Isolation And Identification Of Fungi Associated With The Spoilage Of Some Selected Fruits In Ibadan, South Western Nigeria

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ABSTRACT: The spoilage of Pawpaw (*Carica papaya)*, Orange (*Citrus sinensis*), and Tomato (*Lycopersicon esculentum*) from three selected markets in Ibadan, Oyo State, South Western Nigeria were investigated. Healthy fruits (orange, Pawpaw and Tomato) were brought to the laboratory and allowed to spoil on a laboratory bench. The fruits (Pawpaw, Orange, Pineapple and Tomato) showing spoilage signs were examined for the presence of fungal pathogens inducing spoilage. The isolation of fungi from orange and Pawpaw was carried out on potato dextrose agar (PDA) while that of Tomato fruits was on malt extract agar (MEA). A total of nine (9) fungi isolates were obtained. Of all the samples studied (ripe and unripe Pawpaw fruits), five species of fungi were found to be associated with the fruits decay. The most common fungi found were *Aspergillus flavus*, *A. niger*, *Fusarium solani*, *Penicillium digitatum*, *Rhizopus stolonifer* and yeasts. Three fungal species *Aspergillus niger* (50.0%), *Penicillium digitatum* (100.0%) and *Rhizopus stolonifer* (50.0%); were found associated with deteriorating *Citrus sinensis*. *Aspergilus niger* (50.0%), *Aspergillus flavus* (50.0%) and *Fusarium solani* (50.0%) were associated with *Carica papaya*. The mycoflora found associated with *Lycopersicon esculentum* were *Rhizopus stolonifer* (50.0%), *Fusarium solani* (50.0%) and *Candida tropicalis* (50.0%). Pathogenicity test carried out revealed that all the fungi isolated were pathogenic. The fungi associated with the spoilage of the fruits were identified based on their colonial and morphological characteristics. These fungi species were found in varying degrees. *Aspergillus* species *(A. niger* and *A. flavus*) had the highest rate of occurrence among the isolated fungi (33.3%). This was followed by *Rhizopus stolonifer* and *Fusarium solani* (22.2%) while *Penicillium digitatum* and *Candida tropicalis* were the least encountered (11.1%). Pathogencity tests revealed that all the isolated fungi were pathogenic to the different fruits. It showed that each infected fruit gave the initial organism that caused the spoilage of the fruit. The rot symptoms obtained were similar to those observed previously on the fruits when subjected to identification procedures. The moulds seen were the same as those of the isolated fungi of fresh fruits which were subject to spoilage. The fruits changed colour slightly after infection and became soft thus could easily be punctured with a finger at the point of inoculation. Of all the isolated fungi, *Aspergillus niger* was highly pathogenic leading to rapid disintegration of treated fruits in 3-5 days while *R. stolonifer* and *Fusarium solani* were moderately pathogenic, and *Candida tropicalis*, *Penicillium digitatum* and *Aspergillus flavus* was least pathogenic, and caused the least amount of rot on fruits. This study detected the profile of spoilage fungi which caused pathogenecity of some local fruits in Ibadan city. It showed that fruits decay is caused by fungi. Since fruits were usually infected by pathogenic fungi, to be effective, production, preparation and preservation of food such as fruit salads must be carried out as rapidly and hygienically as possible using good quality equipment, produce and materials.

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1.0 INTRODUCTION

Fruits and vegetables are very important and have high dietary and nutritional qualities. Consumption of fruit and vegetable products has dramatically increased by more than 30% during the past few decades (Barth et al., 2009). During the period 1970–2004, US per capita consumption of fruits and vegetables increased by 19.9%, to 694.3 pounds per capita per year (ERS, 2007). Fresh fruit and vegetable consumption increased by 25.8 and 32.6%, respectively, and far exceeded the increases observed for processed fruit and vegetable products. It is also estimated that about 20% of all fruits and vegetables produced is lost each year due to spoilage (Barth et al., 2009). Raven et al. (2005) reports that 20 new human fungal pathogens are documented each year. Most microorganisms that are initially observed on whole fruit or vegetable surfaces are soil inhabitants, members of a very large and diverse community of microbes that collectively are responsible for maintaining a dynamic ecological

balance within most agricultural systems. Vectors for disseminating these microbes include soil particles, airborne spores, and irrigation water (Barth et al., 2009).

