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FUNGI ASSOCIATED WITH SPOILAGE OF PINEAPPLE (*Ananas comosus L*) FRUIT SOLD IN ANAMBRA STATE, NIGERIA

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author ECF designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors OOJ managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The edible fruit of the tropical plant known as Pineapple (*Ananas comosus*) is commonly grown in Nigeria. Its high sugar content and low pH values make it susceptible to fungi. This study aims to identify those that can cause the spoilage of the fruit in the state. Five markets in Anambra state were visited to collect samples of pineapple (Eke Awka, Nnamdi Azikiwe temporary site (Temp. site), Nnewi, Uli, and Ihiala). They were then transported to a research facility in Awka, where they were studied for the fungi that cause the fruit's spoilage. The samples were treated with the standard procedure. The culture media used for the study were potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA). Both were placed into a conical flask and subjected to an autoclave at a rate of 121ºC at 15psi for 20 minutes. In the study, various fungi that can cause the fruit's spoilage were identified, which included the *Fusarium sp*, *Mucor sp*, *Aspergillus sp* and *Penicillium sp*. Samples from Ihiala had the highest fungal counts (57 x 102 $\pm$ 0.017a cfu/g) on PDA while samples from Temp. site had the highest fungal count (99 x 102 $\pm$ 1.112a cfu/g) on SDA. Also, samples from Uli had the lowest fungal counts (22.00 x102$\pm$ 0.111d cfu/g) on PDA while samples from Nnewi had the highest fungal count (36.00 x102$\pm$ 0.100e cfu/g) on SDA. *Aspergillus sp* (47.91%) had the highest frequency of occurrence from the fungi isolated while *Fusarium sp* (10.42%) had the least occurrence. From the study, *Aspergillus sp* is the fungi specie that is responsible for the spoilage of pineapple fruits in the locations studied in Anambra State although other species of fungi can also affect pineapple fruits.

Keywords: Pineapple; Fungi; nutrition; media.

1. INTRODUCTION

One of the most common vegetatively grown fruits is the pineapple (*Ananas comosus*) which is a semi-fruited pseudo-fruit of the Bromeliaceous family. It has a thick, fleshy, and edible outer surface and a very juicy axis core. This fruit is also known for its inedible, warty skin. The outer surface of the various fruits is only visible on the surface of the syncarp. The central cylinder of the false fruit is also inedible. This axis core is located in the middle portion of the fruit, and it has a thick, woody outer surface [1]. The high

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levels of nutrients and sugars found in the pineapple make it ideal for fungi to thrive. It also helps in the growth and development of pathogens by providing them with vital nutrients and carbohydrates. Unfortunately, the fruit can also be infected by various diseases. These diseases usually appear during the fruit nutrition stage, which is around 20 days before the fruit is harvested. They can then develop and damage the fruit, rendering it unfit for consumption. Black spots on the fruit are believed to be caused by various microorganisms such as the *Penicillium*, *Fusarium*, and Yeasts [2].

In developing countries, such as Nigeria, inadequate transportation and storage facilities can lead to severe post-harvest losses [3]. Pineapple infections can occur during the growing season, handling, and transport of the fruit. Another common source of food contamination is washing water [2]. This process occurs when fungi develop a structure called an appressorium, which allows them to establish themselves inside the host. Once they establish themselves, they can then depolymerize certain cell wall materials used in the cementing of the fruit. The primary cell wall of pineapple is composed of around 90% polysaccharides, 10% proteins, and hemicellulose. It can be divided into three main groups: pectin, hemicellulose, and cellulose. Degradable enzymes can be produced by fungi to break down the cell walls and use them as nutrients [4].

These fungi can also produce an abundant supply of hemicellulases and pectinases, which are known to contribute to the development of soft rot and reduced post-harvest life of pineapples. In addition, they can also produce mycotoxins, which are toxic to humans and animals [5]. The increasing consumption of pineapples in Nigeria has been linked to the country's food-borne illness epidemic. This is because the fruit is cheap and nutritious. It is also easily accessible by street vendors. Despite the lack of training in food hygiene, these individuals still sell the fruit. Aside from being sold on the street, the fruits are also commonly displayed in baskets and benches, which expose them to further food-borne infections [6,7,4].

