Review Article

ISOLATION AND IDENTIFICATION OF FUNGI ASSOCIATED WITH AVOCADO PEAR (Persea americana mill)

Abstract

The aim of this study is the isolation and identification of pathogenic fungi from spilt avocado pear. Healthy avocado pear fruits purchased from Awka, Nnewi and Ihiala were brought to the laboratory and allowed to spoil on a laboratory bench. The fruits showing spoilage signs were examined for the presence of fungal pathogens inducing using potato dextrose agar (PDA) and SDA agar. From the result, there are more growth in PDA media than SDA and there was no significant different ($p>0.005$) among the fungal count of the avocado pear collected from the 3 locations with sample collected from Eke Awka

market showing highest fungal count, (48x102cfu/g), followed by sample from Total market Ihiala (47x102cfu/g) while sample from Nnewi market have the lowest fungal count (45x102cfu/g), The fungi associated with the spoilage of the fruits were identified based on their colonial and morphological characteristics. The study further showed that a total of 5 fungi isolates were obtained from fruits, which were identified as Aspergillus spp, Penicillium spp, Rhizopus sp, Fusarium spp and Candida spp. Of which, Aspergillus niger species were the most frequently isolated fungi (36%). This was followed by Rhizopus spp and Candida with infection rate of 18% each while Penicillium digitatum and Fusarium solani were the least encountered (141%) 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, and Penicillium digitatum were least pathogenic, and caused the least amount of rot on fruits. This study has 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.

Keywords: ISOLATION, IDENTIFICATION, FUNGI, AVOCADO PEAR(Persea americana mill)

INTRODUCTION

1.1Background of Study

Avocado (*Persea americana*) originated from Central America and Southern Mexico. Based on archaeological evidence found in Tehuacán, Puebla (Mexico), it is believed to have appeared ca. 12,000 years ago (Yahia and Woolf, 2011). P. Americana fruit is by itself a complete food containing nine essential amino acids in an unbalanced proportion (Bergh, 2012). Avocado, like many tree crops, is propagated clonally through grafting to preserve commercially desirable varieties. The consumption of avocado fruits has tremendously increased globally and avocado is now an important fruit traded in the international market (Radha and Mathew, 2007). Persea Americana fruits are purple to green in colour with smooth or warty skin depending on variety. The flesh of the fruit is yellowish-green in colour and has the consistency of butter. Each fruit contains one large seed. P. americana trees grown from seed can take 4-6 years to start fruiting, whereas

grafted plants fruit within 1-2 years. The leaves are elongated and could be elliptic, lanceolate, oval, obovate or ovate in shape (Schaffer *et al.*, 2013).

Palmivora (Machado *et al.*, 2012), gummosis and root necrosis caused by Phytophthora nicotianae (Machado *et al.*, 2013). Molecular-based technologies are the most reliable tools for characterizing microorganisms due to the fact that they deal with the genetic composition of organisms. Molecular tools have made it possible to obtain in-depth information on analyses of systems subject to climate changes (Xue *et al.*, 2016), foodstuffs, agriculture, industrial settings and across the environmental sciences (*Long et al.*, 2016). Highly conserved oligonucleotide primers, such as those used for the amplification of the internal transcribed spacer (ITS) region for fungi has been developed (Schoch *et al.*, 2012; Tedersoo *et al.*, 2015).This study was therefore aimed at isolating and identifying fungal species associated with avocado fruits using the samples of spoilt Avocados pear.

1.2 State Of Problem.

It is obvious that fungal infection on Avocados constitute major problem faced by farmers and market dealers in many countries. Several studies on isolation and identification fungi associated with Avocado Pears have been reported in Nigeria and other parts of the world. There is dearth of information on the status of fungal infection of Avocado in Awka, Anambra State, Nigeria. Knowledge of the type of

fungi parasite of Avocado in a particular area is important to identify the risk in the prevention and control of infections

1.3 Aim and objectives

The study is aimed at isolating and identifying fungal species associated with avocado fruits using the samples of spoilt Avocados pear.

Specific objectives;

I. Samples of avocados purchased from a nearby market
II. To determine the diagnostic tools and methods that are available for isolating a fungi from Avocado seed.
III. To identify fungal infection of Avocado seed.

1.4 Justification of study

The study of the isolation and identification of fungi associated with Avocados seed does not only enlighten the possible Fungi but also help in identification and treatment of those parasites. To justify this study, Fungi parasite has to be collected and differentiated according to their different species and how to be tested using a suitable test method and identified and also know the prevalence of those parasites identified.

1.5 Limitation of the study

The study is only limited to the isolation and identification of fungi associated with Avocado pears or seed on the study area.