A fruit is the edible part of a mature ovary of a flowering plant. It is usually eaten raw (Zitter, 1985). Fruits could also be described as the succulent or fleshy covering of a nut which is pulpy, often juicy in character. As they were developed from the flower of a plant, they consist of ripened seed or seeds with some tissues attached (Nagy and Shaw, 1980). Fruits play a vital role in human nutrition by supplying the necessary growth factors such as vitamins and essential minerals in human daily diet and that can help to keep a good and normal health (Al-Hindi et al., 2011). Fruits are widely distributed in nature. One of the limiting factors that influence the fruits economic value is the relatively short shelf-life period caused by pathogens attacked. It is estimated that about 20-25% of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries (Droby, 2006; Zhu, 2006).

Increasing interest in medicinal herbs has increased scientific scrutiny of their therapeutic potentials and safety thereby providing physicians with data to help patients make wise decisions about their use (Oduola et al., 2007). Fruits, apart from being taken as food also have some medicinal importance. The latex from the trunk of the Pawpaw tree is applied externally to speed the healing of wounds, ulcers, boils and warts. The seed is also used to expel worm and the flower may be taken in an infusion to induce menstruation (Oduola et al., 2007). In the Southern part of Nigeria, fruit such as Pawpaw production has improved the diet of the local people, whose diet generally consisted of starch staples lacking essential vitamin and minerals (Baiyewu et al., 2007). These fruits were usually displayed on benches and in baskets for prospective customers in the open markets until sold, thereby exposing them to further microbial infection beside those associated with the fruit surface and those from adjacent infected fruits (Baiyewu et al., 2007; Chukwuka et al., 2010).

Tomatoes are eaten raw or cooked. Large quantities of tomatoes are used to cook soup, juice, sauce, puree, paste and powder, seeds contain 24% oil and this is extracted from the pulp and residues of the canning industry (Chuku et al., 2008; Akinmusire, 2011). Pawpaw fruit can be freshly eaten or cooked. It can also be used in the preparation of jellies, juice and jams. It has great application in the preparation of fruit salad and desserts orange juice is made from fresh healthy oranges. Sweet orange oil is a by-product of the juice industry produced by pressing the peal (Akinmusire, 2011).

The primary cell wall of fruit is composed of approximately 10% proteins and 90% polysaccharides, which can be divided into three groups: cellulose, hemicellulose and pectin (Nathalie, 2006). Numerous cell wall degrading enzymes can be secreted by pathogens to breach and use the plant cell walls as nutrient sources that reduced post-harvest life and finally lead to develop inedible, undesirable quality and soft rot spoilage (Raviyan et al., 2005; Netsanet et al., 2009; Tomassini et al., 2009; Al-Hindi et al., 2011).

In developing countries, postharvest losses are often more severe due to inadequate storage and transportation facilities. Fungal fruits infection may occur during the growing season, harvesting, handling, transport and post-harvest storage and marketing conditions, or after purchasing by the consumer. Fruits contain high levels of sugars and nutrients element and their low pH values make them particularly desirable to fungal decayed (Singh and Sharma, 2007). Studies by Li-Cohen and Bruhn (2002) had shown that fungi can survive and/or grow on fresh produce and that the nutrient content (carbohydrate, protein and fat) of fresh produce support pathogens.

Fruits are however affected by a wide array of microorganisms causing its decay. These microorganisms, under the influence of environmental factors, pose a serious threat to fruits production. Spoilage refers to any change in the condition of food in which the food becomes less palatable, or even toxic; these changes may be accompanied by alterations in taste, smell, appearance or texture (Akinmusire, 2011). Spoilage fungi that typically produce more diverse and greater amounts of extracellular depolymerases successfully attack and spoil both fruits and vegetables (Barth et al., 2009). Fungi in particular produce an abundance of extracellular pectinases and hemicellulases that are important factors for fungal spoilage (Miedes and Lorences, 2004). Some spoilage microbes are capable of colonizing and creating lesions on healthy, undamaged plant tissue (Tournas and Katsoudas, 2005).

