

FUNGI ASSOCIATED WITH DECAYED SWEET ORANGES (*Citrus sinensis*) COLLECTED FROM LAPAI, NIGER STATE, NIGERIA

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ABSTRACT

This study was aimed for isolation, characterization and identification of fungi associated with decayed sweet oranges collected from Lapai, Niger State, Nigeria. Samples of sweet oranges were collected from Police Barrack, Minna Motor Park, Badeggi Market, Soje Garrage and Unguwan Gwari from Lapai. Segments (3-5 cm) of tissue from the margins of decayed *C. sinensis* were cut with sterile scalpel and were placed on the prepared Sabouraud Dextrose Agar in petri dishes and incubated at $28 \pm 1^\circ\text{C}$ for 5 days. The mycelia of isolated fungi were fixed in lactophenol cotton blue on slides and were viewed under the microscope. The fungi isolated were *Fusarium oxysporum*, *Aspergillus niger*, *Candida tropicalis*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Fusarium solani*, *Penicillium chrysogenum* and *P. digitatum*. *A. niger* was the predominant fungus isolated from all the samples collected, while *P. chrysogenum* and *P. digitatum* were the least fungi isolated in this study. *C. sinensis* collected from Soje Garrage had the highest occurrence of fungal isolates 8 (32%) followed by sweet oranges from Minna Motor Park with 5 (20%) of fungal isolates, while sweet oranges from Police Barrack, Badeggi Market and Unguwan Gwari had the least occurrence of fungal isolates 4 (16%) each. The pathogenicity tests showed that the fungal isolates isolated from the decayed sweet oranges were able to produce the same signs in the healthy oranges when re-inoculated.

KEYWORDS : Fungi, decayed, *Citrus sinensis*, Sabouraud Dextrose Agar, Pathogenicity test

Foods, microorganisms and humans have had a long and interesting association that developed long before recorded history. Foods are not only of nutritional value to those who consume them but often are ideal culture media for microbial growth (Prescott et al., 2008). Fruits and vegetable are horticultural products having living tissues with continuing metabolism and thus subject to respiration water loss and cell softening throughout the post harvest system (Kader, 1997). Fruits and vegetables however have serious challenges to their existence. These include changes in climatic condition, pest, inadequate rainfall and microbial attack. Over the years, there has been an increase in that need to identify and isolate the fungi, associated with their spoilage. Spoilage refers to any changes in the condition of food in which the food becomes less palatable, or even toxic; these changes may be accomplished by

alterations in taste, smell, appearances or texture. Different diseases problems arise when crops are harvested because seeds fruits or other storage organs are essentially dormant structures and their cells are physiologically unlike those growing plant. *Citrus* species probably originated from North-Eastern India, Burma and in the adjoining areas. Early in the spread of citrus, some species crossed into China where the sweet orange (*Citrus sinensis*), the mandarins and kumquat developed (Abayomi, 2004). Microbes responsible for fruits and vegetable spoilage include bacteria like *Lactobacillus* sp., *Leuconostoc* sp., *Pseudomonas* sp., and mould such as *Penicillium* sp., *Aspergillus* sp., *Fusarium* sp., *Geotricum* sp., *Rhizopus* sp., and yeast such as *Saccharomyces* sp., and *Rhodotorula* sp..

These microbes render fresh fruits and vegetables unfit for human consumption by causing their deterioration

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and leading to reduction in quality, texture, off flavour development and loss of nutrients (Lund, 1992). Similarly findings revealed by (Voysey, 2011) that *Aspergillus* sp causes black rot in citrus fruits, *Fusarium* sp. causes brown rot of citrus fruits and pineapple, *Geotricum* sp. are responsible for sour rot of citrus and peaches, *Penicillium* sp. causes blue and green mould rots of citrus fruits, apples, grapes, pears and also brown rot of pineapple, *Rhizopus* sp. causes watery, soft rot of apples, pears, stone fruits and grapes.

