



Analysis of Fungal Flora of a Soil Near Biology Laboratory of COOU ULI

Ejimofor Chiamaka Frances ^{a*}, Oledibe Odira Johnson ^b,
Nwakoby Nnamdi Enoch ^c,
Afam-Ezeaku Chikaodili Eziamaka ^b
and Mbaukwu Onyinye Ann ^b

^a Department of Biological Sciences, Chukwuemeka Odumegwu Ojukwu University Uli, Anambra State, Nigeria.

^b Department of Botany, Nnamdi Azikiwe University Awka, Anambra State, Nigeria.

^c Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University Uli, Anambra State, Nigeria.

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

In order to suggest the most effective management strategies, an analysis of the fungal flora in the soil next to the biology laboratory at the COOU Uli campus was conducted. By plating washings from skin surfaces and liquid taken from soil samples in test tubes containing sabour dextrose agar (SDA) and potato dextrose agar (PDA), which had been mixed with streptomycin sulphate to prevent bacterial growth, fungi were isolated, characterised, and identified. 48 hours were spent incubating the inoculated tubes at 28 °C. The genera of the isolated fungus include *Aspergillus* (40%), *Mucor* (30%), *Fusarium* (10%), *Penicillium* (10%), and *Geotrichum* (10%), with *Aspergillus*

*Corresponding author: E-mail: cf.anyaegbu@coou.edu.ng , chiamakanyaegbu@gmail.com;

accounting for the majority of their occurrences. This study revealed that the two common genera of fungi were *Aspergillus* and *Mucor*, and that the number of colony forming units of the two genera in the soil studied exceeded the tolerance limits in foodstuffs specified by the International Commission on Microbiological Specifications for Foods (ICMSF). As a result, it is advised that proper precaution should be taken when interacting with soil samples from the study areas.

Keywords: Soil; flora; food; fungi.

1. INTRODUCTION

A plentiful source of germs that may infect humans is soil [1]. Many fungus in a diverse population that naturally exists in soil have the ability to break down organic pollutants, according to research. Certain microorganisms that previously were unable to breakdown a specific pollutant may also undergo mutations and develop the capacity to do so. In the lab, a colony of bacteria may be exposed to a pollutant and the germs that destroyed it can be isolated [2].

The rhizosphere of soil, which may have more than a million microorganisms per gramme of soil, is where bacteria are most active (2020). Structures of microbial communities can vary and are influenced by things like temperature and pH. Most commonly, faeces from both humans and animals are used to spread infectious illnesses [3-9]. Faecal pollution of water sources will result in the presence of the causative organisms in water if there are current cases or carriers in the community [10].

Another category of soil microorganisms is fungi. Yeast is a fungus that is used in baking and alcohol manufacture. Many antibiotics are produced by several fungus. Quite likely, we have all overheated a loaf of bread and discovered fungus on it. We have encountered or consumed mushrooms, which are certain fungi's fruiting structures [11].

Farmers are aware that fungi are the root cause of several plant diseases, including apple scab, damping-off, downy mildew, and different forms of root rot [12-18]. Furthermore, fungi begin the breakdown of recent organic waste [19]. Bennett as well. They aid in the first stages of the decomposition process by softening organic matter and facilitating the participation of other species. In addition to being the primary lignin decomposers, fungi are also less susceptible to acidic soil conditions than bacteria. No one can survive without oxygen [20-24]. Organic residue buildup at the surface and nearby seems to be encouraged by reduced tillage methods' low soil

disturbance [25]. This tends to encourage the growth of fungi, as it does in many intact natural ecosystems [26].

Several plants have advantageous bonds with fungus that boost the roots' interaction with the soil [27-29]. The roots become infected by fungi, which then release hyphae, which resemble roots Sullivan and Lund [30]. This mycorrhizal fungi's hyphae absorb water and nutrients, which may subsequently be used to nourish the plant. The hyphae are able to reach water and nutrients in confined areas of the soil that may be inaccessible to roots because they are so thin—about 1/60 the diameter of a plant root. In low-phosphorus soils, this is crucial for the phosphorus feeding of plants. The fungus obtain energy in the form of sugars, which the plant creates in its leaves and transfers to the hyphae to assist the plant absorb water and nutrients from the roots [31].