Spoilage microorganisms can be introduced to the crop on the seed itself, during crop growth in the field, during harvesting and postharvest handling, or during storage and distribution (Barth et al., 2009). Those same types of soil-borne spoilage microbes that occur on produce are the same spoilage microorganisms that are present on harvesting equipment, on handling equipment in the packinghouse, in the storage facility, and on food contact surfaces throughout the distribution chain (Barth et al., 2009). Therefore, early intervention measures during crop development and harvesting through the use of good agricultural practices (GAP) will provide dramatic reductions in yield loss due to spoilage at all subsequent steps in the food-to-fork continuum (Eckert and Ogawa, 1988; Barth et al., 2009).

Although available literatures revealed that the importance of fruit is increasing daily, the incidence of microbial attack on this fruit demands attention. Over the years, there has been an increase in the need to identify and isolate the fungi associated with their spoilage. The aim of this study was to isolate and identify the fungi that are associated with the spoilage of Orange, Pawpaw and Tomato fruits sold in some selected markets in Ibadan city, Oyo State, South Western Nigeria.

2.0 MATERIALS AND METHODS

2.1. Fruit source

Fruits (orange, Pawpaw, Tomatoes) both fresh and those found with symptoms of fungal infection were purchased from three different markets located in Ibadan, Oyo State of Nigeria. This study was carried out in Ibadan, Oyo State, located in low humid part of South Western Nigeria. Ibadan city lies 3°5' E and 7°23' N. The city is characterized by low level of environmental sanitation, poor housing, and lack of potable water and improper management of wastes especially in the indigenous core areas characterized by high density and low income populations. The vegetation is typically tropic. The climate is characterized by dry November to April and wet May to October seasons. The mean annual rainfall of 1150 - 1500 mm occurs mainly between April and October with the major peak in June. Higher relative humidity (rH) values (rH 80 - 95%) are recorded during the rainy season than the dry season (rH 20 - 50%).

2.2. Isolation of fungi

A total of 18 randomly selected fungal infected fruits and 18 unblemished, healthy and clean looking fruits were purchased (10 each from each market). Fruits were surface sterilized by exposing them in 1 min 90% ethyl alcohol (BDH chemicals Ltd Poole England) and then 3 min to 1% sodium hypochlorite and then rinsed three times in sterile distilled water. Segments (3 - 5 cm) of tissues from the margins of the rotted areas were cut out with a sterile scalpel and placed on previously prepared potato dextrose agar (PDA, Difco; in the case of orange and Pawpaw) and malt extract agar (MEA, Difco; in the case of Tomato) in Petri dishes and incubated at 28 ± 1^{O}C for 5 days under 12 h photoperiod.

2.3. Control Experiment

Each of the fresh fruits was washed and sterilized with 75% ethanol. Then a little portion of each of the fruits was inoculated onto sterile PDA and MEA plates. All plates were incubated at 30°C for 72 hours.

2.4. Identification of Fungi Isolates

The pure isolated fungi were identified using cultural and morphological features according to the most documented keys in fungal identification (Domsch et al., 1993; Klich, 2002; Samson and Varga, 2007). The isolates were also identified by comparing their characteristics with those of known taxa, as described by Jolt et al. (1994) and Oyeleke and Manga (2008).

2.5. Pathogenicity Tests

This was carried out as described by Baiyewu et al. (2007) and Chukwuka et al. (2010). Each of the fungal isolates was tested on healthy fruits for its ability to induce spoilage. Six healthy fruits (orange, Pawpaw and Tomato) were washed with tap water and rinsed with distilled water after which they were surface sterilized with 75% ethanol. A sterile 4mm cork borer was used to make holes in each of the fruits. A colony of fungi isolate (from each pure culture) was used to inoculate the fruits and the core of the fruits were replaced. The point of inoculation was sealed with petroleum jelly to prevent contamination. Controls consisted of six fruits each of orange, Pawpaw and Tomatoes, wounded with the sterilized cork borer but not inoculated. The inoculated fruits and the controls were placed in clean polyethylene bag (one fruit per bag) each moistened with wet balls of absorbent cotton wool to create a humid environment and incubated at 30 ± 1^{O}C for 5 days. After 72 h, the inoculated fruits were observed for symptom development. The causal agents were re-isolated from the infected Pawpaw fruit and compared with the original isolates. This experiment was replicated three times.