LITERATURE REVIEW

2.1 History and Distribution

The avocado (*Persea americana Mill.*) is a polymorphic tree species that originated in a broad geographical region stretching from the Pacific coast of Central America through Guatemala to the eastern and central highlands of Mexico (Popenoe, 1920). Three distinct and separate taxa or sub-species now termed the Guatemalan, Mexican and West Indian or Antillean races have been selected over millennia (Knight, 2002). Although little is known or recorded about the introduction of avocado to South Africa, it is accepted that the first trees were West Indian race-seedlings planted on the coastal strip around Durban in the late 19th century (Landman, 1930). Fruits from these trees were inferior with regards to storability and it was only until the mid1920's that budded trees of Mexican, Guatemalan and hybrid origin were imported from California, which were more adapted to South African climatic conditions (Malan, 1957). Avocados are now widely distributed throughout South Africa, although production is predominantly in the Limpopo and Mpumalanga provinces in the north and northeast, and to a

lesser extent in the frost-free lowland coastal belts and cooler midlands of KwaZulu Natal. South African avocado production focuses mainly on two cultivars viz. 'Fuerte' and 'Hass', and volumes have increased more than 11-fold from 4700 to 53 800t export-based annually in the years 1961 to 1996 (Knight, 2002). Currently 12400ha are planted with avocado trees in South Africa, with approximately 3015000 trees in production, which could amount to more than 50 thousand tons, of which 36 thousand tons (9 million cartons) are destined for the export market (Retief, 2007).

2.2 Nutritional and Physicochemical Characteristics of Avocado

Avocado is consumed in various forms in northern South America, Central America and Mexico, as puree salads, seasoned with salt, pepper, vinegar and other condiments, as well as being used in the preparation of other dishes (Koller, 1992). In Brazil, the ripe fruit is more appreciated, together with sugar, honey and liqueurs, and consumption is influenced mainly by its sensory and nutritional characteristics (Luíz *et al.*, 2007).

The pulp content in several varieties is between 52.9 and 81.3%, relative to fruit mass (Tango *et al.*, 2004). High lipids and low carbohydrate levels remain in avocado pulp after water removal, thus conferring a high dry matter content to the product. Therefore, it is considered one of the few cultured fruits presenting the

lipid fraction as the major component which can reach up to 25% of the fruit portion (Hierro *et al.*, 1992; Abreu *et al.*, 2009).

The avocado pulp contains from 67 to 78% moisture, 13.5 to 24 % lipids, 0.8 to 4.8% carbohydrate, 1.0 to 3.0% protein, 0.8 to 1.5% ash, 1.4 to 3.0% fiber, and energy density between 140 and 228kcal (Soares & Ito, 2000). Avocado has four (4) times more nutritional value than any other fruit except banana, containing proteins (1 to 3%) and significant levels of fat-soluble vitamins (Francisco & Baptistella, 2005), folic acid, and appreciable amounts of calcium, potassium, magnesium, sodium, phosphorus, sulfur and silicon, and vitamins E, B1, B2, and D (Dembitsky *et al.*, 2011).

The fruit stands out on potassium levels (339mg 100g-1) when compared to other fruits, which regulates muscle activity and protects the body from cardiovascular diseases (Canciam *et al.*, 2008). It also represents a source of glutathione, a powerful antioxidant that acts on potentially carcinogenic compounds (Wang *et al.*, 2012).

2.3 Bioactive Compounds in Avocados

In addition to the important major compounds, avocado contains substantial amounts of bioactive compounds such as phytosterols, especially in the lipid fraction, and the main representative is the β-sitosterol (Salgado *et al.*, 2008b; Santos *et al.*, 2014b). Diets rich in phytosterols can lead to the reduction of the

total cholesterol and LDL cholesterol (Lottenberg, 2002). A 17% decrease average in blood cholesterol levels was observed in a study in Mexico with 45 volunteers who consumed avocado once a day for one week (Borges & Melo, 2011).

Phytosterol is a substance of vegetable origin whose structure is very similar to cholesterol. Its mechanism of action in the body involves the inhibition of intestinal cholesterol absorption and decreased hepatic cholesterol synthesis. According to Brufau *et al.,* (2008), it acts on total plasma cholesterol and LDL cholesterol without affecting HDL and blood triglycerides. The benefit of cholesterol reduction also comes from replacing saturated by unsaturated fats, which promote a decrease in total cholesterol and LDL and an increase in HDL levels (Salgado *et al.,* 2008a).

The β-sitosterol in avocados also has a special effect on immunity, contributing to the treatment of diseases such as cancer, HIV and infections. In relation to cancer, it works by suppressing carcinogenesis and in HIV by strengthening the immune system (Bouic, 2002). This compound enhances lymphocytes proliferation and natural killer cell activity, which inactivates invading microorganisms (Bouic *et al.,* 1996). In addition, studies have shown that the β-sitosterol activity is an aid in weight loss by reducing compulsive eating binge and fat accumulation in the abdominal region (Senai, 2006; Murta, 2013).

The health effects of sterols and stanols have been the subject of several studies. Some authors have demonstrated a 25% reduction in the risk of coronary heart disease with the consumption of 2g of such compounds per day, which are included in the formulations of margarines, spreads, and vegetable oils by etherification without affecting vitamins solubility (Turatti, 2002).

The avocado oil variety Margarida contains a greater diversity of sterols, and β-sitosterol represents 71.8% of the total sterols, besides lower cholesterol levels (0.3%), which can achieve up to 2.3% in other varieties (Salgado *et al.,* 2008b). Santos *et al.*, (2014b) investigated the oil from Fortuna avocado extracted with petroleum ether and subjected to drying under forced air (40°C), and found 87.6% β-sitosterol, 12.41% campesterol, and 0.04% stigmasterol of the total phytosterols. Avocado also has a carotenoid named lutein that helps protect against prostate cancer and eye diseases such as cataracts and macular degeneration (Johnson, 2005).

