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Isolation and Identification of Fungi Associated with Avocado Pear (*Persea americana* Mill)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study's objective is to separate and identify harmful fungus from spoiled avocado pears. Healthy avocado pear fruits were delivered to the lab and left to rot on a lab bench after being purchased from Awka, Nnewi, and Ihiala. Potato dextrose agar (PDA) and SDA agar were used to test the fruits for the presence of fungal infections that cause deterioration. According to the findings, PDA media exhibit greater growth than SDA, and ther45 x 102 CFU/g).e was no statistically significp > 0.005ference (p>0.005) between the fungal counts of the avocado and pear samples collected from the three different sites, with the Eke Awka market sample showing the highes48 x 102 CFUcount (48x102cfu/g), followed by the Total Market47 x 102 CFUample (47x102cfu/g), and the Nnewi market sample showing the lowest fungal count (45x102cf Based on

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their colonial and physical traits, the fungus responsible for the fruit deterioration was identified. The investigation also revealed that a total of 5 isolates of fungus from fruits, including *Aspergillus* spp., *Penicillium* spp., *Rhizopus* sp., *Fusarium* spp., and Candida spp., were collected. Of them, isolates of *Aspergillus niger* species were found most commonly (36%). *Rhizopus* species and Candida were next, each with an infection incidence of 18%, while *Fusarium solani* and *Penicillium digitatum* were the least common (141%). *Aspergillus niger,* one of the identified fungi, was the least harmful and produced the least amount of fruit rot. *R. stolonifer*, *Fusarium solani*, *Candida tropicalis*, and *Penicillium digitatum* were the least pathogenic and caused the most fast disintegration of treated fruits in 3-5 days. This study has demonstrated that fungus is to blame for fruit rotting. This study has demonstrated that fungus is to blame for fruit rotting. Producing, preparing, and preserving foods like fruit salads must be done as quickly and hygienically as possible using high-quality tools, products, and materials since fruits are typically infested by harmful fungus.

Keywords: Isolation; identification; fungi; avocado pear (Persea americana Mill).

1. INTRODUCTION

Southern Mexico and Central America are the original homes of the avocado (*Persea americana*). It is estimated to have first emerged around 12,000 years ago based on archaeological data discovered at Tehuacán, Puebla (Mexico) [1]. The *P. americana* fruit, which contains nine necessary amino acids in an uneven proportion, is a complete diet on its own [2]. To maintain commercially acceptable cultivars, avocado is reproduced clonally by grafting, like many tree crops. Avocado consumption has increased dramatically around the world, and it is now a significant fruit traded on the global market [3]. Depending on the cultivar, *Persea americana* fruits range in color from purple to green and have smooth or warty skin. Fruit flesh has the consistency of butter and is colored yellowish-green. There is a single big seed per fruit. *P. americana* trees planted from seed can start bearing fruit after 4–6 years, whereas grafted plants bear fruit after just one year. Because they deal with the genetic makeup of organisms, molecular-based technologies are the most trustworthy methods for describing microbes [4-9]. Molecular technologies have made it feasible to gather in-depth data on assessments of systems affected by climate change, including those involving food, agriculture, industrial settings, and environmental sciences [9-12]. It has been created to employ highly conserved oligonucleotide primers for amplification of the internal transcribed spacer (ITS) region for fungus. Therefore, the objective of this study was to isolate and identify the fungi that are connected to avocado fruits using samples from spoiled avocado pears [13-16].

It is apparent that growers and market vendors in many nations confront a serious problem as a result of fungal infections on avocados. In Nigeria and other countries throughout the world, several studies on the isolation and identification of fungi connected to avocado pears have been published. In Awka, Anambra State, Nigeria, there is a lack of information on the avocado fungal infection situation. To identify the danger in the prevention and management of infections, it is crucial to know the type of fungal parasite that affects avocados in a certain location [17,18].

Using samples of spoiled avocado pears, the study aims to isolate and identify fungal species linked to avocado fruits.

In addition to shedding information on potential fungi, the study of the isolation and identification of fungi associated with avocado seeds aids in the detection and management of such parasites [19,20]. Fungi parasites must be collected, separated into distinct species, analyzed using an appropriate test technique, identified, and the prevalence of those detected parasites must be known in order for this study to be justified.

The study is exclusively focused on isolating and characterizing the fungus connected to avocado pears or seeds in the study region.

