the developed countries, but such literature on the spoilage fungi of post-harvest oranges and tomatoes in a developing city such as Port Harcourt, Nigeria is very rare. thus, in this study, the fungi associated with the spoilage of post-harvest oranges and tomatoes sold in major marketplaces in Port Harcourt, Nigeria, were isolated, characterized, and identified.

MATERIALS AND METHODS

2.1 Sample collection

Fruit samples were purchased from four different markets in Port Harcourt metropolis. Healthy fruits (orange and tomato) and fruits (orange and tomato) showing symptoms of spoilage were purchased.

2.2 Proximate composition of fruit samples

Following AOAC Kjeldahl's method [6], the protein nitrogen in 0.5g of dried milled fruit pulp sample was converted to ammonium sulphate by digestion with concentrated H_2SO_4, and in the presence of a catalyst (Cu_2SO_4) and Na_2SO_4. This was heated and the ammonia evolved was steam-distilled in 4% boric acid solution, the nitrogen from ammonia was deduced from the titration of the trapped ammonia with 0.1N H_2SO_4 with methyl red indicator until a pink coloration was observed indicating the endpoint of titration. Protein was then calculated by multiplying the deduced value of nitrogen by a protein constant 6.25mg:

$$\%\ \text{Crude protein} = \frac{\text{X} \times 5.6 \times 6.25}{\text{W} \times \text{D.M}}$$

Where; X = mL of 0.1N acid required to neutralize the ammonia after subtracting the blank, W = weight of sample in gram, D.M = dry matter percentage of sample.

2.2.2 Determination of moisture content of fruit samples

The moisture content of the sample was determined using the method of Oladipo and Jadesimi [7]. Two grams (2g) of millet grains sample was put into a previously weighed, washed and dried crucible dish and placed in a thermostatically controlled oven at 105^0C for 1 hour. Then the sample was removed, cooled in a desiccator and weighed; this process was repeated until the weight was constant. The moisture content of the sample was then calculated by difference in weight and expressed as a percentage:

$$\%\ \text{Moisture content} = \frac{W_a - W_b}{\text{W}_c} \times 100$$

Where; Wa = Weight of crucible + sample before drying, Wb = Final weight of crucible + sample after drying, Wc = Weight of sample before drying.

2.2.3 Determination of crude ash content of fruit samples

Following the method of Oladipo and Jadesimi [7], two grams (5g) of homogenized fruit pulp sample was weighed (W) into a previously dried and weighed porcelain crucible (Wa) and the crucibles with its contents was transferred to a muffle furnace at the temperature of 600^0C maintained for 2 hours until whitish-grey color was obtained indicating that all the organic matter content of the sample had been destroyed. Thereafter, the crucible was removed and placed in a desiccator to cool and then weighed (Wb). The ash content was then calculated and expressed as a percentage:

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VI June 2024

$$\% \text{ Ash content} = \frac{W_b - W_a}{\text{W}} \times 100$$

Where; W = Weight of sample, Wa = Weight of crucible, Wb = Final weight of crucible + ash formed.

2.2.4 Determination of crude fibre content of fruit samples

A modified AOAC fritted glass crucible method was followed. 2.0g of homogenized fruit pulp sample was digested in 200mL of 1.25% H_2SO_4, the mixture was boiled for 30min, filtered and washed with hot water to reduce the acidity; this was tested with pH paper and the residue was again digested in 200mL of 1.25% NaOH. The mixture was then heated for 30min, filtered, washed with hot water and dried in an oven; this was transferred to a platinum crucible and weighed (W_a), then heated in a furnace at 550^0C to ash and weighed again (W_b). Percentage crude fibre was then calculated as:

$$\% \text{ crude fibre content} = \frac{W_a - W_b}{\text{W}} \times 100$$

Where; W = Weight of sample used, W_a = Weight of fibre + ash + crucible, W_b = Weight ash + crucible

2.2.5 Determination of crude fat and lipid content of fruit samples

Following the method of Oladipo and Jadesimi [7], with a little modification, cold method of extraction was used to determine fat and lipid. 10g of homogenized fruit pulp sample was accurately weighed into a round bottom flask; 50mL of n-hexane was added and covered for 24h for proper extraction of oil. Then a clean and dried beaker was weighed and the weight noted. Thereafter, the sample was decanted into the beaker, heated to dryness and transferred into a desiccator to cool; weighed and new weight noted. The fat and lipid content will be calculated as a percentage thus:

$$\% \text{ Fat and oil content} = \frac{W_b - W_a}{\text{W}} \times 100$$

Where; W = Weight of sample, W_a = Weight of beaker, W_b = Weight of fat & oil + beaker.