The principle of spread of fungal infection in fruits supports that a single infected orange can be the sources of infection to other oranges during storage and on transit (Jay, 2003). Soil infesting fungi and bacteria that cause loss of fleshy tissue typically infect plants at the time of or just before harvesting. Similarly the contamination of fruits and vegetables by fungi could be as a result of poor handling practices in food supply chain, storage conditions, distribution, marketing practices and transportation (Effiuvwewere, 2000). In making reference to this view, a research was carried out to isolate and identify the fungal species associated with decayed sweet oranges collected from Lapai, Niger State, Nigeria.

MATERIALS AND METHODS

Study Area

The study area where the samples were collected include: Police Barrack, Minna Motto Park, Badeggi Market, Soje Garrage, Unguwan Gwari in Lapai Local Government Area of Niger State. The oranges were mainly brought to the selling spots from States such as Benue, Kogi, Ogun, Ondo and Oyo. These oranges were parked in bags and transported in Lorries through road to Lapai. Lapai is a local government area in Niger State (Latitude 9° 27 North and Longitude 6° 41 East).

Physical Examination of Samples

The physical examinations of spoiled or diseased oranges were identified using the method of (Balali et al., 1995). Various types of spoiled oranges were selected including those that were mechanically wounded or bruised, with purplish to dark brown rot, blue rot, green rot as well as those with black lesions on them. Healthy ones

were also bought to be used for control. Eight samples (5 spoiled and 3 unspoiled) of sweet oranges (*C. sinensis*) was collected into sterile polythene bag from the five sample sites namely: Police Barrack, Minna Motto Park, Badeggi Market, Soje Garrage, Unguwan Gwari area, giving a total of 40 samples collected. These samples were kept unwashed and transported to the Microbiology Laboratory, IBB University, Lapai for the experiments.

Isolation and Identification of Fungi

Isolation of fungi from each of the citrus was carried out using the method of (Amusa et al., 2002) and (Baiyewu et al., 2007). Segment (35cm) of tissue from the margins of the decayed fruit were cut with a sterile scalped and placed on the previously prepared sabourand dextrose agar (SDA) in petri dishes and incubated at 28±1°C for 5 days. The detected fungi were carefully isolated into pure cultures of Sabourand Dextrose Agar in plates and slants. The plates and slants were incubated for seven days at 28°C. Fungal isolates from plates were prepared into mounts in microscopic slides by placing portion of mycelial growth carefully picked with the aid of sterile inoculating needle in a drop of lactophenol cotton blue. The microscopic slide was covered with a cover slip and was examined under the microscope, first with (x10) and then with (x40) objective lens for morphological examination with descriptions by (Samson and Reenen-Hockstra, 1988; Fawole and Oso, 1998; Oyeleke and Manga, 2008). The fungal isolates were identified by comparing their morphology and characteristics with descriptions given by (Samson and Reenen-Hockstra, 1988).

Pathogenicity Tests

Three healthy fruits (sweet oranges) from each sample were surface disinfected with 90% ethanol and incisions were made on them using a sterile 4 mm cork borer; similar sterile cork borer was used to cut pellets of agar containing the cultures of fungal mycelia of the isolates. These fungi were then inoculated into the hole created on the healthy fruits in a laminar flow chamber. The inoculated wound was sealed with petroleum jelly. Two controls with incision but not inoculated were established.

The inoculated fruits and the controls were placed in a clean polythene bag (one fruit per bag) each moistened

with wet balls of absorbent cotton wool to create a humid environment and inoculated fruits were observed for symptom development. The casual agents were re-isolated from the infected fruits and compared with the original isolates.