1.1 Distribution and History

The avocado tree (*Persea americana*), a polymorphic species, is native to a large geographic area that stretches from Guatemala's eastern and central highlands to Central America's Pacific coast. Over the course of millennia, three different and unique taxa or subspecies, currently known as the Guatemalan, Mexican, and West Indian or Antillean races, have been chosen. It is assumed that the first

avocado trees in South Africa were West Indian race-seedlings planted on the coastal strip surrounding Durban in the late 19th century, despite the fact that nothing is known or written about their arrival. Only until the middle of the 20th century were budded trees of Mexican, Guatemalan, and hybrid provenance brought from California, which were better suited to South Africa's climatic conditions. The fruits from these trees were inferior in terms of storability. Avocados are now widely available across South Africa, but production is concentrated in the northern and northeastern provinces of Limpopo and Mpumalanga, as well as, to a lesser degree, in the frost-free lowland coastal belts and colder midlands of KwaZulu Natal. Production of avocados in South Africa is mostly concentrated on two cultivars, Fuerte and Hass, and quantities have risen more than 11-fold, from 4700 to 53,800 metric tons export-based per year, between 1961 and 1996. In South Africa, there are now 12400 ha of avocado trees planted, and there are currently about 3015000 trees producing fruit. This could result in more than 50,000 tons of avocados, with 36,000 tons (9 million cartons) destined for export.

1.2 Nutritional and Physicochemical Properties of Avocado

In northern South America, Central America, and Mexico, avocado is eaten in a variety of ways, including as a puree salad that is seasoned with salt, pepper, vinegar, and other seasonings as well as being used to make other recipes. In Brazil, ripe fruit is more valued than unripe fruit, along with sugar, honey, and liqueurs [21-24]. The sensory and nutritional qualities of the fruit have the most impact on how much is consumed.

Several cultivars have pulp contents that range from 52.9% to 81.3% of the fruit mass. After the water is removed, the avocado pulp still contains high quantities of lipids and low levels of carbohydrates, giving the product a high dry matter content. Consequently, it is regarded as one of the few cultivated fruits that primarily contains a lipid fraction, which can make up to 25% of the fruit portion.

The avocado pulp has an energy density between 140 and 228 kcal and includes between 67 and 78% moisture, 13.5 to 24% lipids, 0.8 and 4.8% carbohydrates, 1.0 and 3.0% protein, and 0.8 to 1.5% ash. With the exception of bananas, avocados have four times the nutritional content of all other fruits combined. It contains proteins (1–3%), considerable amounts of folic acid, and significant amounts of calcium, potassium, magnesium, salt, phosphorus, sulfur, silicon, and vitamins E, B1, B2, and D.

When compared to other fruits, the fruit distinguishes itself for having a higher potassium content (339 mg/100 g), which controls muscular activity and shields the body from cardiovascular disorders. Additionally, it serves as a source of glutathione, a potent antioxidant that combats possibly cancer-causing substances.

1.3 Avocado Bioactive Compounds

Avocados contain significant amounts of bioactive substances, including phytosterols, particularly in the lipid fraction, with -sitosterol serving as the primary representative. Consuming foods high in phytosterols can lower total cholesterol and LDL cholesterol. In research conducted in Mexico with 45 participants who had one avocado per day for one week, blood cholesterol levels dropped on average by 17%.

A molecule of vegetable origin called phytosterol has a structure that is strikingly similar to that of cholesterol. Inhibiting intestinal cholesterol absorption and reducing hepatic cholesterol production are two aspects of its mode of action in the body. It affects total plasma cholesterol and LDL cholesterol without changing HDL or blood triglycerides. The advantage of lowering cholesterol also results from switching from saturated to unsaturated fats, which encourages a decline in levels of total cholesterol and LDL and a rise in levels of HDL.

Furthermore, the -sitosterol in avocados has a unique effect on immunity, aiding in the treatment of diseases such as cancer, HIV, and infections. It combats cancer by reducing carcinogenesis, and it combats HIV by boosting the immune system. This substance promotes the growth of lymphocytes and the activity of natural killer cells, which neutralize invasive pathogens. Additionally, research has demonstrated that sitosterol activity promotes weight reduction by minimizing binges and the formation of belly fat.

Numerous studies have looked into how sterols and stanols affect health. Consuming 2 g of these compounds daily, which are included in the formulations of margarines, spreads, and vegetable oils via etherification without compromising vitamin solubility, has been shown

by some authors to reduce the risk of coronary heart disease by 25%.

Margarida avocado oil contains more sterols and less cholesterol (0.3% vs. up to 2.3% in other types), with -sitosterol accounting for 71.8% of total sterols. Investigation of the Fortuna avocado oil, which was extracted using petroleum ether and dried under forced air (40°C), revealed that 87.6% of the total phytosterols were sitosterol, 12.41% campesterol, and 0.04% stigmasterol. Lutein, another carotenoid found in avocados, aids in the prevention of prostate cancer as well as eye conditions including cataracts and macular degeneration.

