ANALYSIS OF FUNGAL FLORA OF A SOIL NEAR BIOLOGY LABORATORY OF COOU ULI

ABSTRACT

Analysis of fungal flora of a soil near biology Laboratory of COOU Uli campuswas carried out in order to recommend the appropriate control measures. Isolation, characterization and identification of fungi were made by plating washings from skin surface and extracted liquid from soil samples in test tubes containing sabourd dextrose agar (SDA) and potato dextrose agar (PDA) into which *streptomycin* sulphate was incorporated to inhibit bacterial growth. Inoculated tubes were incubated at 28 ± 2°C for 48 hr. The genera of the fungi isolated and their percentage of occurrence include; *Aspergillus* (40%), *Mucor*(30%), *Fusarium* (10%), *Penicillium* (10%), and *Geotrichum* (10%). This study showed that *Aspergillus*and *Mucor* were the two prevalent genera of fungi, 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), therefore, it is recommended that proper care should be taken when coming in contact with soil samples from the study areas.

Keyword: Soil, flora, food, fungi, SDA, PDA.

INTRODUCTION

Soil is a rich source of microorganisms capable of infection in human (Wagner, 2021). Researchers have found that many fungi within a mixed population existing naturally in the soil are capable of degrading organic contaminants. Some microbes which previously were not capable of degrading a given contaminant may also mutate, acquiring the ability to degrade that contaminant. In the laboratory, it is possible to expose a population of bacteria to a contaminant and isolate the microbes which degraded it (Mead, *et al.*,2019).

In soil, microbes are especially active in the rhizosphere, which can contain more than a million microorganisms per gram of soil (Bennettand Harmon, (2020). Microbial community structures are variable and depend on factors such as temperature and pH. Infectious diseases are transmitted primarily through human and animal excreta, particularly faeces. If there are active cases or carriers in the community, then faecal contamination of water sources will result in the causative organisms being present in water (Claus and Berkeley, 2016).

Fungi are another type of soil microorganism. Yeast is a fungus used in baking and in the production of alcohol. Other fungi produce a number of antibiotics. We have all probably let a loaf of bread sit around too long only to find fungus growing on it. We have seen or eaten mushrooms, the fruiting structures of some fungi (Egeonu, 2002).

Farmers know that fungi cause many plant diseases, such as downy mildew, damping-off, various types of root rot, and apple scab. Fungi also initiate the decomposition of fresh organic residues (UmohandOdibo, 2019). They help get things going by softening organic debris and making it easier for other organisms to join in the decomposition process. Fungi are also the main decomposers of lignin and are less sensitive to acid soil conditions than bacteria. None are able to function without oxygen. Low soil disturbance resulting from reduced tillage systems tends to promote organic residue accumulation at and near the surface (Finlay, *et al.,*2020). This tends to promote fungal growth, as happens in many natural undisturbed ecosystems (Thomas, 2010).

Many plants develop a beneficial relationship with fungi that increases the contact of roots with the soil. Fungi infect the roots and send out root-like structures called *hyphae (*Sullivan and Lund, 2019). The hyphae of these *mycorrhizal* fungi take up water and nutrients that can then feed the plant. The hyphae are very thin, about 1/60 the diameter of a plant root, and are able to exploit the water and nutrients in small spaces in the soil that might be inaccessible to roots. This is especially important for phosphorus nutrition of plants in low-phosphorus soils. The hyphae help

the plant absorb water and nutrients, and in return the fungi receive energy in the form of sugars, which the plant produces in its leaves and sends down to the roots (Buzby, (2011).

This symbiotic interdependency between fungi and roots is called a *mycorrhizal* relationship. All things considered, it's a pretty good deal for both the plant and the fungus. The hyphae of these fungi help develop and stabilize larger soil aggregates by secreting a sticky gel that glues mineral and organic particles together.