RESULTS

Presence of Fungal Isolates in Spoiled Sweet Oranges

Fungal species such as *F. oxysporum*, *A. niger* and *Candida tropicalis* were isolated from sweet orange purchased from Police Barrack (Table, 1). *R. stolonifer*, *A. niger*, *C. tropicalis*, *Aspergillus flavus* and *F. oxysporum* were fungal species isolated from *C. sinensis* from Minna Motor Park (Table, 2). *A. niger*, *F. solani*, *R. stolonifer* and *A. flavus* were fungal isolates from sweet orange from Badeggi Market (Table, 3). Fungal isolates such as *P. chrysogenum*, *C. tropicalis*, *A. niger*, *A. flavus*, *F. oxysporum* and *C. tropicalis* were identified from sweet oranges obtained from Soje Garage (Table, 4). While Unguwan Gwari sweet oranges samples had fungal isolates

such as *A. niger*, *C. tropicalis*, *P. digitatum* and *A. flavus* in them (Table, 5).

Occurrence of Fungal Isolates in Spoiled Sweet Oranges

The frequency of occurrence show that *F. oxysporum* had the highest occurrence of 2 (50.0%) followed by *A. niger* and *C. tropicalis* with 1 (25.0%) of occurrence each from orange sample from Police Barrack. *R. stolonifer*, *A. niger*, *C. tropicalis*, *A. flavus* and *F. oxysporum* had 1 (20.0%) frequency of occurrence each from *C. sinensis* obtained from Minna Motor Park. Similarly, *A. niger*, *F. solani*, *R. stolonifer* and *A. flavus* had 1 (25.0%) occurrence each from oranges samples from Badeggi Market. Sweet oranges from Soje Garage had the frequency of occurrence of *A. niger* to be 3 (37.5%), *C. tropicalis* 2 (25.0%) and *P. chrysogenum*, *A. flavus* and *F. oxysporum* had 1 (12.5%) frequency of occurrence each. While *A. niger*, *C. tropicalis*, *P. digitatum* and *A. flavus* isolated from sweet orange obtained from Unguwan Gwari had 1 (25.0%) occurrence each (Table, 6). Twenty five (25) total fungal isolates were obtained out of the twenty five

Table 1: Identification of Fungal Isolates From Spoiled Sweet Oranges From Police Barrack

Spoilage	Macroscopic Examination	Microscopic Examination	Organisms
Whitish–pink mycelia growth, brown rot	Colonies are whitish – pick with purple tinge mycelium extensive and cottony in culture	Micro-conidia, ovoid to ellipsoidal in shape are borne on simple phialides. Macro-conidia are borne on phialides on branched conidiospore. Septate fusiform, slightly curved and pointed at both ends	<i>Fusarium oxysporum</i>
Dark brown discoloration, sunken spots fruits become spongy with gas production	Black colonies with white edges	Conidial heads are large, globose and dark brown, becoming radiate and splitting into several columns with age. Conidiospore stipes is smooth walled. Conidial heads are biserial, and phialides are borne on metula. Conidia are globose and rough walled.	<i>Aspergillus niger</i>
Whitish–pink mycelia growth, brown rot	Colonies are whitish – pick with purple tinge mycelium extensive and cottony in culture	Micro-conidia, ovoid to ellipsoidal in shape are borne on simple phialides. Macro-conidia are borne on phialides on branched conidiospore. Septate fusiform, slightly curved and pointed at both ends	<i>Fusarium oxysporum</i>
Fruit becoming spongy with gas production, sunken spots	Cream white colonies, opaque, smooth, convex	Budding, spherical to elongated cells, forming pseudomycelium.	<i>Candida tropicalis</i>

Table 2: Identification of Fungal Isolates From Spoiled Sweet Oranges From Minna Motor Park