1.4 Avocado Oil

The pulp of the avocado gets the most attention since it has a high lipid content. Lipids range from 5 to 35%, and the majority of them (60–84%) are unsaturated fatty acids. Due to increased pulp output, avocado types with lower core and shell percentages are particularly intriguing for oil extraction. Quintal stands out among these kinds.

The biggest barrier to extracting avocado oil is the high moisture content of fresh pulp, which has an impact on the extraction yield and cost of manufacturing. Hass, Fuerte, and Glória are the cultivars most ideal for oil extraction since they contain 18% lipids and have low moisture levels in the pulp, followed by Collinson, Anaheim, Itzamna, Wagner, Ouro Verde, Carlsbad, and Mayapan.

Solvent extraction has taken over the role of the conventional cold pressing technique for vegetable oils. Although some writers claimed that oil extraction from fleshy pulp could produce 59% when using hexane as the solvent, this figure fell to 12% when acetone was used instead. However, the cake and the extracted oil's hexane residue might be dangerous.

As a function of the drying procedure (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 extraction yield of Fortuna avocado oil was examined. The oil output from the freeze-drying approach was higher than that from oven drying with forced air, according to the authors, who recorded oil levels of between 25 and 33% by cold pressing and between 45 and 57% by solvent extraction. The ecologically friendly alternative extraction method is called enzyme-assisted aqueous extraction.

The pharmaceutical and cosmetics industries make use of the small amount of avocado oil that certain countries now produce in raw form after its unsaponifiable portion is responsible for the healing properties of the epidermis. The cosmetics industry benefits greatly from avocado oil's high absorption capacity of scents and ease of skin absorption. Additionally, it readily creates an emulsion, making it perfect for the production of delicate soaps. In contrast to other vegetable oils, avocado oil is distinguished by having comparatively high quantities of saturated fatty acids, low levels of linoleic acid, and high levels of the monounsaturated fatty acids oleic and palmitoleic acids (palmitic and stearic acids). The cultivars, stage of maturation, anatomical area of the fruit, and growing environment all have an impact on this fatty acid content.

Monounsaturated fatty acids (MFA), which make up 59 to 72% of total fatty acids in avocado oil from the varieties Wagner, Fortuna, Hass, and Fuerte, are present in higher concentrations than saturated fatty acids (SFA), which make up 17 to 23% of total fatty acids, and polyunsaturated fatty acids (PUFA), which are present in lower concentrations and range between 10 and 14%.

Arackal and Parameshwari [25] assessed the impact of the pulp drying procedure (freeze-drying or air circulation: 40 and 70°C) and oil extraction technique to evaluate the fatty acid profile of the Fortuna avocado (solvent or pressing). According to the scientists, oleic fatty acids made up more than half of the raw material's total fatty acids, along with significant levels of unsaturated linoleic and palmitoleic acids. Since the oil produced from the lyophilized pulp included larger quantities of unsaturated fatty acids, they also confirmed that the dehydration of the pulp may influence the fatty acid profile. No discernible impacts of the extraction technique were seen. While the oils from the varieties Rincon, Barker, Waldin, Prince, and Panchoy showed less than 50% of this fatty acid, the oils from the variants Northrop, Duke, Wagner, Quintal, and Fuerte showed more than 63% of this fatty acid. The percentage of palmitic acid in various oils ranged from 15.38% to 32.37%. Palmitic acid and oleic acid levels are therefore influenced by the avocado variety; historically, types with high oleic acid levels had low palmitic acid levels, and vice versa. These oils include additional bioactive minor components, such as tocopherols, squalene, b-sitosterol, campesterol, and cycloartenol acetate, which have good impacts on health in addition to their fatty acid content. Along with the option of

substituting pure avocado oil for olive oil, combining olive oil and avocado oil to displace the typical internal market offerings of olive oil combinations, which mostly use soybean oil, is a viable alternative to lowering the price of Brazilian olive oil imports. To remove the saturated triglycerides that can cloud the oil stored at low temperatures and make it cloudy, avocado oil for salad dressings should be put through winterization.

Despite the fact that the lipid extraction process leads to a significant buildup of pulp residues in the processing industries, the high fiber content of this by-product makes it possible to use it to prepare flour for use in bakery products like cookies, breads, and pasta, increasing the supply of fiber-rich products.