2.4 Avocado Oil

The avocado pulp contains high lipids content, which makes the pulp the portion of greatest interest. Lipids vary from 5 to 35%, being formed mostly by unsaturated fatty acids (60-84%) (Borges & Melo, 2011). The avocado varieties with lower

core and shell percentages are most interesting for oil extraction due to higher pulp yield, and the variety Quintal stands out (Tango *et al.*, 2004).

The high moisture content in fresh pulp is the main obstacle for obtaining avocado oil as it affects the extraction yield and production costs. Thus, the varieties most suitable for oil extraction, considering 18% lipids and low moisture levels in the pulp are: Hass, Fuerte and Glória, followed by the varieties Collinson, Anaheim, Itzamna, Wagner, Ouro Verde, Carlsbad and Mayapan (Tango *et al.*, 2004).

The traditional cold pressing method for vegetable oils was replaced by solvent extraction. Although some authors have reported a yield of 59% in oil extraction from fleshy pulp when using hexane, this value decreased to 12% when acetone was used as solvent (Abreu *et al.*, 2009). However, the hexane residue in the extracted oil and cake may pose risks.

Santos *et al.*, (2013) evaluated the extraction yield of Fortuna avocados oil as a function of the drying process (freeze-drying or air flow: 40 to 70°C) and extraction method (pressing and solvent) of a pulp containing 5 to 6.5% moisture. The authors reported oil contents between 25 and 33% by cold pressing and between 45 and 57% by solvent extraction, while the freeze-drying method showed higher oil yield than the oven drying under forced air. The enzyme-assisted aqueous extraction has emerged as an alternative and environmentally friendly extraction process (Abreu *et al.*, 2009).

The small avocado oil volume currently produced by some countries is used in its raw form by the pharmaceutical and cosmetics industries, once its unsaponifiable fraction is responsible for regenerative properties of the epidermis. Avocado oil is easily absorbed by the skin, with high absorption power of perfumes, which is of great value to the cosmetics industry. In addition, it easily forms an emulsion, ideal for the manufacture of fine soaps (Tango *et al.,* 2004). In comparison to other vegetable oils, avocado oil is characterized by having high levels of monounsaturated fatty acids (oleic and palmitoleic acids), low polyunsaturated fatty acids (linoleic acid), and relatively high levels of saturated fatty acid (palmitic and stearic acids). This fatty acid composition is influenced by the cultivars, maturity stage, anatomical region of the fruit, and geographic location for plant growth (Tango *et al.*, 2004).

Rocha (2008) has reported that avocado oil from the varieties Wagner, Fortuna, Hass and Fuerte had higher levels of monounsaturated fatty acid (MFA) ranging from 59 to 72% of total fatty acids, followed by saturated fatty acids (SFA), from 17 to 23%, and polyunsaturated fatty acids (PUFA) to a lesser extent with levels ranging between 10 and 14%.

Santos *et al.*, (2014a) determined the fatty acids profile of Fortuna avocado, evaluating the effect of the pulp drying process (freeze-drying or air circulation: 40 and 70°C) and oil extraction method (solvent or pressing). The authors reported

that oleic fatty acid represented more than half of the total fatty acids of this raw material, together with substantial amounts of unsaturated linoleic and palmitoleic acids. They also verified that the dehydration of the pulp can affect the fatty acid profile since the oil extracted from the lyophilized pulp contained higher levels of unsaturated fatty acids. With respect to the extraction method, no significant effects were observed.

Avocado oils from the varieties Northrop, Duke, Wagner, Quintal, and Fuerte are characterized by having more than 63% oleic acid, while the oils from the varieties Rincon, Barker, Waldin, Prince and Panchoy showed less than 50% of this fatty acid. Palmitic acid content ranged between 15.38 and 32.37% in oils from different varieties. Therefore, the avocado variety affects the levels of palmitic acid and oleic acid, once varieties with high oleic acid levels had low palmitic acid levels and vice versa (Bleinroth & Castro, 1992). In addition to its fatty acid composition, these oils contain other bioactive minor components such as tocopherols, squalene, b-sitosterol, campesterol, and cycloartenol acetate, with positive effects on health (Dembitsky *et al.,* 2011; Santos *et al.,* 2014b).

Besides the possibility of using pure avocado oil as a substitute for olive oil, the combination of olive oil and avocado oil to replace olive oil mixtures (mainly using soybean oil) usually offered by the internal market is a promising alternative

to reduce costs of Brazilian olive oil imports (Salgado *et al.*, 2008b). Avocado oil for salad dressings should be submitted to winterization to eliminate the saturated triglycerides, which can cloud the oil stored at low temperatures (Salgado *et al.*, 2008b).

Although the lipid extraction process generates large accumulation of pulp residues in the processing industries, the high fiber content of this by-product allows its use for preparation of flour to be used in bakery products like cookies, breads, and pasta thereby increasing the supply of fiber-rich products (Chaves *et al.*, 2013).

2.5 Applications and Forms of Preservation of Avocado Pulp

The processed products of avocado pulp include the paste, puree, and guacamole. Guacamole is a fruit pulp seasoned with salt, onion, lemon, pepper and tomato, being produced not only in an artisanal way but also marketed by some US Companies (Daiuto *et al.*, 2011).