2.2.6 Determination of carbohydrate content of fruit samples

The carbohydrate content of the sample was estimated as the difference obtained after subtracting the values of organic protein, ash, fat/lipid, crude fiber and moisture contents from 100. That is 100 – (organic protein, ash content, fat/oil, crude fibre and moisture).

2.3 Isolation and identification of spoilage fungi from orange and tomato fruits

The method of fungal isolation by Ogofure *et al*. [2] and Sajad and Jamaluddin Abid [8] was applied. Infected samples were washed with running tap water. An appropriate size of spoilt fruits (orange and tomatoes) was carefully cut with the aid of sterile scalpel, then sterilized with 70% ethanol and rinsed in sterile distilled water. Sliced portion were placed on sterile PDA medium plate and 2% tetracycline (30mg/L) was used to inhibit bacterial growth and then incubated at room temperature $27\pm1C^0$. Incubation was carried out in inverted positions of Petri plates for 4-6 days. The colonies developed were then sub-cultured on PDA medium to obtain pure cultures.

2.4 Identification of fungi

The fungi isolates were identified on the basis of macro-morphological and micro-morphological characteristics. The morphological characteristics which include colony growth and colour, presence or absence of aerial mycelium, presence or absence of wrinkles and furrows, presence or absence of pigmentation amongst others were observed under the microscope and recorded. In all cases, a drop of lactophenol blue stain was placed on a clean grease-free sterilized glass slide. Thereafter, a sterile inoculating wire loop was used to

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VI June 2024

pick the mycelium onto the glass slide from the mold culture. The mycelium was then spread evenly on the slide. Teasing was done to separate the mycelium in order to get a homogenous mixture and the mixture was covered with cover slips gently and then allowed to stay for some seconds before observing with the microscope under X40 magnification lens. The microscope examination of actively growing mold was on the basis of structures bearing spores, presence or absence of septa.

2.5 Determination of total count and frequency of occurrence of the isolated fungi

The method applied by Sajad and Jamaluddin Abid [8] was adopted to determine the total count and frequency of occurrences of individual isolated fungus for each fruit sample in the different markets to be studied. The frequencies of occurrences were calculated in percentage (%) using the formular below:

$$\text{Percentage frequency of occurrence} = \frac{\text{Number of times a fungus is encountered}}{\text{Total number of fungal isolate}} \times 100$$

RESULTS

Proximate composition of tomato and orange fruit

The proximate composition of tomato fruit used in the study is presented in Figure 1. The result shows that moisture (92.39 %) was the highest among all the components, followed by protein (13.97 %) and crude fibre (10.72 %). Figure 2 presents the proximate composition of orange fruit used in this study, which shows that moisture (81.51 %) was the highest among all the components, followed by carbohydrate (18.34 %) and protein (1.76 %).