Paste, puree, and guacamole are some of the processed avocado pulp products. Guacamole is a fruit pulp that is seasoned with salt, onion, lemon, pepper, and tomato. Some US companies also offer this condiment. According to the type of packaging utilized, the sensory quality of Hass variety guacamole manufactured without chemical additions and refrigerated was assessed. Comparing the product stored in a container with a gas barrier to that stored in polyethylene packaging, a higher level of customer approval was noted. Although these authors considered the possibility that the heat treatment was beneficial in inactivating polyphenol oxidase, it can also cause bitterness and off flavors to develop in the avocado, altering the texture of the guacamole and making it appear mashed. In order to partially substitute wheat flour and butter, respectively, in whole grain crackers, tested avocado pulp of the Margarida type that had been dried and defatted by cold pressing, as well as avocado oil. According to the scientists, the flour made from avocado pulp often exhibited characteristics that were comparable to those of regular flour and whole wheat flour. The biscuits were well-tolerated by the senses and included increased quantities of fiber and minerals. Avocados and other foods with high vegetable oil content are vulnerable to oxidation, which can lead to rancidity, which can produce unpleasant tastes and cause products to lose quality while stored.

To produce a stable avocado pulp, a number of preservation techniques, including pasteurization, drying, oil extraction, freezing, and freeze-drying, have been investigated. The use of copper chloride and microwave cooking to maintain the color of mashed avocado has also been researched. Additionally, research has been done on high hydrostatic pressure treatment, chemical reducing agents, sequestrants, acids, nitrogen atmospheres, and vacuums.

The underused avocado seed, which makes up a sizable amount of the fruit, can be used as an alternative to lower the price of producing edible oil. However, the presence of harmful phenolic chemicals is the primary issue with using avocado seeds. Studies have indicated that when the chemicals are extracted using ethanol, the seeds may be utilized as feed for monogastric animals. Given that seeds' phenolic content ranges from 2.3% to 5.7%, the extract may exhibit antioxidant action. There are various non-nitrogenous compounds contained in seeds, ranging from 5.1 to 13.2%, in addition to the starch and fiber.

Due to their diuretic qualities, avocado leaves are a common pharmaceutical element in extracts used for medicinal purposes as well as in folk medicine.

Avocado leaves include phytochemicals such as orhamnetin, luteolin, rutin, quercetin, and apigenin that can slow the progression of a number of illnesses caused by oxidative stress.

Yield and quality are the ultimate indicators of a tree's performance. There are several variables that affect avocado tree yield averages, including cultivar, rootstock, environmental variables, tree size, form, and age. The harvest index, or the ratio of photosynthate to fruit, is a measure of seasonal photosynthetic efficiency that ultimately determines production.

Therefore, the impact of Phytophthora root rot on the formation and storage of photosynthate is crucial. Effects of Phytophthora cinnamomi on the body: Avocado tree rashes are quite bad, and afflicted plants have lower water potential, fewer stomatal openings, and less ability to absorb nutrients and water. [26]. Stomata close even when leaves are not under water stress, according to reports by, as long as plant roots are under stress. P. cinnamomi causes severe root mortality in avocado roots under ideal conditions for fungal development, which reduces the plant's ability to absorb water and nutrients. As a result, photosynthesis declines, which lowers the amount of carbon that is partitioned to fruit [27].

A soil-borne pseudofungus of the class Oomycetes in the kingdom Chromista is called