Soil supports an extraordinary diversity of microorganisms; however, surveys of soil indicate that a substantial number of bacteria have not been identified and characterized (Elizabethand Martin, 2013). There has been challenge to link new information on the composition and function of soil bacteria back to the soil processes that have long been the focus of soil fertility. The microbial ecology of fungal flora in soils remains poorly understood; an improved understanding is necessary as processes affecting nutrient availability and loss pathways are microbially mediated. This study therefore, will isolate this pathogenic microbe from soil.

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.This study is significant as it will help to reduce contamination and illness caused by fungal infection.

MATERIALS AND METHOD

The study is limited to the collection of soil samples from ten (10) locations within biology laboratory of COOU Uli campus, Isolation and identification of fungal florafrom the soil sample and determination of antibiotic susceptibility pattern of fungal flora isolated from them against antibiotics.The culture media used for this experiment is the SDA and potato dextrose agar (PDA) which is known to support the growth of only fungi organisms. The media will be prepared according to the manufacturer's directions. All the glass wares used for this study will be sterilized properly in a hot air oven at 160^0C for an hour. Other materials were sterilized by autoclaving at

121^0C for 15minutes. The methods to be used in this experiments will be according to standards recommended by the following researchers (Alexander, 2009, Harrigan, 2008, Dubey and Maheshawi, 2014). 8.5g of the PDA agar will be weighed out with triple beam balance for 100ml of water. The two mixtures will be mixed together and sealed with aluminum foil and autoclaved at 121^0C for 15minutes.

Procedure for serial dilution

Three test tubes containing 9ml of sterile normal saline will be on a rack on the working bench. 1gram of each sample will be dissolved into the first test tubes and mixed thoroughly. 1ml of the sample will be pipette aseptically into the first test tube and mixed, this will be repeated serially to the last tube (10^{-3}). Then 1ml from the last tube will be discarded.

Isolation of fungi

The table will be cleaned with 70% ethanol using cotton wool, the samples bought will be labeled and placed accordingly on the table. The samples will be kept on the table with the Bunsen burner on to keep the working place sterile and free from unwanted organisms. The total of 30 rolls of bread samples were used in this research. Sterile Petri dishes will be aligned and Potato Dextrose Agar (PDA) media already prepared were poured into the Petri dishes, they will be allowed to gel. The plates dried in inverted position, sealed with paper tape and will be incubated for 1 week at 37^0C for colony formation. The count will be determined by counting the corresponding colonies that were observed after the 1ml of the serially diluted samples. Spread plating techniques will be used for discrete colonies within the 1ml inoculums. The count was recorded in colony forming unit per ml (CFU/ ml) (Cheesbrough, 2020).

Sub-culturing of the fungal

A small portion of each sub cultured colony will be cut out using a sterile dissecting blade. It will be picked up with a sterile forceps and placed on a new, sterile glass slide; the slide will be covered with a cover slip, kept in a slant in new petri dishes. The petri dishes will be left on the bench for 5 days. The

cover slips will be carefully picked respectively with forceps and dropped on the slides containing lacto-phenol.

The slide preparation will be carefully covered with cover slips with the exclusion of air bubbles. Blotting paper will be used to remove excess stain coming through the edge of the cover slip. Slides of each colony will be made and observed under the low power objective (x10) and high power objective (x40) lens of the compound microscope. The spore type, surface texture, pigmentation and the pigmentation of the reverse side of the plate; the colonies formed were also recorded.

Identification of the fungal isolates

The identification of the fungal species will be carried out using a number of observations such as macroscopic studies, microscopic examination, morphology (size and shape) and also using gram staining characteristics for yeasts as described by Cheesebrough (2020). Slides of the fungal isolates will be prepared and observed under the microscope and the organisms identified on the basis of their appearance with reference to Yamada, *et al.*,2018 and Samson,*et.al*., 1984.