This study aims to identify the fungi associated with the spoilage of pineapple fruits in a different location in Anambra state, Nigeria.

2. MATERIALS AND METHODS

2.1 Sample Collection

The pineapple samples were purchased from five (5) markets in Anambra state (Eke Awka, Nnamdi Azikiwe temporary site (Temp. site), Nnewi, Uli and Ihiala). Laboratory and other facilities used in the practical work were obtained from Alpha research laboratory Awka, Anambra state.

2.2 Fungal Isolation

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.

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 a 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. The PDA medium was used to culture the cotyledons and testa.

SDA media preparation: About 65g of the medium was suspended in one litre of distilled water, mixed well, and dissolved by heating to boiling, with frequent agitation. After heating for one minute and dissolving the solution, it was sterilized in an autoclave at 118-121^0C for 15 minutes. This was followed by the addition of 500 mg streptomycin antibiotic while the solution was still in a molten state. If all the solution was not used at that moment, the remainder was stored in the refrigerator at 8 – 150 C until when needed.

2.3 Isolation of Fungi

Spoilt pineapple surfaces were washed with distilled water to enhance the removal of dirt. A small portion of the spoilt area of the fruits was cut in and out using a sterile scalpel and inoculated onto a freshly prepared PDA and SDA agar and incubated at room temperature for three (3) days.

2.4 Sub-culturing Techniques

This was done following the method by Chuku et al., 2017. The resulting colonies were then sub-cultured onto Potato dextrose agar (PDA), process was repeated whenever more than a single colony of fungi was observed in the Petri-dishes until pure cultures were obtained.

2.5 Identification of isolated Fungi

All the various species of fungi isolated were identified, both macroscopic and microscopic features and their various characteristics studied, (i.e) colour, texture, a form of hyphae, form of conidia, presence of conidiophores, shape of conidial heads. The microscopic identification was aided by appropriate taxonomic keys [8,9].

2.6 Determination of Fungal Frequency (%)

The fungal frequency will be determined location-wise, as well as cultural 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:

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

2.7 Statistical Analysis

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

3. RESULTS

The morphological characteristics of the fungal isolates are shown.

Table 1. Morphological characteristics of fungal isolates

S/N	Isolate Code	Colour of Spores	Reverse of the agar	Aerial hypae	Abundance	Growth	Pigmentation
1	SD1	Black	Light green	Powdery, spores embedded	Abundant	Fast	No
2	PD1	White	Cream	Fluffy, raised a little	Abundant	Fast	No
3	SD3	White	Orange	Fluffy raised	Abundant	Fast	No
4	PD2	Light - green	Creamy green	Embedded	Abundant	Fast	Yes

Table 2. Identification of Fungi

S/N	Isolate code	Description	Probable identity
1	SD1	Conidiophores are usually black, and they are produced from a long, broad, and thick-walled foot cell. They have tall conidiophores and radiate heads.	*Aspergillus sp*
2	PD1	The foot cells are usually whitish to olivaceous-buff, and they have an odorous taste. The sporangiophores are short and tall, and the spores have an ellipsoid or subglobose surface.	*Mucor sp.*
3	SD3	These colonies are growing fast and have sparse aerial mycelium, which often becomes felted or peach-colored, and a characteristic scent of lilae.	*Fusarium sp.*
4	PD2	These colonies are fast-growing, and they have a zonate appearance. They are usually light green, and they have reversed colorless conidiophores. They have a smooth walled, pencilli-like structure, and they have an odorous taste and fruit. The conidia subglobose have an ellipsoid surface.	*Penicillium sp.*

Table 3. Mean fungi count in PDA and SDA for spoilt pineapple fruit samples

Samples	Mean total fungi count in PDA (cfu/g)	Mean total fungi count in SDA (cfu/g)
Eke-Awka	$38 \times 10^2 \pm 0.111^c$	$55 \times 10^2 \pm 0.110^c$
Temp. site	$45 \times 10^2 \pm 0.310^b$	$99 \times 10^2 \pm 1.112^a$
Ihiala	$57 \times 10^2 \pm 0.017^a$	$75 \times 10^2 \pm 0.121^{ab}$
Nnewi	$25.00 \times 10^2 \pm 0.027^d$	$36.00 \times 10^2 \pm 0.100^e$
Uli	$22.00 \times 10^2 \pm 0.111^d$	$43.00 \times 10^2 \pm 0.001^d$