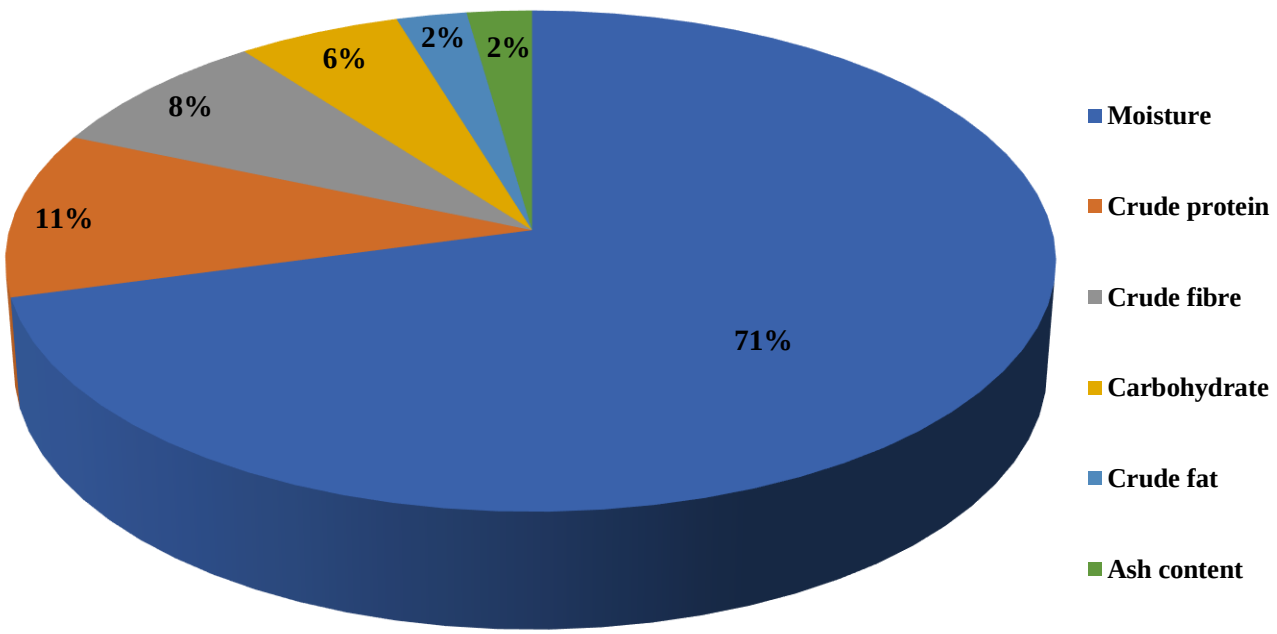


Figure 1: Proximate composition of tomato fruit

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VI June 2024

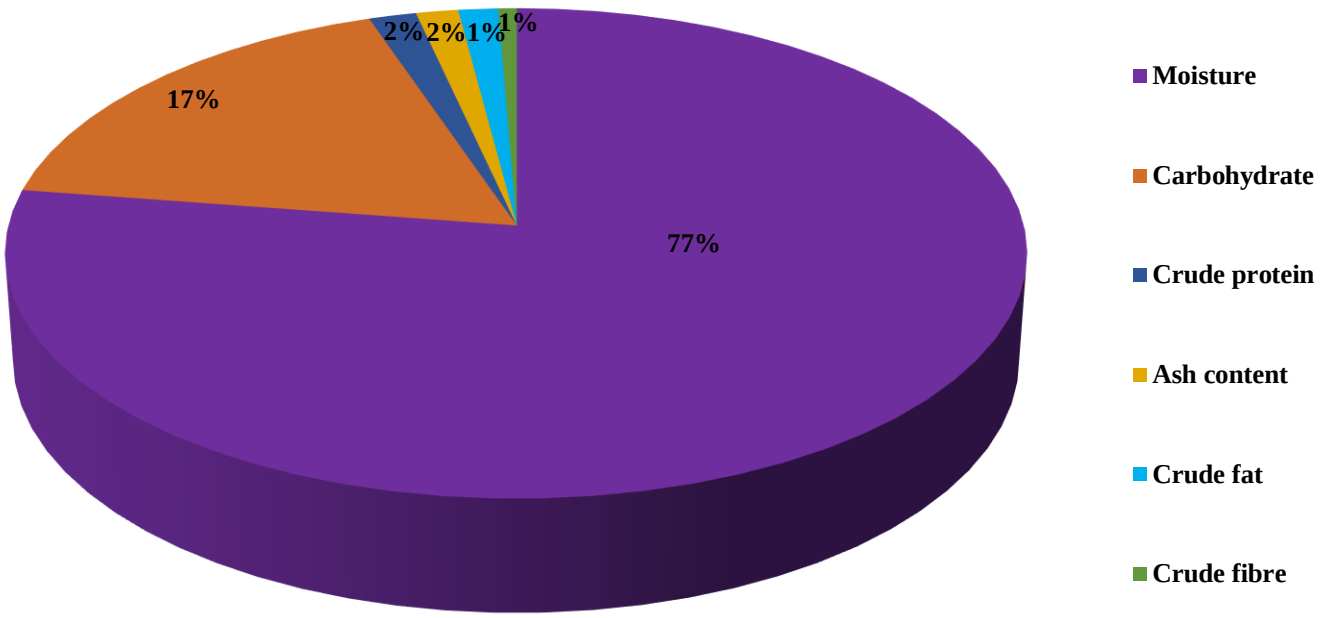


Figure 2: Proximate composition of orange fruit

Cultural and microscopic characteristics of the fungal isolates

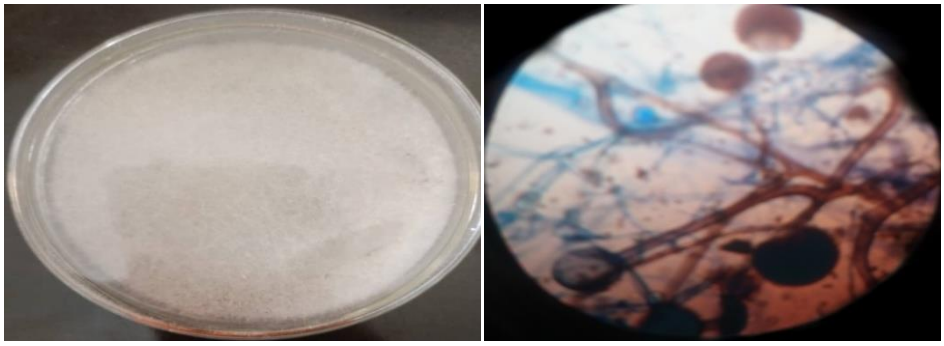
Cultural characteristics of and pictorial representation of spoilage fungal isolates from tomatoes and orange fruits is presented in Table 3 and Figure 3 respectively. The major fungi identified were *Rhizopus, Alternaria, Penicillium, Fusarium* species and *Aspergillus flavus*.

Table 1: Cultural characteristics of spoilage fungal isolates from tomatoes and orange fruits