Mycorrhizal relationships are described as fungus and roots interacting symbiotically. When everything is taken into account, it's a very good bargain for the fungus and the plant [32-36]. This fungi's hyphae secrete a goeey gel that binds organic and mineral particles together, aiding in the development and stabilisation of larger soil aggregates. The diversity of microorganisms found in soil is astounding, yet studies show that many of the bacteria present there have not yet been identified and classified [37]. It has been difficult to relate new knowledge about the make-up and function of soil bacteria to the soil processes that have long been the subject of soil fertility research [38-41]. Currently, little is known about the microbial ecology of the fungal flora in soils; since mechanisms regulating nutrient availability and loss routes are mediated by microbes, a better understanding is required. Soil will be used in this investigation to identify this dangerous microorganism.

The main aim of this study is the isolation and identification of fungal flora from soil samples collected from biology laboratory of COOU Uli campus.

1.1 Specific Objectives

1. To isolate fungal flora from soil samples from biology laboratory of COOU Uli campus.
2. To characterize fungal flora isolated from soil sample
3. To determine the antibiotic susceptibility pattern of the isolated fungal flora against antibiotic

1.2 Scope of Study

The collection of soil samples from ten (10) locations within the COOU Uli campus biology lab, isolation and identification of the fungal flora from the soil sample, and assessment of the fungal flora's antibiotic susceptibility pattern against antibiotics are the only activities covered by this study.

1.3 Significance of Study

This work is important because it will contribute to a decrease in pollution and disease brought on by fungus. It was intended to establish the foundation for strengthening traditional traditions and understanding using contemporary drug development methods.

The outcome of this effort will assist in identifying the microbial compositions of soil samples, which will help in the development of antibiotics resistant to these microorganisms in order to significantly lessen their attack.

Lastly, this effort will create a strong platform for future research, advancing our understanding of fungal infections locally, nationally, and worldwide.

2. MATERIALS AND METHODS

2.1 Equipment / Apparatus

Petri dish, autoclave, inoculating wire loop, forcep, Bunsen burner, Conical flask, Antibiotic discs, Weighing balance, Test tube rack, plastic pipette, wire loop, Microscope, Incubator, beakers, glass slide, sterile cotton wool, test tube rack, universal container.

2.2 Sample Collection

The biological lab at the COOU Uli campus was used to gather soil samples from various sites.

2.3 Media Preparation

SDA and potato dextrose agar (PDA), which are known to promote the development of only fungal species, are the culture mediums employed for this experiment. In accordance with the manufacturer's instructions, the media will be prepared. The appropriate sterilisation of all the glassware utilised for this investigation will take place in a hot air oven at 160°C for an hour. Additional items were autoclaved at 121°C for 15 minutes to disinfect them. The research criteria to be followed in this experiment are those suggested by the following researchers (Alexander, 2009; Harrigan, 2008). (Dubey and Maheshawi, 2014). For every 100ml of water, 8.5g of PDA agar will be weighed out using a triple beam balance. The two mixes will be combined, wrapped in aluminium foil, and autoclaved for 15 minutes at 121°C.

2.4 Procedure for Serial Dilution

On a rack on the workbench, there will be three test tubes with 9ml of sterile normal saline in each. In the first test tubes, 1 gramme of each sample will be dissolved and properly mixed. Pipette 1 ml of the sample aseptically into the first test tube, mix it, and then repeat to fill the last tube (10⁻³). The final tube's 1ml will then be discarded.