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

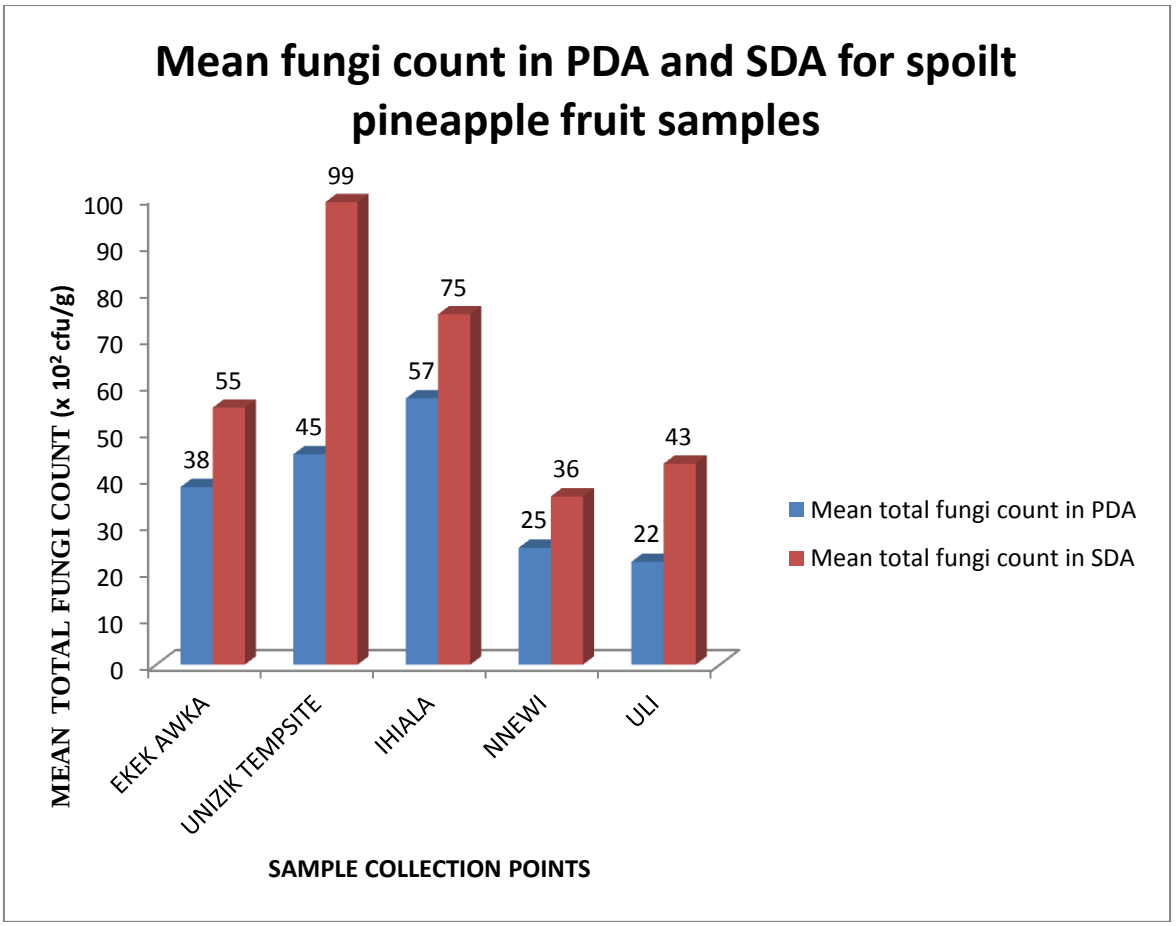

Plate 1. Mean total Fungi Count (x 10^2 cfu/g)

Table 4. Percentage occurrence of fungal isolate

Fungi Isolate	Number isolated	Frequency of occurrences (%)
Aspergillus spp	23	47.91
Mucor spp	8	16.67
Fusarium spp	5	10.42
Penicillium spp	12	25.00
Total	48	100