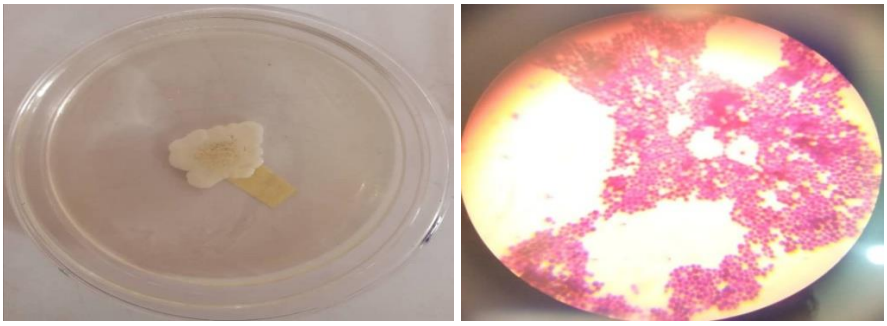
Isolate code	**Colour**	**Surface characteristics**	**Edge**	**Reverse**	**Colony diameter (mm)**	**Tentative identity of isolate**
F1	White	Cottony	White, irregular	White	22.0	*Rhizopus* spp.
F2	Cream	Granular	Irregular, flexuous	Cream	2.0	*Alternaria* spp.
F3	Cream	Cream	Flexuous	Cream	2.0	*Alternaria* spp.
F4	Bluish-green	Powdery	White, circular	Brownish	10.0	*Penicillium* spp.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VI June 2024

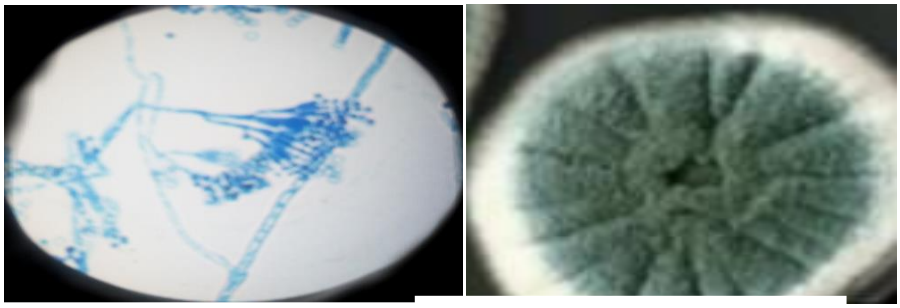
				yellow		
F5	Whitish-pink	Cottony	White, circular	Whitish pink	20.0	*Fusarium* spp.
F6	Bluish-green	Powdery	White, circular	Cream	31.0	*Penicillium* spp.
F7	Green	Powdery	White, irregular	Amber	25.0	*Aspergillus* spp.