Spoilage	Macroscopic Examination	Microscopic Examination	Organisms
Watery, soft rot, wrinkled appearance with depression	Whitish becoming brown black with age	None septate mycelia sporangiospore, are directly opposite to the branched rhizoids. Sporangia are subglobose. Sporangiospore are ovoid in shape and columella are subglobose.	<i>Rhizopus stolonifer</i>
Dark brown discoloration, sunken spots fruits become spongy with gas production	Black colonies with white edges	Conidial heads are large, globose and dark brown, becoming radiate and splitting into several columns with age. Conidiospore stipes is smooth walled. Conidial heads are biserial, and phialides are borne on metula. Conidia are globose and rough walled.	<i>Aspergillus niger</i>
Fruit becoming spongy with gas production, sunken spots	Cream white colonies, opaque, smooth, convex	Budding, spherical to elongated cells, forming pseudomycelium.	<i>Candida tropicalis</i>
Black rot, sunken spots fruit become spongy with gas production	Whitish colony then light yellow-green becoming dark yellow-green	Conidial heads are radiate, later splitting into several loose columns. Conidiospores hyaline, coarsely roughened vesicle in globose and flask-shaped phialides that produces chains of rough conidia are borne directly on the vesicle.	<i>Aspergillus flavus</i>
Whitish – pink mycelial growth, brown rot	Colonies are whitish – pink with purple tinge mycelium extensive and cottony in culture	Micro-conidia, ovoid to ellipsoidal in shape are borne on simple phialides. Macro-conidia are borne on phialides on branched conidiospore. Septate fusiform, slightly curved and pointed at both ends.	<i>Fusarium oxysporum</i>

spoiled sweet orange samples analysed. *A. niger* had the highest occurrence 7(28%) from all locations of sampling and *F. solani*, *P. chrysogenum* and *P. digitatum* had the least frequency of occurrence (1) each from different location of sampling (Figure, 1).

Pathogenicity of Fungal Isolates

The pathogenicity tests showed that the isolated fungal species were pathogenic. In that, they were able to produce the same spoilage signs and indications in the healthy *C. sinensis* fruits into which they were re-introduced/re-inoculated under the same conditions (Table,7).

DISCUSSION

The spoiled sweet oranges sampled from Police Barrack, Minna Motor Park, Badeggi Market, Soje Garage and Unguwan Gwari of Lapai Local Government Area in Niger State were found to be massively infected with five genera of fungi namely *Fusarium* sp., *Aspergillus* sp., *Candida* sp., *Rhizopus* sp. and *Penicillium* sp. This is similar to the findings of (Bukar et al., 2009) who reported that diseased oranges sampled from Na'ibawa yan Lemu Market in Kano were found to be massively infected with six genera of fungi namely *Aspergillus* sp., *Penicillium* sp., *Mucor* sp., *Rhizopus* sp. and *Alternaria* sp. *Aspergillus*

Table 3: Identification of Fungal Isolates From Spoiled Sweet Oranges From Badeggi Market

Spoilage	Macroscopic Examination	Microscopic Examination	Organisms
Dark brown discoloration, sunken spots fruits become spongy with gas production	Black colonies with white edges	Conidial heads are large, globose and dark brown, becoming radiate and splitting into several columns with age. Conidiospore stipes is smooth walled. Conidial heads are biserial, and phialides are borne on metula. Conidia are globose and rough walled.	<i>Aspergillus niger</i>
Whitish – pink mycelial growth, brown rot	Colonies are whitish – pink with purple tinge mycelium extensive and cottony in culture	Micro-conidia, ovoid to ellipsoidal in shape are borne on simple phialides. Macro-conidia are borne on phialides on branched conidiospore. Septate fusiform, slightly curved and pointed at both ends.	<i>Fusarium solani</i>
Watery, soft rot, wrinkled appearance with depression	Whitish becoming brown black with age	None septate mycelia sporangiospore, are directly opposite to the branched rhizoids. Sporangia are subglobose. Sporangiospore are ovoid in shape and columella are subglobose.	<i>Rhizopus stolonifer</i>
Black rot, sunken spots fruit become spongy with gas production	Whitish colony then light yellow-green becoming dark yellow-green	Conidial heads are radicate, later splitting into several loose columns. Conidiospores hyaline, coarsely roughened vesicle in globose and flask-shaped phialides that produces chains of rough conidia are borne directly on the vesicle.	<i>Aspergillus flavus</i>