Phytophthora cinnamomi [28]. It is the most significant and harmful disease to affect avocados globally [27]. From young nursery trees to big bearing trees, it affects them all and kills them by damaging the delicate feeder roots. Free soil water encourages the fungus's reproduction, development, and dissemination. As a result, the mobility of contaminated soil is crucial to the propagation of this fungus [28]. P. cinnamomi was originally identified in 1929 on 10 avocado trees in Puerto Rico, where it produced severe root rot. Rands first identified it in 1922 as the causative organism of a cinnamon tree stem canker in Sumatra [29]. Over 1000 plant species have been found to harbor it [30], and hosts include blue-gums, chestnuts, pineapples, macadamias, peaches, kiwi fruit, peaches, pears, and many native Australian and South African plants [27]. Arentz and Simpson [31] and have proposed that the fungus originated in Papua New Guinea and spread to other tropical and subtropical areas of the world as a result of human activity. The major barrier to productive avocado production in nations like Australia, South Africa, and the USA has been Phytophthora root rot. The disease is thought to be responsible for up to 70% of commercial orchard losses in the US, which amounts to a US$30 billion yearly loss [32]. R45,000,000 has been lost in South Africa as a result of avocado tree Phytophthora root rot. Fine feeder roots decay due to Phytophthora cinnamomi, killing host plants [33]. Larger root invasion has also been documented [33,27], and it may result in the development of brown lesions in the wood. Peeling of the bark or a weeping canker at the base of the tree, below the soil line, that may extend one meter up the trunk are possible symptoms of this [27]. However, the infection mostly affects the tiny feeder roots, which become black and brittle before dying off. Under trees with severe root rot, feeder roots could be hard to locate. Due to the trees' inability to absorb rainwater due to the lack of feeder roots, the soil beneath such trees tends to stay damp [27]. Depending on the degree of the root rot, the foliage becomes wilted and chlorotic, the leaves fall, and the branches quickly wither. There is very little new leaf growth, and any leaves that do appear are tiny and light green. In trees with root rot, fruit set is typically constrained, and fruit is typically tiny. Unnatural nutrient distribution in plant tissue and obstructions to nutrient absorption can also cause visible symptoms in the tree. Chloride builds up in leaves and may reach dangerous levels because roots are unable to regulate salt intake, leading to the burning of leaf edges and tips. According to Labanauskas et al. [34], Phytophthora infection changes how nutrients are distributed throughout plant sections [35].

Avocado trees frequently exhibit a modest tolerance without a decline in aerial tree health [36]. However, root rot-afflicted plants might show reduced photosynthesis, transpiration, and stomatal conductance before these obvious aerial signs develop [26,35].

1.5 Cycle of a Disease and Epidemiology

According to Zentmyer et al. avocado Phytophthora root rot is more severe and develops more quickly in soils with inadequate drainage. Particularly in warm, wet, well-aerated soils and if feeder roots are abundant, the disease has a rapid generation time and a high reproductive capacity, and inoculum can develop from low, frequently undetectable levels to high levels within days [30]. Due to enhanced sporangial production and favorable circumstances for zoospore release, motility, and transport to feeder roots, high soil moisture worsens infection. Oospore generation can take place in less than 48 hours, which explains why epidemics see fast colonization [37]. They are delicate and transit through soils for just minutes to hours at a time, depending on energy supplies and other ecosystem-affecting conditions [38]. Chlamydospores may endure for long periods of time in soil and root waste. At soil temperatures reaching 15 °C, they begin to germinate by generating a number of germ tubes. Oospores are uncommon, and even though they could live for a long time, they most likely don't play a significant part in the progression of illness [30].

2. MATERIALS AND METHODS

2.1 Sample Collection

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

2.2 Fungal Isolation

2.2.1 Culture media

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

medium, and Sabouraud Dextrose Agar (SDA), which is a modification of Dextrose Agar.

2.2.2 PDA media preparation

39 g of the medium were suspended in one liter of distilled water, heated over a Bunsen flame while being stirred frequently, and allowed to boil for one minute to thoroughly dissolve the medium and its contents. The solution was autoclaved for 15 minutes at a temperature of 1210°C and one atmosphere of pressure (15 psi). Allow ten minutes to cool after removing from the autoclave.To act as antibiotics, 500 mg of streptomycin sulfate were added to the molten solution.

2.2.3 SDA media preparation

65 g of the medium were suspended and dissolved in 1 liter of distilled water by heating to boiling and stirring frequently. It was heated for one minute to dissolve the solution, and then sterilized for 15 minutes at 1210 C in an autoclave. After that, while the solution was still molten, 500 mg of the antibiotic streptomycin was added.

2.3 Isolation of Fungi from Samples

In this work, the isolation method from Onuh et al., [24] was used. 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. A small section of infected avocado pear fruit containing the advancing margin of rot and adjoining healthy tissue was cut using a sterilized scalpel and cork borer. In a laminar airflow cabinet, the tuber pieces were dried by blotting with sterile filter paper. Four portions of each cut sample were individually inoculated (90°apart) on solidified potato dextrose agar (PDA) and sabouard dextrose agar (SDA) plates using sterile forceps. For each sample, two replicas were created. For 72 hours, the plates were kept in an incubator at a temperature of 28 to 30 °C. There were fungi found that were connected to avocado and pear fruit deterioration.