3.0 RESULTS ANALYSIS

Different colonies were observed at the end of the procedure necessary for the isolation and identification of fungi associated with the spoilage of orange, Pawpaw and Tomato. The fungal colonies spoiled the orange, Pawpaw and Tomato fruits causing their deterioration. Mixed colonies were obtained when the fungi were first isolated on potato dextrose and malt extract agar. Pure cultures of the spoilage fungi were observed afterwards when each colony of the fungi was subcultured on freshly prepared medium.

3.1. Observed Symptoms on the Spoilt Fruits

Physically observation of the diseased fruits revealed brownish, necrotic patches on the skin of the orange, Pawpaw and Tomato fruits. The patches were a bit sunken and turned black by the third day in the case of Tomato. The patches on the orange and Pawpaw fruits took 7 days to turn black. A mass of mycelia growing on the surface of the fruits was also observed. Table 1 shows the colonial, morphological and cellular characteristics of the fungi isolated.

3.2. Frequency of Occurrence of Fungi Isolates Associated with the Spoilage of Fruits

The frequency of occurrence of fungi isolates associated with the spoilage of fruits is shown in Table 2. It showed that a total of 9 fungi isolates were obtained from fruits, which were identified as *Aspergillus flavus*, *A. niger*, *Fusarium solani*, *Penicillium digitatum*, *Rhizopus stolonifer* and *Candida tropicalis*. Of which, *Aspergillus niger species (A. niger* and *A. flavus*) were the most frequently isolated fungi (33.3%). This was followed by *Rhizopus stolonifer* and *Fusarium solani* with infection rate of 22.2% while *Penicillium digitatum* and *Candida tropicalis* were the least encountered (11.1%) as shown in Table 2. Three fungal species *Aspergillus niger* (50.0%), *Penicillium digitatum* (100.0%) and *Rhizopus stolonifer* (50.0%); were found associated with deteriorating *Citrus sinensis*. *Aspergillus niger* (50.0%), *Aspergillus flavus* (50.0%) and *Fusarium solani* (50.0%) were associated with *Carica papaya*. The mycoflora found associated with *Lycopersicon esculentum* were *Rhizopus stolonifer* (50.0%), *Fusarium solani* (50.0%) and *Candida tropicalis* (50.0%) as shown in Table 2.

3.3. Control Results

Control experiment showed no growth of fungi in the plates of Potato Dextrose Agar (PDA) and Malt Dextrose Agar (MEA) on which the healthy fruits were inoculated.

3.4. Pathogenicity Test

All the fungi isolates were found to be pathogenic on all fruits. The rot symptoms obtained were similar to those observed previously on the fruits when subjected to identification procedures by examining their morphological, colonial and cellular characteristics. The moulds seen were the same as those of the isolated fungi of fresh fruits which were subject to spoilage. The fruits changed colour slightly after infection and became soft thus could easily be punctured with a finger at the point of inoculation. The pathogenicity test showed that each infected fruit gave the initial organism that caused the spoilage of the fruit. Of all the isolated fungi, *Aspergillus niger* was highly pathogenic leading to rapid disintegration of treated fruits in 3-5 days while *R. stolonifer* and *Fusarium solani* were moderately pathogenic, and *Candida tropicalis*, *Penicillium digitatum* and *Aspergillus flavus* was least pathogenic, and caused the least amount of rot on fruits.