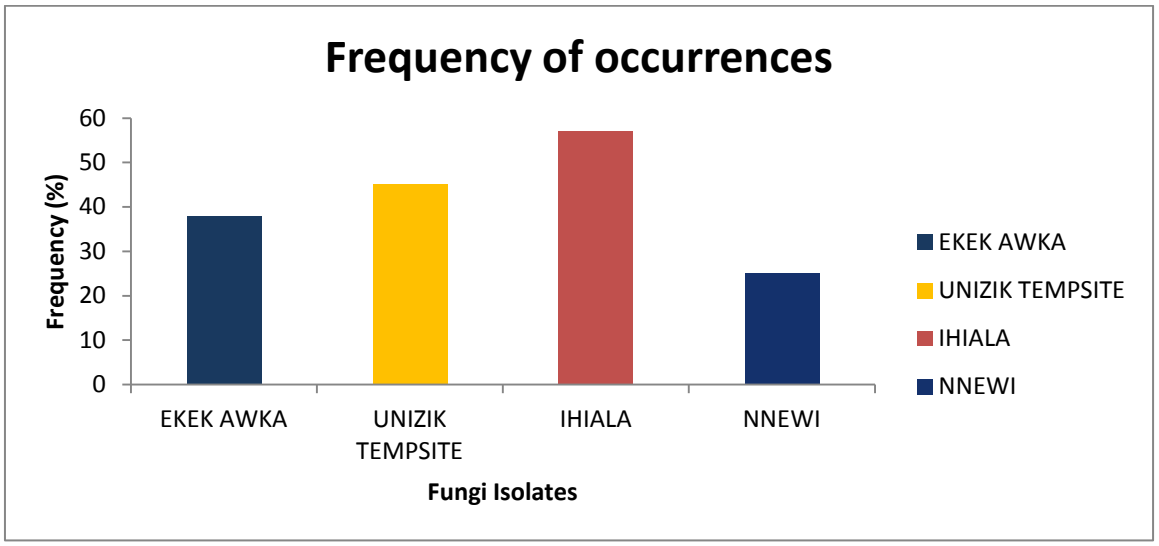

Plate 2. Percentage occurrence of fungal isolate

The percentage occurrence of the fungal isolates is shown in Table 2 respectively. Fungi identified were *Aspergillus sp, Fusarium sp, Mucor sp* and *Penicillium sp*.

Table 3 showed the mean total fungi count (TFC) in the PDA and SDA of each Pineapple sample. Ihiala showed the highest fungal counts ($57 \times 10^2 \pm 0.017^a$) on PDA and Temp. site ($99 \times 10^2 \pm 1.112^a$) on SDA respectively while the least fungal counts were Uli ($22.00 \times 10^2 \pm 0.111^d$) on PDA and Nnewi ($36.00 \times 10^2 \pm 0.100^e$) on SDA respectively. Table 3 above, showed that there were significant differences in fungal.

4. DISCUSSION

In a study conducted in different locations in Anambra state, it was discovered that the fruits samples in these locations were contaminated with a vast number of fungi. The fungal counts ranged from fungal counts ranged from (22, 25, 38, 45 and 57) x102 cfu/g in PDA and (36, 43, 55, 75 and 99) x102 cfu/g in SDA.

The study revealed that the fungi that were responsible for the spoilage included various types of fungi. The study identified the various fungi as *Mucor sp*, *Fusarium sp*, *Penicillium sp*, and *Aspergillus sp*. These fungi can be harmful to the health of people who eat fresh fruits. This is in agreement with other studies; Jolaosho et al., [10], isolated a type of fungi known as Rhizopus stolonifer from a batch of pineapples in Ogun State. In 2011, Ademusire isolated fungi from a batch of pineapples in Maiduguri, which were produced in North-East Nigeria. In 2000, Oyelade and Effiuvwevwere noted that the fungi *Aspergillus sp* were responsible for the rotting of the fruit.

The presence of these microorganisms in the country could be attributed to the fact that they can produce spores that are resistant to high temperatures. In 1984, Emifoniye and Uzuegbu noted that the fungi were also responsible for the spoilage of vegetables and fruits in Nigeria.

The presence of these fungi in fresh pineapple fruits can be a health hazard to consumers. Aside from water contamination, other factors such as the improper handling and processing of the fruit can also contribute to their presence [11-12]. The presence of these fungi in the fruit can also lead to the development of secondary metabolites in the plant tissues. These could be harmful to humans and animals. Pathogenic fungi, on the other hand, can cause allergic reactions and infections.