2.4 Fungus Identification

To create a pure culture, isolated fungi were further subcultured. According to Marthur and Kongsdal [39], identification was then completed based on colony traits, morphology, and microscopic features. Using morphological traits and matching the results to established keys as given by Nwachukwu and Osuji [40], fungi were identified. Each isolate was examined using a colony and a microscope, and their morphological characteristics were noted and documented. Based on growth patterns, mycelia color, and microscopic investigations of vegetative and reproductive structures, morphological traits were explored. A little piece of mycelia was taken from the area between the colony's center and edge using a sterile inoculating needle, and it was then put on a spotless microscopic slide with cotton blue lactophenol. The mycelia were evenly spread across the slide using the sterile needle and a cover slip that was gently and slightly pressed to remove air bubbles. The slide was heated by the steam from some boiling water in order to better preserve the fungal formations on it. Using sterile blotting paper, the cover slip's excess lactophenol was removed from the margins. The microscope's slide was attached, and the 10 and 40 objectives were used to view it.

2.5 Determination of Fungal Frequency (%)

Fungal frequency will be determined location-wise as well as culture- and 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 fungus frequency percentage determination:

$$Fungal\ Frequency\ (\%) = \frac{Number\ of\ specific\ fungus\ colonies\ observed\ in\ plates\ multiplied\ by\ 100}{Total\ number\ of\ colonies\ of\ all\ fungi}$$

2.6 Pathogenicity Test

For the pathogenicity test, Ogbo and Agu's [41] methodology will be used. Nine wholesome avocado pears were thoroughly cleaned with tap water before being rinsed with distilled water. The corms' surfaces were then cleaned with 75% ethanol. The degree of rot is determined by both the size of the infection and the rate at which the rot-causing fungus becomes pathogenic. The ultimate weight of each yam tuber was measured after the rotten sections of the complete tubers were removed. Clean polyethylene bags were used to contain the unvaccinated controls. The severity of spoiling percentage (Sr%) was computed as follows:

$$Sr\ \% = \frac{FW - wx100}{w}$$

where,

FW denotes the final weight of infected yam tuber.
w denotes the weight of the rotted tuber portion.

2.7 Statistical Analysis

Fungal colony percentages and means were computed. When significant at the 5% level of probability, the data were subjected to Analysis of Variance (ANOVA), and the Duncan Multiple Range Test (DMRT) was used to differentiate the treatment means.

3. RESULTS

3.1 Isolation of Spoilage Fungi

At the conclusion of the method required for the isolation and identification of fungi linked to the spoiling of avocado and pear fruit, several colonies were seen. Fruits began to deteriorate because of the fungal colonies that ruined them. When the fungi were originally isolated on potato dextrose and SDA agar, mixed colonies were produced. After each colony of the spoilage fungus was subcultured on freshly produced media, pure cultures of the fungi were seen.

Colony-forming unit per gram (cfu/g) measurements were used to determine the type of fungal growth and the total number of fungi, as indicated in Tables 1 and 2.

Using a colony counter and Table 2, the total number of fungi found in the fungal growth on PDA and SDA agar medium was calculated in colony forming units per gram (cfu/g). According to the results, PDA is a better substrate for isolating spoilage fungi from avocado and pear as it supports higher growth than SDA. The fungal count of the avocado and pear samples taken from the three different locations did not differ significantly ($p>0.005$), with the sample taken from Eke Awka market having the highest fungal count (48x102cfu/g), followed by the sample taken from Total Market Ihiala (47x102cfu/g), and the sample taken from Nnewi market having the lowest fungal count (45x102cfu/g).

Table 1. 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 ($p < 0.05$)*

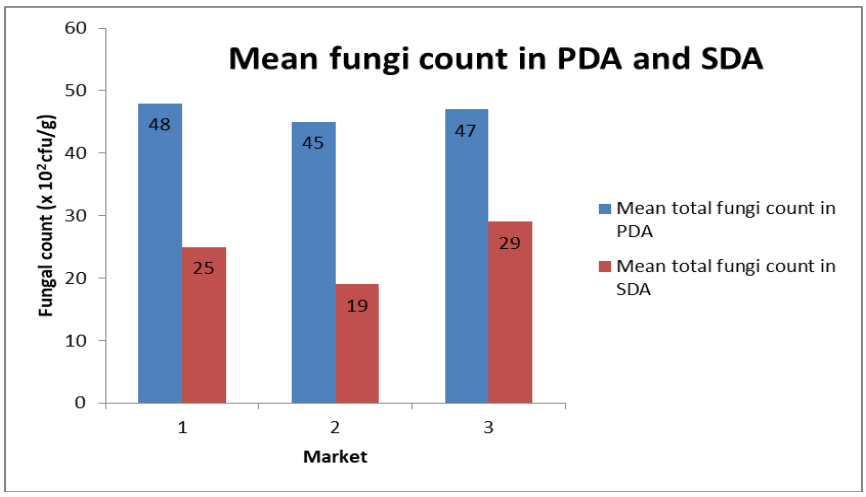

Chart 1. Mean fungi count in PDA and SDA for spoilt avocado pear fruit samples

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

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

When examined physically, the infected avocado pear fruits had reddish, necrotic spots on their skin. It took 7 days for the avocado pear fruit patches to become black. It was also noted that the fruits' surface was covered in a mass of mycelia. The colonial, morphological, and cellular characteristics of the isolated fungus are displayed in Table 3.