Table 1: Colonial, Morphological and Celullar Characteristics of Fungi Associated with the Spoilage of Fruits

Isolates	Colonial Characteristics	Morphology And Cellular Structure	Organism
O_1	Colonies velvety yellow, Green.	Conidiophores smooth, relatively short. Penicillia mycelia arranged very irregular and asymmetrical with branches of various lengths. Sparse and irregular metulae with phialides on them, conidia smooth and ellipsoidal.	*Penicillium digitatum*
O_2	Colonies light grey, growing extreme rapidly and filling the petri dish with dense cottony mycelia producing mass of sporangia.	A bundle of sporangiophore was formed. Sporangiophore is smooth-walled, aseptate, light brown, simple (arising in groups of 3-5 from stolons opposite rhizoids). Sporangia globose or sub-globose with some flattened base, contained many spores (white at first, then turned black)	*Rhizopus stolonifer*
O_3	Colonies with loose white to yellow mycelium rapidly becoming dark brown to black on the development of conidia.	Vesicles light yellow brown. Phialides growing radially along the whole periphery of phialides. Primary phialides and secondary vesicles both are brown.	*Aspergillus niger*
P_1	Colonies with loose white to yellow mycelium, rapidly turning dark brown and eventually black on the development of conidia.	Vesicles were light yellow-brown. Phialides growing radially along the periphery of vesicles. Primary phialides and secondary phialides are both brown.	*Aspergillus niger*
P_2	Mycelium grey-white with sparse floccose.	Oval microconidia. Microcnidia produced on richly branched conidiophores. Cylindrical to facilitate.	*Fusarium solani*
P_3	Colonies light green-yellow. At maturity conidia is straw-like and yellow-green.	Conidiophores growing from substrate, hyphae long with thickened finely denticulate wall. Conidia typically radial. Vesicle elongated. Phialides in two layers: primary and secondary.	*Aspergillus flavus*
T_1	Shiny, creamy, white colonies.	Single-celled structures	Yeast
T_2	Colonies light grey, growing rapidly and filling the petri dish with dense cottony mycelium, producing mass of sporangia.	A bundle of sporangiophores was formed. Sporangiophores smooth-walled, aseptate, light brown, simple, arising in groups of 3-5 from stolons opposite rhizoids. Sporangia globose and sub-globose with some flattened base (white at first turning black afterwards) and many spores.	*Rhizopus stolonifer*
T_3	Growth moderately rapid, covering agar plate within 4 days with sparse, floccose greyish-white mycelium. A bluish to bluish-brown discoloration developed in the agar.	Oval microconidia produced. Microconidia produced on richly branched conidiophores. Cylindrical to facilitate.	*Fusarium solani*

Key: O = Orange; P = Pawpaw; T = Tomato

Table 2: Frequency of Occurrence of Fungi Isolates Associated with the Spoilage of Fruits

Isolates	No. (%)	Orange (%)	Pawpaw (%)	Tomatoes (%)
Penicillium digitatum	1(11.1)	1(100.0)	0(0.0)	0(0.0)
Rhizopus stolonifer	2(22.2)	1(50.0)	0(0.0)	1(50.0)
Aspergillus spp. (A. niger & A. flavus)	3(33.3)	1(50.0)	2(50.0)	0(0.0)
Fusarium solani	2(22.2)	0(0.0)	1(50.0)	1(50.0)
Candida tropicalis	1(11.1)	0(0.0)	0(0.0)	1(100.0)
Total	**9(100.0)**	**3(33.3)**	**3(33.3)**	**3(33.3)**

4.0. DISCUSSION

The findings of this study showed that *Aspergillus flavus*, *A. niger*, *Fusarium solani*, *Penicillium digitatum*, *Rhizopus stolonifer* and yeasts were found in fruits sold in major markets in Ibadan, Oyo State, South Western Nigeria. *Penicillium digitatum*, *Rhizopus stolonifer*, and *Aspergillus niger* were found to be associated with spoilage or deterioration of orange fruits. These pathogens have been reportedly isolated from Pawpaw fruits in Nigeria (Baiyewu et al., 2007; Chukwuka et al., 2010). All the five organisms isolated were confirmed to be pathogenic on the fruits but in varying degrees. It showed that of all the isolated fungi, *Aspergilus niger* was highly pathogenic leading to rapid disintegration of treated fruits in 3-5 days while while *R. stolonifer* and *Fusarium solani* were moderately pathogenic, and *Candida tropicalis*, *Penicillium digitatum* and *Aspergillus flavus* was least pathogenic, and caused the least amount of rot on fruits. When these isolates were aseptically inoculated into healthy susceptible fruits, the characteristic symptoms originally observed were also noticed again. All the five organisms were successfully taking part in the decay and are thus confirmed as the causal organism of fruit decay (Baiyewu et al., 2007; Chukwuka et al., 2010).