2.5 Isolation of Fungi

The samples will be labelled and arranged appropriately on the table after being cleaned with 70% ethanol and cotton wool. In order to maintain a sterile environment and keep the workplace free of unwelcome organisms, the samples will be stored on the table with the Bunsen burner on. In this study, a total of 30 rolls of bread samples were used. After being lined up and filled with already prepared Potato Dextrose Agar (PDA) medium, sterile Petri dishes are left to set up. The plates will be cultured for one week at 37°C to promote colony development after drying inverted and being sealed with paper tape. By counting the matching colonies that were seen after the 1ml of the serially diluted samples, the count will be calculated. For distinct colonies inside the 1ml inoculums, spread plating methods will be utilised. Colony forming unit per ml (CFU/ml) was used to measure the count [42].

2.6 Sub-culturing of the Fungal

Each subcultured colony will have a little section taken out of it using a sterile dissecting blade. It

will be held at a slant in fresh Petri dishes after being picked up with sterile forceps and placed on a fresh, sterile glass slide. The slide will then be covered with a cover slip. Five days will pass with the Petri dishes on the workbench. Using forceps, the cover slips will be gently picked up and placed on the lacto-phenol-containing slides. With the exclusion of air bubbles, the slide preparation will be properly coated with cover slips. To remove extra discoloration that has seeped through the edge of the cover slip, use blotting paper.

Each colony will be represented on a slide and examined using the compound microscope's high power objective (x40) and low power objective (x10) lenses. The type of spore, surface texture, pigmentation, and pigmentation of the plate's backside were all noted, along with the colonies that developed.

2.7 Identification of the Fungal Isolates

The high power objective (x40) and low power objective (x10) lenses of the compound microscope will be used to look at each colony as shown on a slide. Together with the colonies that formed, the type of spore, surface texture, pigmentation, and pigmentation of the plate's back were all documented.

2.8 Determination of Fungal Frequency (%)

The frequency of fungi will be assessed in terms of both geography and media culture, and its relationship with the percentage of diseases identified by symptoms will then be examined. For calculating the proportion of fungi, use the following formula:

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

2.9 Determination of the Antimicrobial Activity

The Agar well diffusion technique will be used to assess the antibiotic activity on the bacterial isolates. The extracts will be reconstituted at a 10 mg/ml concentration. On a Mueller-Hinton agar plate, four (4) holes or wells will be drilled using a sterile cork borer with a 6 mm diameter. Each of the four wells will receive a tiny amount of the plant extract (50 l) at concentrations of 10 mg/ml, 5 mg/ml, 2.5 mg/ml, and 1.25 mg/ml. 50 l of the extraction solvents will be added to the negative control culture plate. Inoculated plates will be cultured for bacteria at 25–28°C for 72 hours and at 37°C for 24 hours. Zones of inhibition for every examined organism will be noted.

3. RESULTS AND DISCUSSION

3.1 Results

The total fungi count was measure in spore-forming unit per gram (sfu/g) and shown in Table 1.

Table 1. Mean fungi count of fresh soil samples

Sample	Mean total fungi count (cfu/g)
1	2.50 x10 ⁷
2	2.80 x10 ⁷
3	2.00 x10 ⁷
4	2.45 x10 ⁷
5	1.90 x10 ⁷

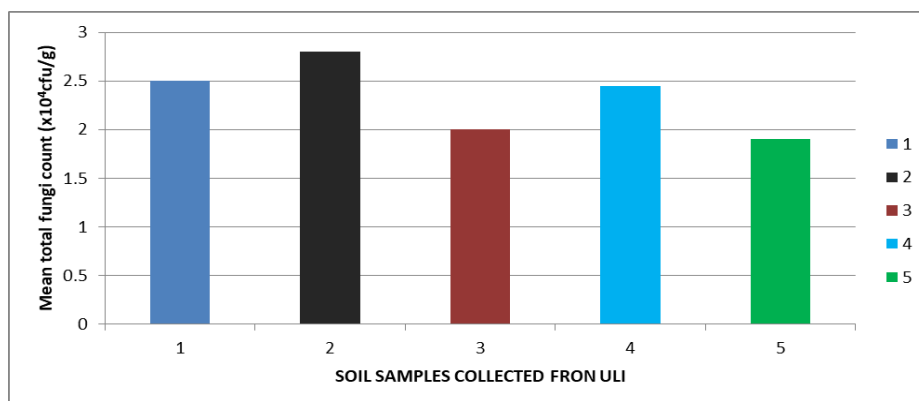


Fig. 1. Mean total fungi count (cfu/g)

The morphological characteristics of the fungal isolates are shown.