The sensory quality of guacamole of Hass variety made without chemical additives and stored under refrigeration was evaluated according to the type of packaging used. A greater consumers' acceptance was observed for the product stored in container with gas barrier when compared to that stored in polyethylene package

(Daiuto *et al.*, 2011). Although these authors have also considered that the heat treatment may have been effective on the polyphenol oxidase inactivation, it can result in the development of bitterness and off-flavors in avocado, which changes the guacamole texture, negatively contributing to a mashed appearance. Chaves *et al.*, (2013) studied avocado pulp Margarida variety dehydrated and defatted by cold pressing and avocado oil to partially replace wheat flour and butter, respectively, in whole grain crackers. The authors reported that the flour from avocado pulp, in general, showed characteristics similar to those of conventional flour and whole wheat flour. The biscuits had higher minerals and fiber levels, with good sensory acceptance. Products that contain high levels of vegetable oil, like avocado, are sensitive to oxidation, resulting in rancidity and hence production of undesirable flavors and loss of quality during storage. Elez-Martinez *et al.*, (2005) demonstrated the possibility to control oxidative rancidity in processed avocado puree with the use of α-tocopherol and ascorbic acid.

Several preservation methods have been studied to obtain a stable avocado pulp, including pasteurization, drying, oil extraction, freezing, and freeze-drying (Palou *et al.*, 2000; Soliva *et al.*, 2001; Soliva-Fortuny *et al.*, 2004). Use of microwave heating and copper chloride to preserve color of mashed avocado has been also investigated (Guzmán *et al.*, 2002). Furthermore, chemical reducing agents,

sequestrants, acids, nitrogen atmosphere and vacuum (Soliva *et al.,* 2001) and high hydrostatic pressure treatment have been studied

2.6 By-Products

Avocado seed is underutilized and represents a large portion of the fruit, thus its use can be an alternative to reduce the production cost of edible oil. However, the main problem in the use of avocado seeds is the presence of phenolic compounds that exhibit toxicity. Studies have shown that the seeds can be used in feed for monogastric animals after extraction of these substances with ethanol (Ichimaru *et al.,* 1982). The extract may present antioxidant activity, once the phenolics levels in seeds vary from 2.3 to 5.7%. In addition to the starch and fiber, there are other non-nitrogenous substances present in seeds, ranging from 5.1 to 13.2% (Salgado *et al.,* 2008a).

Avocado leaves are a pharmaceutical ingredient widely used in extracts for therapeutic purposes, and also as teas in folk medicine (Vendruscolo & Mentz, 2006), probably due to the diuretic properties (Wright *et al.,* 2007).Phytochemicals as orhamnetin, luteolin, rutin, quercetin, and apigenin have been isolated from avocado leaves, which can help prevent the progress of various diseases related to oxidative stress (Owolabi *et al.,* 2010).

2.7 Plant Morphology and Physiology

Tree performance is ultimately measured by yield and quality. Average yields of avocado trees are determined by numerous factors including cultivar, rootstock, environmental factors, tree size, shape and age. However, the ultimate factor controlling yield is seasonal photosynthetic efficiency, and in particular the harvest index, i.e. the measure of photosynthate proportioned to fruit (Wolstenholme, 1987). 8 Consequently, the effect of Phytophthora root rot on photosynthate accumulation and storage is of major importance. Physiological effects of Phytophthora cinnamomi Rands on avocado trees are severe and infected trees have lower water potential, reduced stomatal openings, and reduced water and nutrient uptake (Sterne *et al.*, 1977, 1978; Whiley *et al.*, 1986). Reports by Davies *et al.,* (1986) indicated that stomata close even when leaves are not experiencing water stress, provided plant roots are stressed. Under optimal conditions for fungal growth, avocado roots become severely infected by P. cinnamomi, leading to severe root death, and thus a loss in water and nutrient uptake. This leads to a drop in photosynthesis resulting in a reduction of carbon partitioning to fruit (Pegg *et al.,* 2002).

2.8 Phytophthora Root Rot Disease of Avocado

Phytophthora cinnamomi is a soilborne psuedofungus of the Class Oomycetes in the Kingdom Chromista (Hardy *et al.*, 2001). It is the most important and destructive disease of avocado worldwide (Pegg *et al.*, 2002). It attacks trees of all ages, from nursery trees to large bearing trees, killing them by destroying the fine feeder roots. Reproduction, growth and spread of the fungus are favoured by free soil water. Consequently, movement of infected soil plays an important role in the spread of this fungus (Hardy *et al.*, 2001). P. cinnamomi was first described by Rands as the causal organism of a stem canker of cinnamon trees in Sumatra in 1922, and it was first reported in 1929 on 10 avocado in Puerto Rico where it caused severe root rot (Tucker, 1929). Its presence has now been reported in over 1000 plant species (Zentmyer, 1980), and hosts include pineapple, macadamia, peach, pear, kiwi fruit, chestnut, blue-gums, and many native Australian and South African plants (Pegg *et al.*, 2002). It has been postulated by Arentz and Simpson (1986) and Linde *et al.*, (1997) that the fungus originated in Papua New Guinea, and was moved by the activities of people into other tropical and subtropical regions of the world. Phytophthora root rot has been the main economic factor limiting successful avocado production in countries such as Australia, South Africa and the USA. In the US, where it is estimated that up to 70% of commercial orchards are affected, the annual loss attributed to the disease has been estimated at

US$ 30 000 000 (Coffey, 1987). The loss in South Africa due to Phytophthora root rot of avocado trees amounts to R45 000 000.