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:

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

Determination of the antimicrobial activity

The antimicrobial activities will be evaluated on the bacterial isolates using Agar well diffusion method. The extracts will be reconstituted into a concentration of 10 mg/ml. Four (4) holes or wells will be bored on Mueller Hinton agar plate using a 6 mm diameter sterile cork borer. A small portion of the plant extract (50 µl) at concentrations of 10 mg/ml, 5 mg/ml, 2.5 mg/ml and 1.25 mg/ml will be filled in each

of the four wells. The negative control culture plate will be filled with 50 µl of the solvents used for the extraction. Inoculated plates will be incubated at 37^0C for 24 hrs and 25-28^0C for 72 hours for bacteria. Zones of inhibition will be recorded for each tested organism.

RESULT AND DISCUSSION

4.1 RESULT

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	**2.50×10^7**
2	**2.80×10^7**
3	**2.00×10^7**
4	**2.45×10^7**
5	**1.90×10^7**

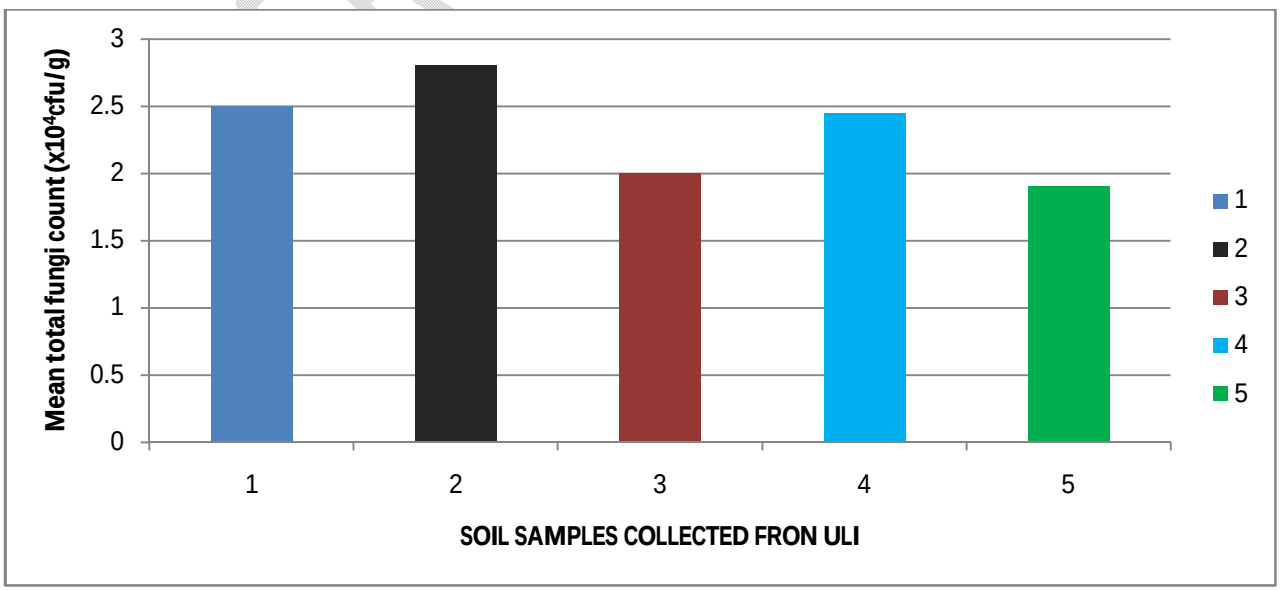

Fig. 1. Mean total fungi count (cfu/g)

The morphological characteristics of the fungal isolates are indicated in Table 2.

Table 2. Morphological characteristics of fungal isolates

S/N	Isolate Code	Colour of Spores	Reverse of the agar	Aerial hypae	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 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.	*Aspergillusniger*
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	*Aspergillusniger*
3	AF3	Colonies spread thinly, blue-green with strictly columnar conidial heads. Pigmented conidiophores present with clavate vesicles arising from clearly differentiated thick-walled foot cells. Conidia are absent.	*Aspergillusfwnigatus*
4	AF4	Colonies are whitish to olivaceous-buff, odour aromatic; in the dark differentiated into tall and short sporangiophores. Sporangia blackish with ellipsoidal, pyriform or subgloblose. Chlamydospores absent.	*Mucor sp.*
5	AF5	Colonies are whitish to olivaceous-buff, odour aromatic; in the dark differentiated into tall and short sporangiophores. Sporangia blackish with ellipsoidal, pyriform or	*Mucor sp.*