Table 2. Morphological Characteristics of Fungal Isolates

S/N	Isolate Code	Colour of Spores	Reverse of the agar	Aerial hyphae	Abundance	Growth	Pigmentation
1	KF1	Black	Light green	Powdery, spores embedded	Abundant	Fast	No
2	KF2	Black	Light green	Powdery, spores embedded	Abundant	Fast	No
3	KF3	Blue –green	Cream	Powdery, spores embedded	Abundant	Fast	No
4	KF4	White	Cream	Fluffy, raised a little	Abundant	Fast	No
5	KF5	White	Cream	Fluffy not raised	Abundant	Fast	No
6	KF6	White	Yellowish brown	Fluffy not raised	Abundant	Fast	No
7	KF7	White	Orange	Fluffy raised	Abundant	Fast	No
8	KF8	Brown	Cream	Powdery, raised	Abundant	Fast	No
9	KF9	Light - green	Creamy green	Embedded	Abundant	Fast	Yes
10	KF10	Light –brown	Dark-brown	Powdery, not raised	Abundant	Fast	Yes

Table 3. Identification of Fungi

S/N	Isolate code	Description	Probable identity
1	AF1	They are normally powdery black, with tall conidiophores emerging from long, wide, thick-walled, occasionally branching foot cells. Conidia are big, globose, irregularly roughed, and have radiating heads.	<i>Aspergillus niger</i>
2	AF2	They are typically powdery black, Conidiophores arising from long, broad, thick-walled, sometimes branched foot cell, it has tall conidiophores. Conidia are large with radiating heads, mostly globose and irregularly roughed	<i>Aspergillus niger</i>
3	AF3	Colonies were thinly spaced, blue-green, and their conidial heads were absolutely columnar. Conidiophores that are pigmented have clavate vesicles that come from thick-walled foot cells that are clearly distinguished. Conidia don't exist.	<i>Aspergillus fwnigatus</i>
4	AF4	Colonies are whitish to olivaceous-buff in colour, with a fragrant odour and tall and short sporangiophores that may be distinguished in the dark. Sporangia with ellipsoidal, pyriform, or subglobose features are blackish. Absence of chlamydo spores.	<i>Mucor sp.</i>
5	AF5	Colonies are whitish to olivaceous-buff in colour, with a fragrant odour that may be distinguished in the dark between tall and short sporangiophores. Sporangia that are ellipsoidal, pyriform, or subglobose in shape. Without chlamydo spores.	<i>Mucor sp.</i>
6	AF6	Colonies have a smoke-grey colour in the dark and a yellowish brown colour in the light. They have large sporangiopores and a denser layer of small sporangiopores that are frequently branched. Sporangiopores having granular contents and thick walls.	<i>Mucor mucedo</i>

S/N	Isolate code	Description	Probable identity
7	AF7	Colonies are rapidly expanding, with sparse to abundant, floccose aerial mycelium that becomes felted and is either white or peach in colour with a hint of violet. Aromatic scent that is distinctive and suggests lilae.	<i>Fusarium sp.</i>
8	AF8	Colonies expand quickly and change colour from cinnamon to orange-brown. It seems silky to the eye. Conidiophores have hemispherical vesicles and lengthy, smooth walls. Metulae are present, and the smooth-walled conidial heads rigidly columnar conidia seem globose to slightly ellipsoidal.	<i>Aspergillus terreus</i>
9	AF9	Colonies are loosely synematous, rapidly developing conidiophores in new isolates that give the colony a zonate look. Colonies are light green, inverted colourless, yellow-brown conidiophores that are typically smooth walled, penciilli that are 2-3 stages branching, and conidia that are subglobose to ellipsoidal smooth-walled. The odour is fragrant, fruity, and reminiscent of apples.	<i>Penicillium sp.</i>
10	AF10	Has fast-growing colonies, membranous or white butyrous skin, and a fruity odour. Diagonally branching moving hyphae. Conidia in the conidial chain are mostly aerial, predominantly nucleate, and do not advance as dichotomously branching hyphae.	<i>Geotrichwn sp.</i>

The percentage occurrence of the fungal isolates is shown in Tables 4 respectively. Among the isolates *Aspergillus* has the highest frequency among fungal isolates.