2.9 Symptoms

Phytophthora cinnamomi causes rot of fine feeder roots, leading to death of host plants (Anon, 2004). Invasion of larger roots has also been reported (Anon, 2004; Pegg *et al.*, 2002) and may lead to brown lesion formation in the wood. This may result in symptomatic peeling of bark, or cause a weeping canker at the tree base, below the soil line, possibly extending up the trunk for 1m (Pegg *et al.*, 2002). However, infection is mostly limited to the fine feeder roots, which become black and brittle and eventually die off. Feeder roots may be difficult to find under trees with advanced root rot. Beneath such trees soil tends to remain damp, as the absence of feeder roots prevents trees from absorbing moisture (Pegg *et al.*, 2002). Foliage becomes wilted and chlorotic, leaves fall and branches rapidly die back depending on root rot severity. New leaf growth is minimal, and if leaves form, they are small and pale green. Fruit set is usually limited in root rot affected trees, and fruit are small. Visible symptoms in the tree can also result from unnatural distribution of nutrients in plant tissue and interference with nutrient uptake.

Because roots are unable to control salt uptake, chloride accumulates in leaves and may reach toxic levels, resulting in scorching of leaf margins and tips (Whiley *et al.,* 1987). Labanauskas *et al.,* (1976) reported that Phytophthora infection affects the distribution of nutrients within plant parts (Ploetz and Schaffer, 1989).

A moderate tolerance is often observed in avocado trees without degradation of aerial tree health (Ploetz and Parrado, 1988). Reduced photosynthesis, transpiration and stomatal conductance can however be detected in root rot affected trees before these visible aerial symptoms appear (Sterne *et al.*, 1978; Ploetz and Schaffer, 1989).

2.10 Disease Cycle and Epidemiology

Zentmyer *et al.,* (1994) reported Phytophthora root rot of avocado to be more severe and develop more rapidly in soils with poor drainage. The disease has a short generation time and high reproductive capacity and inoculum can increase from low, often undetectable levels, to high levels within days, particularly in warm, moist and well aerated soils, and if feeder roots are in abundance (Zentmyer, 1980). High soil moisture increases infection due to increased sporangial production and favourable conditions for zoospore release, motility and movement to feeder roots. Oospore production can occur in less than 48h and thus

are responsible for the rapid colonization observed during epidemics (Zentmyer and Mircetich, 1966). They are fragile, short-lived and only motile in soils for periods of minutes to hours, depending on energy reserves and factors affecting encystment (Zentmyer *et al.,* 1994). Chlamydospores survive for considerable periods in root debris and soil. They germinate by producing several germ tubes at soil temperatures above 15°C. Oospores occur infrequently and, although they may survive for long periods of time, they probably do not play an important role in the disease cycle (Zentmyer, 1980). Disease development is optimal in wet soil at temperatures from 21-30°C, whereas little or no infection occurs above 33°C, or below 13°C (Zentmyer et al., 1994).

Zentmyer and Mircetich (1966) reported the non-pathogenic stage of P. cinnamomi to be more significant than previously thought. Saprophytic tests indicate persistence of this fungus for long periods in the absence of a viable host, showing moderate mycelial growth through non-sterile soil, and appreciative invasion of dead organic matter, especially under moist conditions.

MATERIALS AND METHODS

3.1 Sample Collection

The spoilt avocado pear fruit were purchased from three (3) markets in within the study area. Other apparatus, agar media and reagents were obtained from Alpha research Laboratory Awka.

3.2 Fungal isolation

3.2.1 Culture Media

Two commercially available media were used in this work. These were Potato Dextrose Agar (PDA), which is a general purpose culture media, and Sabouraud Dextrose Agar (SDA), which is a modification of Dextrose Agar

3.2.2 PDA media preparation

In one litre of distilled water, 39g of the medium was suspended, heated over a Bunsen flame with frequent agitation, and allowed to boil for one minute to completely dissolve the medium/contents. The solution was autoclaved at temperature of 121^0 C for 15 minutes, at a pressure of one (1) atmosphere (15 PSI).After removing from the autoclave, allowed to cool for 10 minutes. Five

hundred (500 mg) streptomycin sulphate was added into the molten solution to serve as antibiotics.

3.2.3 SDA media preparation

In one litre of distilled water, 65g of the medium was suspended and dissolved by heating to boil, with frequent agitation. After heating for one minute and dissolving the solution, it was sterilized in an autoclave at 121^0C for 15 minutes. This was followed by the addition of 500mg streptomycin antibiotic while the solution was still in a molten state.

3.3 Isolation of Fungi from Samples

The isolation technique of Onuh, Shiriki, Ubwa, and Shambe, (2015) was adopted in this study. A small section of infected avocado pear fruit containing the advancing margin of rot and adjoining healthy tissue were cut using sterilized scalpel and cork borer while the surfaces were sterilized by dipping completely in a concentration of 40% hypochlorite solution for 60 seconds; the sterilized sections to be inoculated were then removed and rinsed with three changes of sterile distilled water. The tuber pieces were made to dry by blotting with sterile filter paper in a laminar airflow cabinet. With the aid of a sterile forceps four pieces of

each cut samples were separately inoculated (90° apart) on solidified potato dextrose agar (PDA) and sabouard dextrose agar (SDA) plates. Two replicates for each sample were made. The plates were incubated a temperature of 28-30 °C in an incubator for 72 hours. Fungi associated with the avocado pear fruit spoilage were observed.