Table 4 displays the frequency of occurrence of fungus isolates linked to fruit deterioration. It revealed that a total of five isolates of fungi—Aspergillus spp., Penicillium spp., Rhizopus sp., Fusarium spp., and Candida spp.—were taken from fruits. Of them, isolates of Aspergillus niger species were found most commonly (36%). Rhizopus species and candida were the next most common pathogens, each with an infection rate of 18%, while *Penicillium digitatum* and *Fusarium solani* were the least common (141%), as indicated in Table 4.

3.2 Pathogenicity Test

On every fruit tested, every fungal isolate was shown to be harmful. When the fruits were submitted to identification processes by looking at their morphological, colony, and cellular properties, the rot symptoms produced were comparable to those previously seen on the fruits. The molds that were seen were identical to the isolated fungus on spoiled fresh fruit. After infection, the fruits took on a little color change and softened, making it simple to pierce them with a finger at the injection site. According to the pathogenicity test, each fruit that was infected produced the original organism that led to the fruit's deterioration. *Aspergillus niger*, one of the identified fungi, was the most harmful and caused the fast disintegration of treated fruits in 3-5 days. *R. stolonifer*, *Candida tropicalis*, *Penicillium digitatum,* and *Fusarium solani* were the least harmful, and they caused the least amount of fruit rot.

The pathogenicity test results are displayed in the table above for each of the infected fruits, which helped to pinpoint the original microorganism that caused the fruit to deteriorate. One of the discovered fungi, *Aspergillus niger*, was the least dangerous and caused the least amount of fruit rot. *R. stolonifer*, *Fusarium solani*, *Candida tropicalis*, and *Penicillium digitatum* generated the fastest disintegration of treated fruits in 3-5 days while being the least harmful.

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)

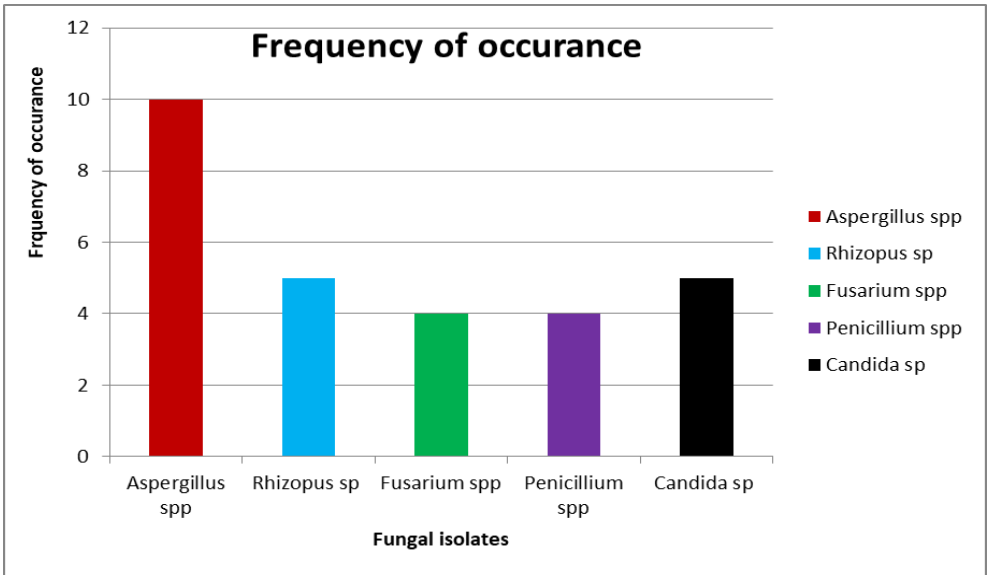

Chart 2. The frequency of occurrence of fungi isolates

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

4. DISCUSSION

Fruits and vegetables have reportedly suffered significant degradation due to microbes, particularly fungal ones. Some of these microorganisms cause the fruits to rot, turn brown, or ferment, which affects how long they can be preserved. This study shows that several fungi attack avocado and pear fruits, leading to degradation. In the current investigation, a large number of yeast and filamentous fungus were isolated from avocado pears that were procured from several markets in the Anambra State cities of Awka, Nnewi, and Ihiala.