Table 4. Percentage Occurrence of fungal isolate

Isolate	Frequency	Percentage
<i>Aspergillus spp</i>	4	40
<i>Mucor</i>	3	30
<i>Fusarium</i>	1	10
<i>Penicillium</i>	1	10
<i>Geotrichum</i>	1	10
<i>Total</i>	10	100

3.2 Discussion

Many illnesses in humans and animals have been linked to microorganisms, notably fungus. Several of these microorganisms lead to tomato fruit rotting, discolouration, or fermentation, which affects tomato fruit preservation. This study demonstrated that several fungus attack tomato fruit, causing degradation. In the present study, many filamentous and yeast fungi were isolated from the soil collected from various locations in Uli. The discovery of filamentous and yeast fungus in soil gathered from markets suggests that the Uli campus's soil sickness was caused by the isolated mycoflora.

The discovery of these pathogens supported Nweze's [43], Runner et al., [44], De Boer, [45] Ochei, & Kolhat [46] research that *A. niger* discovered to be connected to tomato fruit rotting are extremely pathogenic and result in noticeable losses in tomato fruit at post-harvest. Together with other diseases, *Fusarium spp.*, *A. flavus*, and *Rhizopus spp.* were also isolated by Baiyewu (2014) from tomato fruit. 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 tomato fruit [47]. Fruit is improperly handled and transported after harvest (WHO) [48]. Because of the nature of the transportation infrastructure that exist in rural areas, the majority of tomato fruit typically does not reach the big cities in time.

This suggests that consumers face a significant risk of aflatoxin and other mycotoxins. Aflatoxin M1 was found in the urine of the women who had tomato fruit containing aflatoxin, according to a research by Sage et al. (2002). In order to assess the existence and potential risk of such

metabolites in the majority of working class people in this South Western part of Nigeria, Amoah [49] claims that no tests have been done to see if aflatoxins are in the urine and blood. The majority of people may not develop hepatoma or aflatoxicosis, but it does not mean the toxic metabolite does not present in their bodies [50].

The outcomes of the pathogenicity tests conducted reveal that every organism was pathogenic, was the real cause of the spoiling of various tomato fruits, and could also infect various tomato fruits in addition to their original host. The experiments also proved that, as Salamitou, et al.,2020 reported, fungi cause the garri to deteriorate when they entered through mechanical damage such bruising and wounds. When compared to the other fungi, *Aspergillus niger* caused the garri to deteriorate at a higher pace because it grew more quickly than the other fungal isolates. It was also observed that *Aspergillus niger* appeared on the garri before the other fungi. Also, the health of those who ingest this Pawpaw garri may be seriously endangered by the presence of these fungal pathogens.

It is acknowledged that the fungal rot of tomato fruit can 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 vary in toxicity and toxicity levels [51-53].

4. CONCLUSION

On soil samples taken from the ULI campus, this study identified the fungus profile. Also, it demonstrated that fungus were important players in food deterioration and infection-causing agents. Nonetheless, food spoiling can be avoided by using the following techniques: Crops that have been harvested should be washed with clean or pure water. Warehouses should be cleaned and sanitised properly. Packaging and shipping containers should be disinfected. Fungus development may be inhibited by storing food under refrigeration or by using fungicides. To prevent contamination and the consumption of tainted tomato fruit, it is crucial that both the farmer who processes or sells the tomato fruit into bags for transportation, the marketers, and the consumers take the appropriate precautions. Nonetheless, this will help to lower the likelihood

that these study-isolated fungus would develop aflatoxin and other mycotoxins that are harmful to human health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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