Aside from being harmful to humans and animals, the presence of fungi in fresh pineapple fruits can also cause food poisoning. *Aspergillus niger* is also known to produce ochratoxins, which can cause food intoxication in humans and other animals. This toxin is very important because it can affect animal health and human health [13-15]. The activity of the *Penicillium sp* fungi can also have a remarkable effect on how much fruit is valued by the food industry. This is in line with the findings of Amusa et al. [16], which stated that the presence of these microorganisms in fresh pineapple fruits can be harmful to the public. It is therefore important that the fruit industry takes measures to prevent the spread of these microorganisms.

5. CONCLUSION

Proper handling and hygiene practices are required to prevent the development of harmful fungi on the pineapple fruits. This issue can lead to waste and unacceptability since the high moisture content of the fruit prevents it from being preserved properly.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Osei-Kofi F, Amoatey HM, Lokko Y. Improvement of pineapple using biotechnology and mutation breeding techniques. In: Report of 2 FAO/IAEA Research coordination meeting on 'invitro techniques for selection of randiation-induced mutant adapted to adverse environmental conditions.' 1997;23-27.
2. Kochhar S. Economic botany in the tropics, Macmillian Press, India. 1994;203.
3. Droby S, Chaluz E, Wilson CL, Nisneiweski M. Characterization of the biocontrol of Debaryomyces hensenii in the control of Penicillium digitatum on gapefruit. Can. J. Microbiol. 2006;35:794-800.
4. Nwachukwu E, Ezeama CF, Ezeanya BN. Microbiology of polythene packaged sliced pineapples sold by street vendors in Nigeria. Afr. Journal Microbiology Res. 2008;2:192-195.
5. Al- Hindi RR, Al-Najada AR, Mohammed SA. Isolation and identification of some fruits spoilage fungi African journal of microbiology Research. 2011;5(4):443-449.
6. Agrois G. Plant pathology (4th edition). Academic press, New York. 1997;63.
7. Debnath P, Dey P, Chanda A, Bhakta T. A survey of pineapple and its medicinal value. Scholar Acad. Journal Pharmacology. 2012; 1(1):143-149.
8. Onuorah SC, Udemezue OI, Uche JC, Okpoli IC. Fungi associated with spoilage of pineapple fruit. The Bioscientist. 2013;1(1):22- 27.
9. Hemalatha R, Anbuselvi S. Physicochemical constituents of pineapple pulp and waste. J. Chem. Pharm. Res. 2013;5(2):240-242.
10. Jolaosho AA, Kareem SO, Ogunmuyiwa SLO, Ajayi JO, Oseifeso OO. microbial analysis of sliced pineapple and paw paw in Ogun State, Nigeria. Journal of Medical and Applied Biosciences. 2010;2:112.
11. Akinmusire OO. Fungi species associated with spoilage of some edible fruits in Maiduguri. Advances in Environmental Biology. 2011; 5(1):157-161.
12. Chuku EC, Ogbonna DN, Onuegbu BA, Adeleke MTV. Comparative Studies on the Fungi and Bio-Chemical Characteristics of Snake Gaurd (*Trichosanthes curcumerina* linn) and Tomato (*Lycopersicum esculentus* mill) in Rivers state, Nigeria. Journal of Applied Sciences. 2008;8(1):168-172.
13. Effiuvwevwere BJO. Microbial Spoilage Agents of Tropical and Assorted fruits and Vegetables (An Illustrated References Book). Paragraphics publishing company, Port Harcourt. 2000;1-39.
14. Uzuegbu JO, Emifoneye AT. Post harvest Fungal Spoilage some Nigeria Fruits and Vegetables. Nigeria Food Journal. 1984;2 (1):153–155.
15. Varge J, Frisvad J, Samson R. A Reappraisal of fungi producing aflatoxins. World Mycotoxin J. 2009;2(3):263-277.
16. Amusa N, Shaye O, Oladapo M, Kafaru O. Preharvest deteriorations of soursop at Ibadan South Western Nigeria and its effect on nutrient composition. Afr. J. Biotechnol. 2003;2:23-25.