Generally, spoiling fungi are considered toxigenic or pathogenic (Al-Hindi et al., 2011). Toxigenic fungi have been isolated from spoiling fruits (Al-Hindi et al., 2011). During refrigeration some moulds may produce mycotoxins (Tournas and Stack, 2001). 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 (Eaton and Groopman, 1994; Baiyewu et al., 2007). A good example is Aflatoxin which has been associated in cancer of the liver (heptatoma), aflatoxicosis and also with acute hepatitis in humans, especially in the developing world (Krogh, 1992; Prasad, 1992; Eaton and Groopman, 1994; Muhammad et al., 2004; Baiyewu et al., 2007). Pathogenic fungi, on the other hand, could cause infections or allergies (Monso, 2004). *Aspergillus* spp. are known to produce several toxic metabolites, such as malformins, naphthopyrones (Pitt and Hocking, 1997) 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 (Peraica et al., 1999; Petzinger and Weidenbach, 2002) thus extra care should be taken during personnel handling of these fruits; such as harvesting, cleaning, sorting, packaging, transport and storage (Al-Hindi et al., 2011).

Aspergillus spp. were widespread among all examined spoilage fruits. Several fruit spoilage fungi from different region has been isolated and identified (Al-Hindi et al., 2011). *A. niger* is a fungus commonly found on grapes (Chulze, 2006), apples (Oelofse, 2006) and tomatoes (Yildz and Baysal, 2006). Bali et al. (2008) reported that black mold *A. niger* were caused post harvest spoilage in sweet orange and acid lime at field. Okereke et al. (2010) indicated that the fungi species isolated from the infected mangoes included *A. niger, Fusarium* sp and *A. Flavus* and that *Fusarium* sp and *A. Flavus* could not prove pathogenicity when inoculated into healthy mango fruits. The *A. flavus* was investigated by incubating inoculated mango fruits at different temperature showed that at 35°C and 100% relative humidity, A. *flavus* rot severity was maximum (Gadgile and Chavan, 2010; Al-Hindi et al., 2011).

Microorganisms are naturally present on all foodstuffs and can also be brought in by outside elements (wind, soil, water, insects, animals, human handling. They can become contaminated during growing, harvesting and transport of the raw materials, and/or processing into finished products (Lelieveld *et al.*, 2003). It is therefore necessary and important that both the farmer who harvests the fruits into bags for transportation, the marketers and consumers take necessary and appropriate precautions in preventing contamination and eating of contaminated fruits (Baiyewu et al., 2007). This will however reduce the risk of mycotoxins associated with fungi contamination which are deleterious to human health (Baiyewu et al., 2007; Chukwuka et al., 2010).

In this study, *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus stolonifer* were found to be associated with the spoilage of Pawpaw (*Carica*

papaya). This is in agreement with the findings of previous studies. Gupta and Pathak (1986) had earlier reported that *A. niger* and *A. flavus* among others were responsible for post harvest losses in Pawpaw in South Western Nigeria. In consonant with Oyeniyi (1992) who identified *A. niger* isolated from rhizosphere of Pawpaw tree. Baiyewu et al. (2007) had also reported that *Rhizopus nigricans*, *Aspergillus flavus*, *Aspergillus niger* and *Fusarium moniliforme* among others, were responsible for post harvest losses in Pawpaw in South Western Nigeria. Chukwuka et al. (2010) recently reported that *R. nigricans*, *A. flavus, A. niger, Fusarium sp.,* and *Mucor sp.* were responsible for with Pawpaw fruits decay from a farm in Oyo State, South Western Nigeria. Krogh (1992) has earlier reported that most microbes infecting plant tissues often produced secondary metabolites in their hosts, which are known to be hazardous to animals including man. Fungi associated with Pawpaw roots were studied by Oluma (1992) while Oyeniyi (1992) carried out a survey of microflora in the rhizosphere of Pawpaw. It is however note-worthy to intensify efforts in combating the production constraints associated with Pawpaw fruits spoilage caused by microbes. Researches have shown that the recent disruption of the global food supplies is predominantly due to post-harvest losses associated with microbes (Chukwuka et al. 2010). In line with the assertions of Krige et al. (2006) and Chukwuka et al. (2010), since Pawpaw fruits were usually infected by pathogenic organisms, to be effective, production, preparation and preservation of food such as fruit salads made with Pawpaw must be carried out as rapidly and hygienically as possible using good quality equipment, produce and materials.