3.4 Identification of fungi

Isolated fungi were further sub-cultured to obtain a pure culture. Identification was then done based on colony characteristics, morphology and microscopic features according to Marthur and Kongsdal (2013). Fungal identification was done using morphological characteristics and comparing the findings with established keys as described by Nwachukwu and Osuji (2018). Each isolate was subjected to colony and microscopic examinations during which their morphological features were observed and recorded. Morphological features studied were based on growth patterns, color of mycelia and microscopic examinations of vegetative and reproductive structures. A sterile inoculating needle was used to get a small portion of mycelia from between the colony centre and the edge and placed on a clean microscopic slide containing lacto phenol in cotton blue. The mycelia were spread well on the slide using the sterile needle and a cover slip gently placed with little

pressure to eliminate air bubbles. The slide was placed above some boiling water to steam it for better staining of fungal structures. The excess lactophenol on the edges of the cover slip was wiped using sterile blotting paper. The slide was mounted on the microscope and observed with ×10 and ×40 objective lenses.

3.5 Determination of Fungal Frequency (%)

Fungal frequency will be determined location wise as well as culture media wise and later its correlation will be observed with the Percent Disease index calculated based on symptoms. The following formula will be used for fungal frequency percentage determination:

$$\text{Fungal Frequency (\%)} = \frac{\text{Number of particular fungus colony observed in plates} \times 100}{\text{Total number of colonies of all fungi}}$$

3.6 Pathogenicity test

The method of Ogbo and Agu, (2015) will be use for the pathogenicity test. Nine healthy avocado pear fruit were properly washed with tap water and then rinsed with distilled water. Then the surfaces of the corms were dis-infected with 75% ethanol. The severity of rot seeks to measure the magnitude of the infection as well as the rate of the pathogenicity of the rot-causing fungi. This was determined by obtaining the rotted portions off the whole tubers and taking the final weight of the

individual yam tuber. Un-inoculated controls were placed in clean polyethylene bags. The percentage severity of spoilage (Sr %) was calculated thus:

$$Sr\ \% = \frac{FW - w}{w} \times 100$$

where,

FW = Final weight of infected yam tuber,

w = weight of rotted tuber portion.

3.7 Statistical analysis: Percentages and means of fungal colonies were calculated. Data obtained was subjected to Analysis of Variance (ANOVA), and Duncan Multiple Range Test (DMRT) was used to separate the treatment means when significant at 5 % level of probability.

RESULTS

4.1 Isolation of spoilage fungi

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

The nature of fungal growth and total fungi count was measure in colony-forming unit per gram (cfu/g) and shown in table 1 and 2.

Table1: Nature of fungal growth in PDA and SDA for spoilt avocado pear fruit samples

SAMPLE	NATURE OF GROWTH

Sample 1 + PDA	**Heavy growth**
Sample 2 + PDA	**Heavy growth**
Sample 3 + PDA	**Heavy growth**
Sample1 + SDA	**Moderate growth**
Sample 2 + SDA	**Heavy growth**
Sample 3 + SDA	**Moderate growth**

Table 2: Mean fungi count in PDA and SDA for spoilt avocado pear fruit samples

Sample	Mean total fungi count in PDA (x 10^2 cfu/g)	Mean total fungi count in SDA (x 10^2 cfu/g)
1	48 ± 0.111^a	25 ± 0.110^b
2	45 ± 0.310^c	19 ± 1.112^c
3	47 ± 0.017^b	29 ± 0.121^a

***Values are mean scores ± Standard deviation of three (3) replicates**
***Data in the same column bearing different superscript differ significantly ($p < 0.05$).**