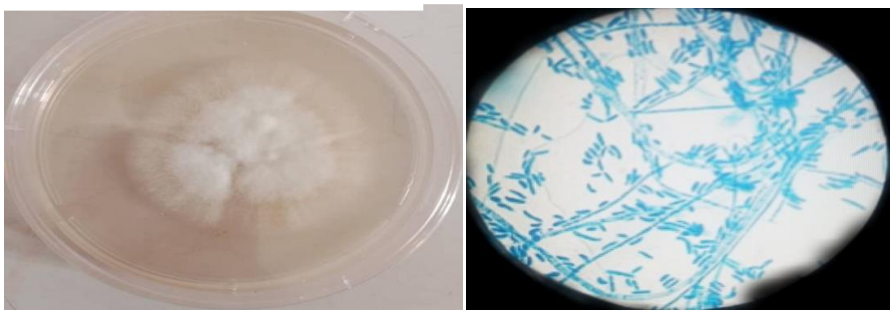
F1(a): (*Rhizopus* sp.) **F1(b): (*Rhizopus* sp.)**



F2 (a): (*Alternaria* sp.) **F2 (b): (*Alternaria* sp.)**

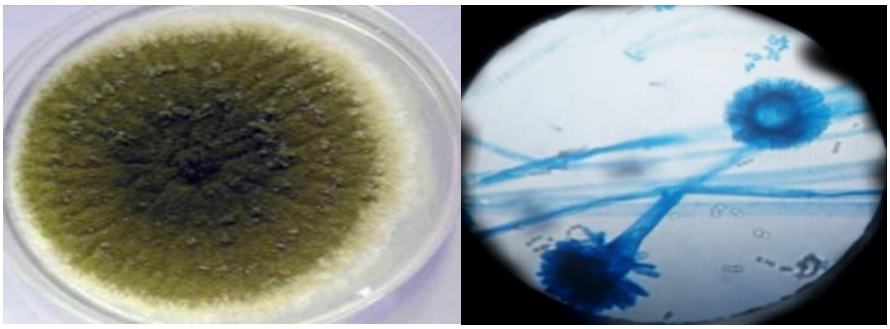


F4(a): (*Penicillium* sp.) **F4(b): (*Penicillium* sp.)**



F5 (a): (*Fusarium* sp.) **F5 (b): (*Fusarium* sp.)**

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VI June 2024



F7 (a) : (*Aspergillus* flavus) **F7 (b): (*Aspergillus* flavus**

Figure 3: Cultural and microscopic representation of fungi isolated from tomato and orange fruit

Keys: (a) = Surface and cultural characteristics

(b) = Microscopic characteristics

Total count and frequency of occurrence of the isolated spoilage fungi from tomatoes and Spoilage fungal isolates

Table 2 shows the distribution of fungal isolates associated with 50 spoilt tomatoes from Choba, Rumuokoro, D/Line, Mile 1, and Creek Road market. *Alternaria* sp. had the highest frequency of 42.7 % followed by *Aspergillus flavus* which recorded frequency of 23.6 % while the fungi with the least frequency was *Penicillium* sp. recording 5.6 %.

The frequencies of occurrence of the fungal isolates from 50 spoilt orange samples is presented in Table 3. *Aspergillus flavus* showed the highest frequency of 30.9 % across the 50 sample of spoilt tomatoes followed by *Alternaria* sp. which recorded frequency of 25.5 % while *Rhizopus* sp., recorded the least frequency (5.5 %).