In this study, three fungal species *Aspergillus niger*, *Penicillium digitatum* and *Rhizopus stolonifer*; were found associated with deteriorating Orange (*Citrus sinensis*). In a study by Al-Hindi et al. (2011), eighty-three percent of the citrus fruit samples showed fungal growth at levels ranging from 25 to 100% of tested fruits and *Fusarium* spp. were the most common fungi in citrus fruits (Tournas and Katsoudas, 2005). Orange had been studied for fungal decay in storage and its relation to shop (local storage places) and a number of *Aspergillus* spp., *A. nigei, A. nidulans, A. variecolour, A. fumigatus, A. Candidus* had been isolated (Sinha, 1946; Al-Hindi et al., 2011). The preponderance of the isolated moulds from Orange (*Citrus sinensis*) belongs to *Aspergillus* species and other genus, and this confirms their prevalence in foods and fruits exposed to tropical humid climate thus constituting potential health risks to consumers of this fruit and it's by products (Niji *et al*., 1997). *Aspergillus niger and Candida tropicalis*. were found associated with deterioration of orange; this is in line with the work of Niji *et al.* (1997) who reported that *Aspergillus* Sp. is the predominant organism associated with the spoilage of orange.

In the case of Tomato fruits (*Lycopersicon esculentum*), *Rhizopus stolonifer*, *Fusarium solani* and *Candida tropicalis* was implicated in its spoilage. These agree partly with the findings of Mitra (1997) who discovered that the species of fungi associated with the spoilage of orange, Pawpaw and Tomato fruits include species of *Aspergillus*, *Fusarium*, Yeast, *Penicillium*, and *Rhizopus*. *A. flavus* and *A. Fumigatus* caused tomato spoilage were also investigated by Adisa (1993) and Al-Hindi et al. (2011). Other studies on the fungi associated with tomato rot showed seven fungi associated with fruit rot of tomato including *Fusarium equiseti, A. flavus* and *A. niger*, they were all pathogenic on tomato fruits (Oladiran and Iwu, 1993; Al-Hindi et al., 2011). It has been also rported that fungi affecting Tomatoes (Lycopersicum esculentus) includes *Fusarium Oxysporium, Fusarium moniliform, Aspergillus niger* and *Rhizopus Stolonifer*. They are responsible for Tomato soft rot, as was isolated by Onuegbu (2002) and Akinmusire (2011). Result on the percentage incidence and rot shows that *Rhizopus Stolonifer* caused the greatest rot on tomato fruit. A lot of breading works have been carried on Tomato up to the point where we now have Tomato hybrids that could withstand adverse environmental condition and are resistant to diseases and pests (Chuku *et al.*, 2008). The isolation of *Fusarium oxysporum, Rhizopus stolonifer* and *Mucor* Sp. from tomato confirmed the studies of Efiuvwerwere (2000), Chuku *et al*. (2008) and Akinmusire (2011) who reported that *Fusarium* Sp, and *Rhizopus stolonifer* is responsible for the soft rot of tomato. Onyia *et al.* (2005) also reported that *Fusarium monilifome*, *Aspergillus niger* and *Rhizopus stolonifer* were isolated from rotten tomato fruits.