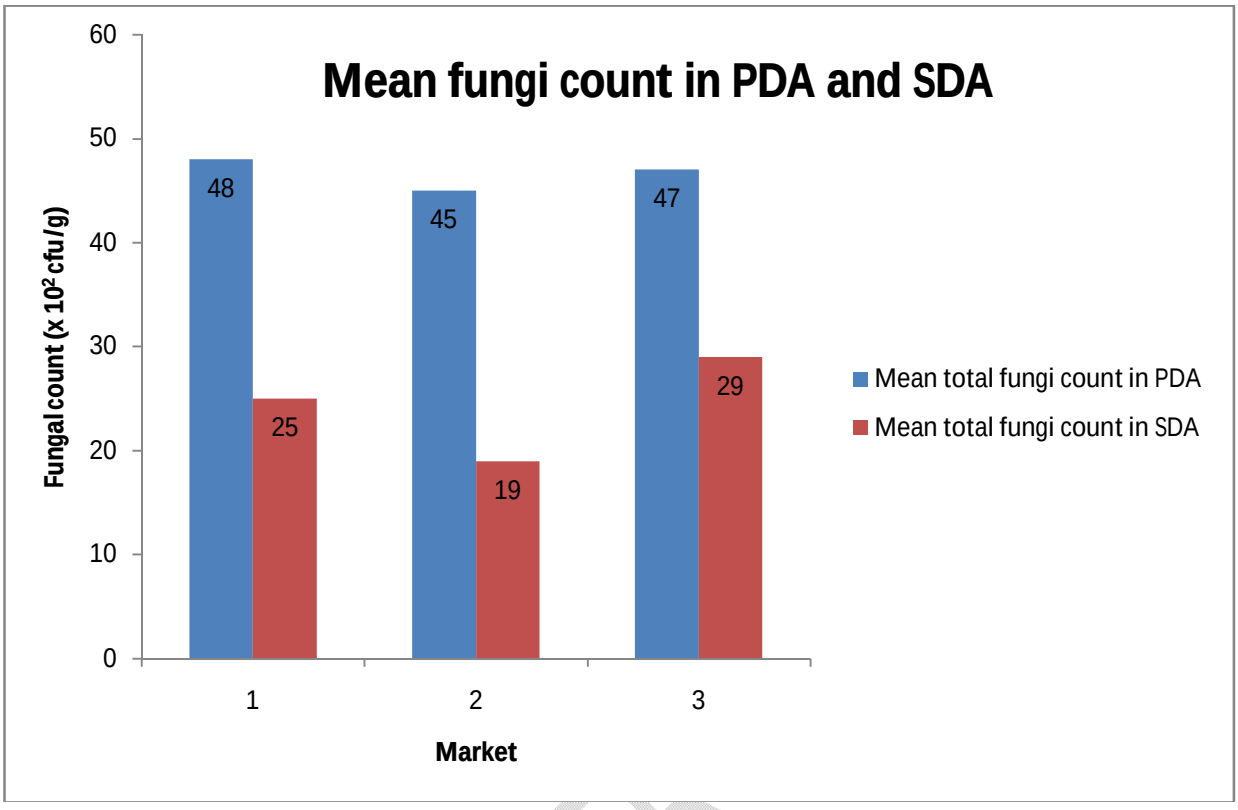

Chart 1: Mean fungi count in PDA and SDA for spoilt avocado pear fruit samples

The total fungi count of the fungal growth in PDA and SDA agar media were determined in colony forming unit per gram (cfu/g) using a colony counter and presented in table 2 above. From the result, there are more growth in PDA media than SDA indicating PDA as better media for isolating spoilage fungi from avocado pear. There was no significant different ($p>0.005$) among the fungal count of the avocado pear collected from the 3 locations with sample collected from Eke Awka market showing highest fungal count, ($48x10^2$cfu/g), followed by sample from

Total market Ihiala ($47x10^2$cfu/g) while sample from Nnewi market have the lowest fungal count ($45x10^2$cfu/g).

Table 3 :Colonial and Morphological features of the fungi isolated from the spoilt avocado pear fruits.

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Isolate	**Colonial Features**	**Morphological Features**	**Suspected Organism**
1	Black Colonies with white edges	Conidia heads are large, globose,dark brown and biseriate. Conidia are globose and rough walled. Conidiophores are smooth walled	Aspergillus sp
2	Whitish colonies, growing rapidly and filling the petridish with dense cottony mycelium and becoming brownish-black with age	Non-septate mycelia. Sporangiophores are smooth walled. Sporangia and columella are subglobose. Sporangiospores are ovoid in shape.	Rhizopus sp
3	Green and velvety	Colonies are smooth and ellipsoidal Conidiophores are smooth and short. Mycelia are arranged irregularly with branches of various lengths.	Penicillium sp
4	Pink and cottony colonies	Microcondia are ovoid in shape. Macroenidia are borne on phialides on branched conidiophores. Septate fusiform, slightly curved and pointed at both ends is present.	Fusarium sp

5	Creamish, smooth, convex and opaque colonies with a yeasty odour	Budding, spherical to elongated cells, forming pseudomycelium	Candida sp

Physically observation of the diseased fruits revealed brownish, necrotic patches on the skin of the avocado pear fruits. The patches on the avocado pear fruits took 7 days to turn black. A mass of mycelia growing on the surface of the fruits was also observed. Table 3 shows the colonial, morphological and cellular characteristics of the fungi isolated.

Table 4: Frequency of Occurrence of Fungi Isolates Associated with the Spoilage of avocado Fruits

Fungi Isolate	Market 1	Market 2	Market 3	Total (%)
Aspergillus spp	**2**	**3**	**5**	**10 (36.00)**
Rhizopus sp	**3**	**0**	**2**	**5 (18.00)**
Fusarium spp	**3**	**0**	**1**	**4 (14.00)**
Penicillium spp	**0**	**0**	**3**	**4 (14.0)**
Candida sp	**2**	**2**	**1**	**5 (18.0)**
Total	**10**	**5**	**12**	**27 (100)**