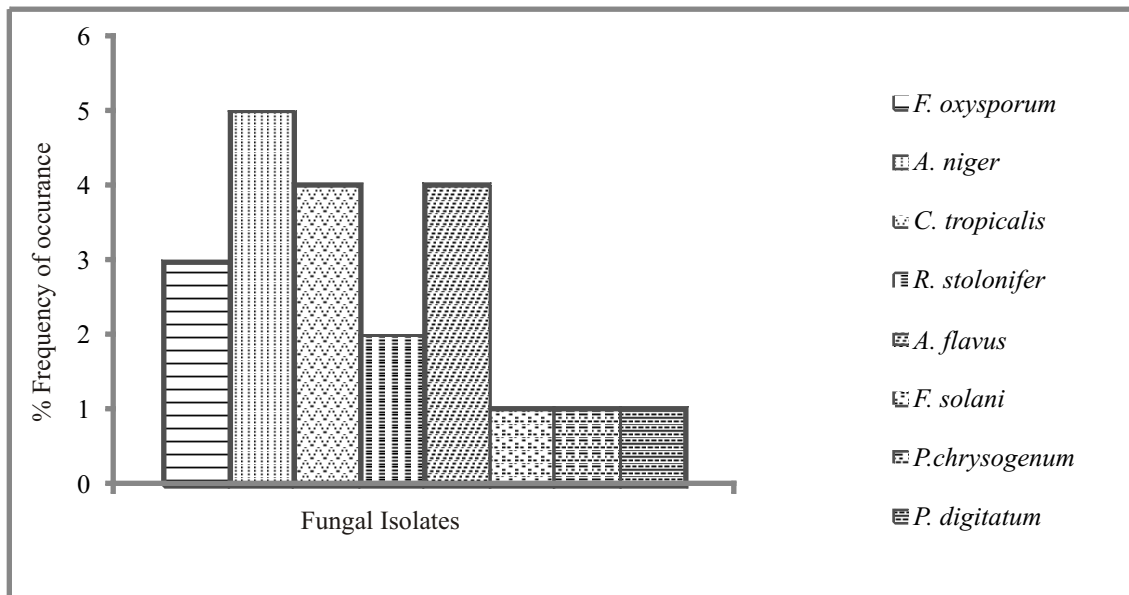


Figure 1: Occurrence of Fungal Isolates In Decayed Sweet Oranges (*C. sinensis*)

niger were predominately isolated in all sample locations of the spoiled and unspoiled sweet oranges in this study and this might be due to the poor hygiene of the handlers or poor storage methods adopted. This is inline with (Nijis et al., 1997) who reported that *Aspergillus* sp. are predominant

organism associated with spoilage of oranges. *P. chrysogenum* and *P. digitatum* were least fungal isolated in this study. This might be due to the inability of the species to grow under the given condition which the sweet orange were handled or stored. *Fusarium* sp, *Candida* sp., *Rhizopus*

Table 4: Identification of Fungal Isolates From Decayed Sweet Oranges From Soje Garrage

Spoilage	Macroscopic Examination	Microscopic Examination	Organisms
Blue, green rots	Blue green colonies	The mycelium consist of branched network of multinucleate, septate, colourless hyphae, constricted conidiospore.	<i>Penicillium chrysogenum</i>
Fruit becoming spongy with gas production, sunken spots	Cream white colonies, opaque, smooth, convex	Budding, spherical to elongated cells, forming pseudomycelium.	<i>Candida tropicalis</i>
Dark brown discoloration, sunken spots fruits become spongy with gas production	Black colonies with white edges	Conidial heads are large, globose and dark brown, becoming radiate and splitting into several columns with age. Conidiospore stipes is smooth walled. Conidial heads are biserial, and phialides are borne on metula. Conidia are globose and rough walled.	<i>Aspergillus niger</i>
Dark brown discoloration, sunken spots fruits become spongy with gas production	Black colonies with white edges	Conidial heads are large, globose and dark brown, becoming radiate and splitting into several columns with age. Conidiospore stipes is smooth walled. Conidial heads are biserial, and phialides are borne on metula. Conidia are globose and rough walled.	<i>Aspergillus niger</i>
Black rot, sunken spots fruit become spongy with gas production	Whitish colony then light yellow-green becoming dark yellow-green	Conidial heads are radicate, later splitting into several loose columns. Conidiospores hyaline, coarsely roughened vesicle in globose and flask-shaped phialides that produces chains of rough conidia are borne directly on the vesicle.	<i>Aspergillus flavus</i>
Whitish – pink mycelial growth, brown rot	Colonies are whitish – pink with purple tinge mycelium extensive and cottony in culture	Micro-conidia, ovoid to ellipsoidal in shape are borne on simple phialides. Macro-conidia are borne on phialides on branched conidiospore. Septate fusiform, slightly curved and pointed at both ends.	<i>Fusarium oxysporum</i>
Fruit becoming spongy with gas production, sunken spots	Cream white colonies, opaque, smooth, convex	Dark brown discoloration, sunken spots fruits become spongy with gas production Budding, spherical to elongated cells, forming pseudomycelium.	Black colonies with white edges <i>Candida tropicalis</i>