According to the research's findings, major marketplaces in the study region sold fruits that included *Aspergillus* spp., *Penicillium* spp., *Rhizopus* sp., *Fusarium* spp., and *Candida* spp. According to reports, several infections have been found in Nigerian pawpaw fruits. All five of the isolated organisms were shown to be harmful in varying degrees to the fruits. According to the results, *Aspergillus niger* was the least pathogenic and caused the least amount of fruit rot of all the isolated fungi, while *R. stolonifer, Fusarium solani, Candida tropicalis,* and *Penicillium digitatum* were the most pathogenic and caused the most rapid disintegration of treated fruits in 3-5 days. The distinctive

symptoms that were first noted reappeared when these isolates were aseptically injected into healthy, susceptible fruits. All five species effectively participated in the deterioration, confirming that they are the cause of fruit rot.

It has been noted that the fungi identified in this investigation create secondary metabolites in plant tissues. These metabolites might be toxic to both people and animals. A great example is aflatoxin, which has been linked to human acute hepatitis, aflatoxicosis, and liver cancer (heptatoma), particularly in impoverished countries. On the other hand, pathogenic fungus could result in allergies or infections. Because Aspergillus spp. are known to produce a number of toxic metabolites, including malformins and naphthopyrones, as well as the mycotoxin ochratoxin A (OTA), which poses a risk to both human and animal health, extra caution should be exercised when handling these fruits by workers during harvest, cleaning, sorting, packaging, and transport.

All of the spoiled fruits tested positive for *Aspergillus* species. Many fruit-rotting fungi have been isolated and identified from different regions. *A. niger* is a fungus that frequently affects tomatoes, apples, and grapes. Black mold *A. niger* was responsible for post-harvest spoiling of sweet orange and acid lime in the field.

All foods naturally include microorganisms, and they can also be introduced by external factors such as wind, soil, water, insects, animals, and human touch. They may get polluted when the raw materials are being grown, harvested, transported, or processed into finished goods. The farmer who gathers the fruits into bags for shipment, the marketers, and the customers must thus take the required and suitable procedures to prevent contamination and the consumption of tainted fruits. However, this will lessen the possibility of mycotoxins linked to fungal contamination that are harmful to human health. The discovery of these pathogens supported that the pathogens *Rhizopus* spp. and *A. niger* discovered associated with avocado fruits are highly pathogenic and cause significant disease. Along with other pathogens, *Fusarium* sp., *A. flavus*, and *Rhizopus* sp. were also isolated.

It's possible that improper handling procedures in the food supply chain, storage conditions, distribution, marketing strategies, and transportation led to the fungi's contamination of avocado fruits [42]. Fruit is improperly handled and transported after harvest. Due to the nature of the transportation infrastructures that exist in rural regions, the majority of the fruits that are picked typically do not reach the larger cities in time. Although fruit with bruising is not separated from fruit without bruising, creating cross-infections, consumers are typically provided with partially rotting fruits. This suggests that consumers face a significant risk of aflatoxin and other mycotoxins. Aflatoxin M1 was found in the urine of the Philippine women who had eaten peanut butter containing aflatoxin, according to research by Sage et al., published in 2002.

Fruits that have been spoiled by fungus are known to pose a risk to both human and animal health. This is because they produce mycotoxins, which are naturally occurring dangerous chemicals, sometimes with an aromatic structure, and which can cause mycotoxicoses in people when ingested or inhaled. They differ in their degree and manner of toxicity [43,42].

5. CONCLUSION

This study identified the pathogenic fungal profile that caused several native fruits, such as avocados, in Abuja to spoil. It also demonstrated that many fruits were damaged by fungus. Mechanical wounds like cuts or bruises sustained during or after harvesting, grading, or packaging might serve as infection sites for microorganisms that cause deterioration. However, the following procedures can be used to prevent fruit deterioration: Using clean or pure water to wash the gathered fruit; properly cleaning and sanitizing the warehouses; sanitizing the packing and transit containers; and treating the fruit properly during harvest to avoid bruising, scars, or other mechanical damage; fungicide usage; and refrigerated storage to reduce storage temperatures and prevent the growth of fungus. As a result, it is critical that the farmer, who collects the fruits and places them in bags for transportation, as well as the marketers and consumers, take the necessary precautions to prevent fruit contamination and consumption. However, this will help to lower the likelihood that these study-isolated fungi would develop aflatoxin and other mycotoxins that are harmful to human health.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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