Table 2: Percentage frequency of occurrence of isolated spoilage fungi on tomato

Fungal isolates	**Markets sampled**					
	Choba (n=10)	**Rumoukoro (n=10)**	**D/line (n=10)**	**Mile 1 (n=10)**	**Creek Road (n=10)**	**Total prevalence (N=50)**
Rhizopus sp.	4(23.5)	4(23.5)	2(11.8)	6(35.3)	1(5.9)	17(19.1)
Alternaria sp.	6(15.8)	9(23.7)	7(18.4)	10(26.3)	6(15.8)	38(42.7)
Penicillium sp.	1(20)	1(20)	0(0)	3(60)	0(0)	5(5.6)
Fusarium sp.	1(12.5)	2(25)	0(0)	4(50)	1(12.5)	8(9.0)
Aspergillus flavus	4(19)	5(23.8)	2(9.5)	6(28.6)	4(19)	21(23.6)
Total	**16 (18)**	**21(23.6)**	**11(12.4)**	**29(32.6)**	**12(13.5)**	**89 (100)**

n=Number of samples analyzed in each market, N=Total number of samples analyzed for each sample

Table 3: Percentage frequency of occurrence of isolated spoilage fungi on orange fruit

Fungal isolates	**Markets sampled**					
	Choba (n=10)	**Rumoukoro(n=10)**	**D/line (n=10)**	**Mile 1 (n=10)**	**Creek Road (n=10)**	**Total prevalence (N=50)**
Rhizopus sp.	0(0)	1(33.3)	0(0)	1(33.3)	1(33.3)	3(5.5)

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VI June 2024

Alternaria sp.	1(10)	5(35.7)	2(14.3)	5(35.7)	1(7.1)	14(25.5)
Penicillium sp.	2(18.2)	1(9.1)	1(9.1)	5(45.5)	2(18.2)	11(20)
Fusarium sp.	2(20)	1(10)	1(10)	5(50)	1(10)	10(18.2)
Aspergillus flavus	5(29.4)	3(17.6)	2(11.8)	5(29.4)	2(11.8)	17(30.9)
Total	**10 (18.2)**	**11(20)**	**6(10.9)**	**21(38.2)**	**7(12.7)**	**55 (100)**

n=Number of samples analyzed in each market, N=Total number of samples analyzed for each sample

DISCUSSION

The result showing *Alternaria* species as the most frequently occurred (72.51%) spoilage fungi on tomato agrees with the result obtained by Sajad, and Jamaluddin [8] who found *A. alternata* and *A. solani* in their study as the two most frequently occurred out of seventeen (17) fungi associated with the spoilage of post-harvest tomato fruits in different markets of Jabalpur, Madhya-Pradesh, India.

The result obtained in this study shows that *Aspergillus flavus* (30.9 %) were the most frequently occurred spoilage fungi isolated from orange followed by *Alternaria* spp. (25.5 %), *Penicillium* spp. (20 %) and *Fusarium* spp. (18.2 %). The result conformed with the work of Onuorah and Orji [11], Akinmusire [12] and Ibrahim et al [13]. They reported that *Aspergillus* species had high rate of occurrence in the orange and tomato fruits, and studied and concluded that the fungus may be the major organism responsible for the spoilage of tomato fruits. However, Oviasogie *et al*. [9], in their study of spoilage fungi on *Citrus sinensis* sold in five markets in Benin metropolis got the following fungi in decreasing order frequency of occurrence top (*Aspergillus* spp.), *2nd* (*Rhizopus, Mucor & Candida* spp.) and 3rd (*Alternaria* & *Saccharomyces* spp.). Tafinta *et al*. [10], in their study on spoilage fungi on orange in Sokoto metropolis isolated the following with their frequency of occurrence in percentage *Rhizopus stolonifer* (36.0%), *Aspergillus flavus* (25%), *Aspergillus fumigatius* (22.0%) and *Aspergillus niger* (17.0%). The geographical variations across the different countries may be responsible for the differences in the microbial counts and frequency of occurrence (Adetunji *et al.*, 2019).

The fungi that were isolated in this study are sources of potent mycotoxins that are harmful to health. For example, Aspergillus flavus is a source of aflatoxin, which is regarded as a potent carcinogen. Consequently, it is imperative to discard spoilt oranges and tomatoes, as their consumption could have a negative impact on one's health [11].

CONCLUSION

The present study showed that five fungal pathogens (*Rhizopus, Alternaria, Penicillium, Fusarium* species and *Aspergillus flavus*) are associated with tomato and orange spoilage in Port Harcourt. *Aspergillus flavus* and *Alternaria* spp. appeared to be the most active of all the pathogens that result in economic loses of tomato and orange fruits in Port Harcourt. Hence, it is recommended that farmers and marketers of these fruits take the necessary precautions during the harvesting, transportation, storage, and sale of tomatoes to mitigate the risk of these organisms and other contaminants that are detrimental to health.