Table 5: Identification of Fungal Isolation From Spoiled Sweet Oranges From Unguwan Gwari

Spoilage	Macroscopic Examination	Microscopic Examination	Organisms
Dark brown discoloration, sunken spots fruits become spongy with gas production	Black colonies with white edges	Conidial heads are large, globose and dark brown, becoming radiate and splitting into several columns with age. Conidiospore stipes is smooth walled. Conidial heads are biserial, and phialides are borne on metula. Conidia are globose and rough walled.	<i>Aspergillus niger</i>
Fruit becoming spongy with gas production, sunken spots	Cream white colonies, opaque, smooth, convex	Budding, spherical to elongated cells, forming pseudomycelium.	<i>Candida tropicalis</i>
Green rots with patches	Smooth green colonies.	Thallus multinucleate, hyphae colourless individually produce conidiospore individual.	<i>Penicillium digitatum</i>
Black rot, sunken spots fruit become spongy with gas production	Whitish colony then light yellow-green becoming dark yellow-green	Conidial heads are radiate, later splitting into several loose columns. Conidiospores hyaline, coarsely roughened vesicle in globose and flask-shaped phialides that produce chains of rough conidia are borne directly on the vesicle.	<i>Aspergillus flavus</i>

sp., *Aspergillus* sp. and *Penicillium* sp. identified in this study were found to be responsible for the spoilage of the sweet oranges. This could be due to the presence of their spores, which in turn release toxins into the oranges or even release enzymes which could contribute to the deterioration of the oranges. This is similar to the report of (Akinmusire, 2011) who emphasized that *A. niger* and *C. tropicalis* were found associated with deterioration of oranges during his study on fungi species associated with spoilage of some edible fruits. *C. sinensis* sample from Soje Garage had the highest occurrence of fungal isolates 8 (32%). This might be due to the contaminations from passers-by or even the handlers or method of storage in bags and sacks, or means of transportation while sweet oranges from Police Barrack, Badeggi Market and Unguwan Gwari had the least occurrence of fungal isolates 4 (16%) each and this could be as a result of improved personal hygiene of handlers or good storage methods. In conformity with this research, (Effiuvwevwere, 2000)