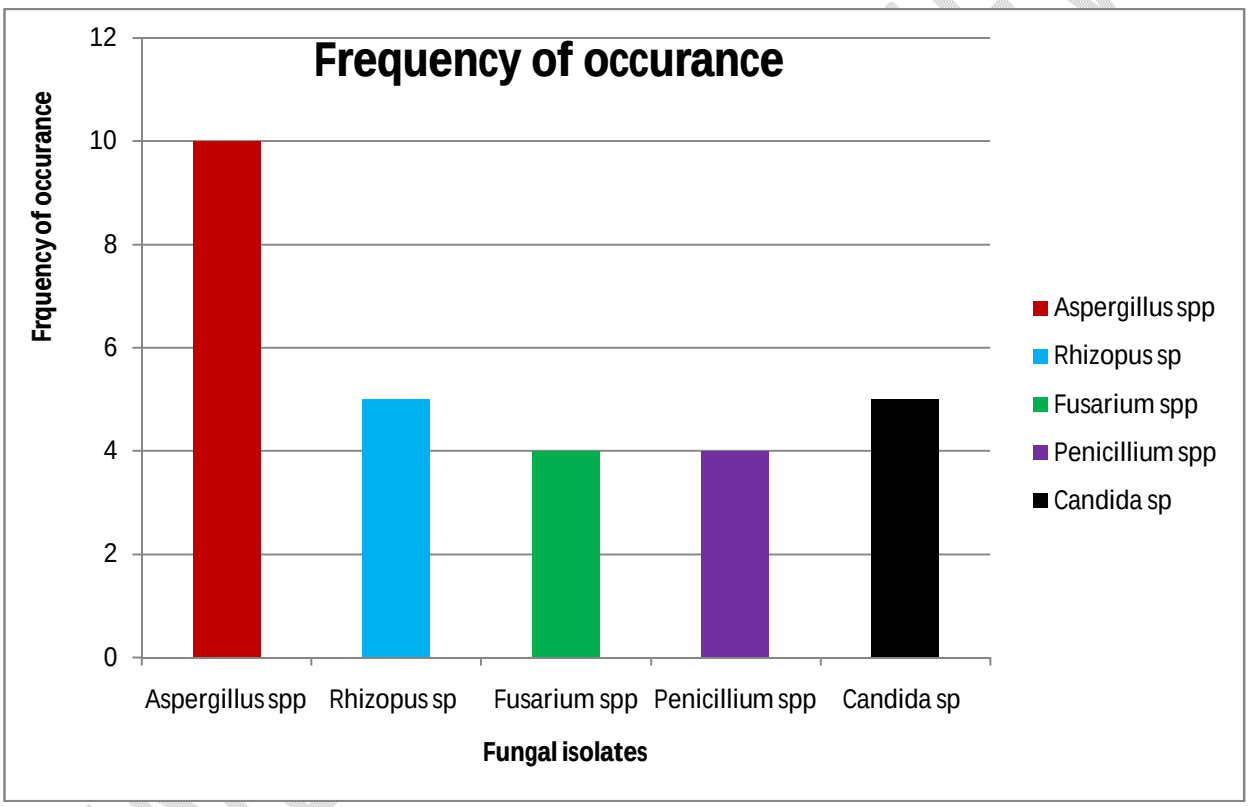

Chart 2: **The Frequency of Occurrence of Fungi Isolates**

The frequency of occurrence of fungi isolates associated with the spoilage of fruits is shown in Table 4. It showed that a total of 5 fungi isolates were obtained from fruits, which were identified as *Aspergillus spp*, *Penicillium spp*, *Rhizopus sp*, *Fusarium spp* and *Candida spp.* Of which, *Aspergillus niger species* were the

most frequently isolated fungi (36%). This was followed by *Rhizopus spp* and candida with infection rate of 18% each while *Penicillium digitatum* and *Fusarium solani* were the least encountered (141%) as shown in Table 4.

4.2 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, and Penicillium digitatum were* least pathogenic, and caused the least amount of rot on fruits.

Table 5: Result of the Pathogenicity Test On The Healthy Avocado Fruits

Sample	Aspergillus sp	Rhizopus sp	Penicillium sp	Fusarium sp	Candida sp
1	+	+	-	+	+
2	+	-	-	-	+
3	+	+	+	+	+

+ = detected
- = not detected

The table above shows the pathogenicity test on each of the infected fruit and this 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, and Penicillium digitatum were* least pathogenic, and caused the least amount of rot on fruits.

DISCUSSION AND CONCLUSION

5.1 Discussion

Micro- organisms especially fungi have been reported to cause extensive deterioration of fruits and vegetables (Fajola, 2009; Amadioha and Uchendu, 2013). Some of these micro-organisms cause rotting, discoloration or fermentation of the fruits which affect their preservation. This study showed that the avocado pear fruits *are* attacked by various fungi which cause deterioration. In the present study, many filamentous and yeast fungi were isolated from the avocado pear collected from various market in Awka, Nnewi and Ihiala in Anambra State.

The findings of this study showed that *Aspergillus spp, Penicillium spp, Rhizopus sp, Fusarium spp* and *Candida spp.* were found in fruits sold in major markets in the study area. 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, *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 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.

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).

The isolation of these pathogens confirmed the studies of Gupta and Pathak (2016), Baiyewu and Amusa (2009), Baiyewu *et al.* (2007) and Chukwuka *et al.* (2010) that *Rhizopus spp.*, and *A. niger* found associated with avocado fruits are highly pathogenic causing appreciable losses in avocado pear fruits at post harvest. Baiyewu (2014) also

isolated *Fusarium* spp., *A. flavus*, and *Rhizopus* spp. among other pathogens from avocado fruits.

The contamination of avocado fruits by fungi could be as a result of poor handling practices in food supply chain, storage conditions, distribution, marketing practices and transportation (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 (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).

5.2 CONCLUSION

This study detected the profile of spoilage fungi which caused pathogenicity of some local fruits in such as avocado fruits in Abuja 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.

5.3 Recommendations

This study revealed high fungal presence in the different avocado fruits investigated. This tends to reflect the level of bio security and hygienic practices in the harvesting, handling and storing of the avocado pear fruits.. These findings emphasize the need for constant quality assessment of these avocado pear fruits on sales.

Prompt removal of over-ripe fruits under the *Avocado pear* is recommended to reduce inoculums levels of the pathogenic fungi. Careful handling is also recommended to ensure minimal damage of the fruit.

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