The results of the pathogenicity tests carried out show that all the organisms were pathogenic and were the actual causal agents of spoilage of the different fruits and can also infect different fruits other than their original host. The tests also established the fact that fungi cause deterioration of the fruits when they gained entrance into them through mechanical injuries such as bruises and wounds as noted by Zitter (1985). *Aspergillus niger* grew at a faster rate than the remaining fungal isolates which was evident in its cause of spoilage in the fruits at a faster rate when compared to the other fungi. *Aspergillus niger* was also noted to appear first on the fruits before the other fungi. Also, the presence of these fungi pathogens in theses Pawpaw fruits could pose a serious threat to the health of its consumers.

The isolation of these pathogens confirmed the studies of Gupta and Pathak (1986), Baiyewu (1994), Baiyewu and Amusa (1999), Baiyewu et al. (1994, 2007) and Chukwuka et al. (2010) that *Rhizopus spp.,* and *A. niger* found associated with rotten Pawpaw are highly pathogenic causing appreciable losses in Pawpaw fruits at post harvest. Baiyewu (1994) also isolated *Fusarium* spp., *A. flavus,* and *Rhizopus* spp. among other pathogens from Pawpaw fruit. In our studies, the pathogenicity analysis revealed that all isolated fungi *A. niger* proved highly pathogenic causing a rapid disintegration of inoculated fruits in three to five days while *R. stolonifer,* Yeasts, *Penicillium digitatum* and *Fusarium* sp. were moderately pathogenic and the least pathogenic was *A. flavus*. However, from the result of this study, *A. flavus* was likely to be a pathogen of Pawpaw fruit but rather contaminants was mostly claimed by Baiyewu et al. (2007) and Chukwuka et al. (2010). Hence, necessary precaution in preventing contamination of this produce by these bacteria and fungi will enhance the microbial quality of the produce (Baiyewu et al., 2007; Chukwuka et al., 2010).

The contamination of fruits and vegetables by fungi could also be as a result of poor handling practices in food supply chain, storage conditions, distribution, marketing practices and transportation (Effiuvwevwere, 2000; Akinmusire, 2011). Post harvest handling and transport of fruit is inadequate (Baiyewu et al., 2007). Therefore most of the fruits harvested do not usually get to the major cities in time due to the nature of transport systems existing in the rural areas. While fruit with bruises are not isolated from the unbruised ones and thereby causing cross-infections, consumers are supplied mostly with partly rotten fruits (Baiyewu et al., 2007). This portends a great risk of aflatoxin and other mycotoxins to the consumers. This is confirmed in a study by Sage et al. (2002) who reported that Aflatoxin M1 was detected in the urine of the Philippine women that consumed peanut butter containing aflatoxin. According to Baiyewu et al. (2007), no tests have been conducted if aflatoxins are in the urine and blood to determine the presence and risk of such metabolites in most working class people in this South Western region of Nigeria. However, the fact that most people have not been diagnosed as having hepatoma or aflatoxicosis does not mean that the toxic metabolite does not exist in their body system (Muhammad et al., 2004; Baiyewu et al., 2007).

The occurrence of fungal spoilage of fruits is also recognized as a source of potential health hazard to man and animals. This is due to their production of mycotoxins (naturally occurring toxic chemical often of aromatic structure) compounds which are capable of including mycotoxicoses in man following ingestion or inhalation. They differ in their degree and manner of toxicity (Effiuvwevwere, 2000; Akinmusire, 2011).

5.0. CONCLUSION

This study detected the profile of spoilage fungi which caused pathogenecity of some local fruits in Ibadan city. It also showed that fungi were involved in the spoilage of many fruits. Mechanical injuries such as bruises or cuts that occur during harvesting or post-harvesting, grading and packing could provide infection sites for spoilage pathogens. Fruit spoilage however can be controlled by the following practices: Washing of harvested fruit with clean or pure water; Proper cleaning and sanitation of warehouses and disinfection of packaging and transit containers; Proper handling of the fruit during harvest to prevent bruises and scars or other mechanical injuries; Inhibition of fungal growth by lowering storage temperatures through storage under refrigeration and the use of fungicides. It is therefore important that both the farmer who harvests the fruits into bags for transportation, the marketers and consumers take necessary precaution in preventing contamination and eating of contaminated fruits. This will however, enhance reduction the risk of aflatoxin and other mycotoxins that are deleterious to human health which are produced by these fungi that have been isolated in this study.

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