Table 6 : Frequency of Occurrence of Fungal Isolates From Different Locations

Locations	Number of samples with fungal isolates	Fungal species	Percentage frequency (%)
Police Barrack (PBK)	2	<i>Fusarium oxysporum</i>	50.0
	1	<i>Aspergillus niger</i>	25.0
	1	<i>Candida tropicalis</i>	25.0
Minna Motor Park (MMP)	1	<i>Rhizopus stolonifer</i>	20.0
	1	<i>Aspergillus niger</i>	20.0
	1	<i>Candida tropicalis</i>	20.0
	1	<i>Aspergillus flavus</i>	20.0
	1	<i>Fusarium oxysporum</i>	20.0
Badeggi Market (BDM)	1	<i>Aspergillus niger</i>	25.0
	1	<i>Fusarium solani</i>	25.0
	1	<i>Rhizopus stolonifer</i>	25.0
	1	<i>Aspergillus flavus</i>	25.0
Soje Garage (SJG)	1	<i>Penicillium chrysogenum</i>	12.5
	2	<i>Candida tropicalis</i>	25.0
	3	<i>Aspergillus niger</i>	37.5
	1	<i>Aspergillus flavus</i>	12.5
	1	<i>Fusarium oxysporum</i>	12.5
Unguwan Gwari (UGG)	1	<i>Aspergillus niger</i>	25.0
	1	<i>Candida tropicalis</i>	25.0
	1	<i>Penicillium digitatum</i>	25.0
	1	<i>Aspergillus flavus</i>	25.0

Table 7: Pathogenicity Test Result of Sweet Organs (*Citrus sinensis*)

Fungal species	Location	Spoilage Patern Produced
<i>F. Oxysporum</i>	Police Barrack Minna Motor Park Soje Garage	Whitish – pink mycelia growth
<i>A. niger</i>	Police Barrack Minna Motor Park Badeggi Market Soje Garage Unguwan Gwari	Dark brown discoloration, sunken spots, fruits become spongy with gas production
<i>C. tropicalis</i>	Police Barrack Minna Motor Park Soje Garage Unguwan Gwari	Fruit becoming spongy with gas production, sunken spots
<i>R. stolonifer</i>	Minna Motor Park Badeggi Market	Watery, soft rot wrinkled appearance with depression
<i>A. flavus</i>	Minna Motor Park Badeggi Market Soje Garage Unguwan Gwari	Black rot, sunken spots fruit become spongy with gas production
<i>F. solani</i>	Badeggi Market	Pink mycelia growth
<i>P. chrysogenum</i>	Soje Garage	Wrinkled appearances
<i>P. digitatum</i>	Unguwan Gwari	Wrinkled appearances with sunken spots

reported that contamination of fruits and vegetables by fungi could be as a result of poor handling practices in food supply chain, storage condition, distribution, marketing practices and transportation.

Different spoilage types were observed on re-infection of healthy oranges with pure isolate of fungi species. This could be as a result of the ability of the fungi species to survive inside the oranges, producing their spores, then toxin production and enzymes. This is similar to the findings of (Bukar et al., 2009) who reported that different spoilage types were observed when the healthy oranges were re-inoculated with the pure isolates of the pathogens. Some however, did not cause spoilage on re-inoculation. *Aspergillus* sp., *Fusarium* sp., *Candida* sp., *Rhizopus* sp. and *Penicillium* sp. were the fungi that caused spoilage of the sweet oranges in this study. This is in conformity with (Bukar et al., 2009) who revealed that *Aspergillus* sp., *Penicillium* sp., *Mucor* sp., *Rhizopus* sp. and *Fusarium* sp. were the fungal that were able to cause re-infection in the healthy oranges after the pathogenicity tests. They further revealed that *Aspergillus* sp. had about 20% occurrence rate on re-infection, as well as *Penicillium* sp., *Mucor* sp. and *Rhizopus* sp. and *Fusarium* sp. had about

10% on re-infection while *Alternaria* sp. shared no growth on re-inoculation of the healthy oranges.

CONCLUSION

The observed spoilage in the sweet Oranges examined was as a result of the favorable moist conditions for the growth of the fungi in the Oranges. The presence of these fungi species on the sweet Oranges resulted in production of toxic substances/ enzymes that influenced the spoilage. Prevention of microbial spoilage of oranges could be achieved by the use of quality raw materials, good storage for the food type, allocation of appropriate shelf-life, Hazard analysis and critical control point (HACCP), other quality systems, Hygiene of processing environment, use of predictive methods, Training and Educating personals involve in handling of the food products and enlightening the populace on the health dangers of consuming spoiled *C. sinensis*. It's therefore recommended that more research be focused on the methods of prevention and control of spoilage of sweet Oranges by fungal isolates as well as bacteria isolates.

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