REFERENCES

1. Yusuf HZ, Muhammad A, Mukhtar MD. Point of marketing survey for fungi associated with Spoilage of oranges distributed at some depots in kano Nigeria. Bayero Journal of Pure and Applied Sciences. 2022; 13(1): 192 – 197.
2. Ogofure AG, Oviasogie, FE, Beshiru A, Ode JN, and Omeje F. I. Assessment of fungal pathogens associated with orange spoilage. African Journal of Microbiology Research. 2015; 9(29):1758-1763. DOI: 10.5897/AJMR2014.7246.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue VI June 2024

3. Sani NM, Salihu Y, Sai du AY, Yusouf S, Nuhu DZ, Rabiu GA. Isolation of Fungi associated with Spoilage of Selected Vegetables sold in Dutse-Ultra Modern Market, Jigawa State, Nigeria. Dutse Journal of Pure and Applied Sciences (DUJOPAS). 2018; 4 (2): 349-356.
4. Kuyu CG, Tola YB. Assessment of banana fruit handling practices and associated fungal pathogens in Jimma town market, southwest Ethiopia. Food Science and Nutrition. 2018; 6(3):609-616. doi: 10.1002/fsn3.591.
5. Muhammad NO, Soji-Omoniwa O, Usman LA and Omoniwa BP. Antihyperglycemic activity of leaf essential oil of Citrus sinensis (L.) Osbeck on alloxan-induced diabetic rats. Annua Research and Review in Biololgy. 2013; 3(4): 825-834.
6. AOAC. AOAC Official Methos of Analysis (15th ed.). Association of Official Analytical Chemists, Inc. Suite 400, 2200 Wilson Boulevard, Arlington, Virginia 22201 USA. 1990
7. Oladipo I C and Jadesimi P D. Microbiological analysis and nutritional evaluation of West African soft cheese (wara) produced with different preservatives. American Journal of Food and Nutrition. 2012; 3(1): 13-21. DOI:10.5251/ajfn.2013.3.1.13.21.
8. Sajad AM, Jamaluddin Abid H. Fungi Associated with the spoilage of post-harvest tomato fruits and their frequency of occurrences in different markets of Jabalpur, Madhya-Pradesh, India. International Journal of Current Research Review. 2017; 9(5):12-16.
9. Oviasogie FE, Ogofure AG, Beshiru A, Ode JN, and Omeje, FI. Assessment of fungal pathogens associated with orange spoilage. African Journal of Microbiology Research, 9(29):1758-1763. DOI: 10.5897/AJMR2014.7246
10. Tafinta IY, Shehu K, Abdulganiyyu H, Rabe AM, Usman A. 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. 2013; 21(3): 193-196**.** DOI: http://dx.doi.org/10.4314/njbas.v21i3.4.
11. Onuorah S, Orji, MU. Fungi associated with the Spoilage of Post-harvest Tomato Fruits Sold in Major Markets in Awka, Nigeria**.** Universal Journal of Microbiology Research. 2015; 3(2): 11-16, http://www.hrpub.org DOI: 10.13189/ujmr.2015.030201
12. Akinmusire. OO. Fungi species associated with the spoilage of some edible fruits in Maiduguri, North eastern Nigeria. Advances in Environmental Biology. 2011; 5(1): 57-161, 2011. http://www.aensiweb. Com/old/aeb/2011/157-161.
13. Ibrahim AD, Musa K, Sani A, Aliero AA, Yusuf BS. Microorganisms associated with the production of volatile compounds in spoilt tomatoes. Research in Biotechnology, 2011; 2(2):82-89, 2011. http://www.unilorin.edu.ng/publications/sania/28.pdf
14. Mailafia S, Okoh GR, Olabode HOK, Osanupin R. Isolation and identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria. Veterinary World. 2017; 10(4): 393–397.
15. Thiyam B, Sharma G.D. Isolation and identification of fungi associated with local fruits of Barak Valley, Assam. Current World Environment. 2013; 8(2):319–322.
16. Afsah-Hejri L, Jinap S, Hajeb P, Radu S, Shakibazadeh S. A review on mycotoxins in food and feed: Malaysia case study. Comprehensive Reviews in Food Science and Food Safety. 2013;12(6):629–651.