		subgloblose. Chlamydospores absent.	
6	AF6	Colonies smoke-grey in dark, yellowish brown in the light: odour aromatic, it has wide sporangiopore and a denser layer of short repeatedly branched sporangiopores. Sporangiopores thick-walled with granular contents.	*Mucormucedo*
7	AF7	Colonies are fast growing, aerial mycelium sparse to abundant and floccose, becoming felted, white or peach, but with a violet tinge. Characteristic aromatic odour suggesting lilae.	*Fusarium sp.*
8	AF8	Colonies growing rapidly, appearing cinnamon to orange-brown. It appears velvety in appearance. Conidiophores are long smooth walled, hyaline, with hemispherical vesicles. Metulae are present, conidial heads strictly columnar conidia appear globose to slightly ellipsoidal and it is smooth walled.	*Aspergillusterreus*
9	AF9	Colonies are fast growing conidiophores in fresh isolate typically loosely synematous, giving the colony a zonate appearance. Colonies are light green, reversed colourless, yellow-brown conidiophores usually smooth walled, pencilli 2-3 staged branched with numerous usually oppressed mutulae, conidia sub-globose to ellipsoidal smooth-walled, odour aromatic, fruity and suggesting apples.	*Penicillium sp.*
10	AF10	Has fast growing colonies, white butyrous or membraneous, odour often fruity. Advancing hyphae	*Geotrichwn sp.*

		dichotomously branched. Conidial chain mostly aerial, conidia, mostly nucleate, it has no dichotomously branched advancing hyphae.	

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**
Aspergillusspp	**4**	**40**
Mucor	**3**	**30**
Fusarium	**1**	**10**
Penicillium	**1**	**10**
Geotrichum	**1**	**10**
Total	**10**	**100**

DISCUSSION

Micro- organisms especially fungi have been reported to cause extensive infections in man and animal. Some of these micro-organisms cause rotting, discoloration or fermentation of the tomato fruit which affect their preservation. This study showed that the tomato fruit *are* attacked by various fungi which

causedeterioration. In the present study, many filamentous and yeast fungi were isolated from the soil collected from various locations in Uli. Filamentous and yeast fungi were isolated from the soil collected from markets and this is an indication that the isolated mycoflora were responsible for the soil disease in Uli campus.

The isolation of these pathogens confirmed the studies of Nweze, 2020,Runner, *et al.*,2020 andDe Boer, *et al.*,2021. OcheiandKolhat, 2021 reported that*A.niger*found associated with spoilage of tomato fruit are highlypathogenic causing appreciable losses in tomato fruit at post harvest. Baiyewu (2014) also isolated *Fusarium* spp., *A. flavus*, and *Rhizopus* spp. among other pathogens from tomato fruit. The contamination of tomato fruit by fungi could be as a result of poor handling practices in food supply chain, storage conditions, distribution, marketing practices and transportation (Owegh*et al.*,2021). Post harvest handling and transport of fruit is inadequate (WHO, 2021). Therefore, most of the tomato fruit do not usually get to the major cities in time due to the nature of transport systems existing in the rural areas.

This portends a great risk of aflatoxin and other mycotoxins to the consumers. This is confirmed in a study by Sage *et al.*, (2002) reported that Aflatoxin M1 was detected in the urine of the women that consumed tomato fruit containing aflatoxin. According to Amoah, 2022 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 (Agaisse, *et al.*,2019).

The results of the pathogenicity tests carried out show that all the organisms were pathogenic and were the actual causal agents of spoilage of the different tomato fruit and can also infect different tomato fruit other than their original host. The tests also established the fact that fungi cause deterioration of the *garri*when they gained entrance into them through mechanical injuries such as bruises and wounds as noted by

Salamitou, *et al.*,2020.*Aspergillusniger* grew at a faster rate than the remaining fungal isolates which was evident in its cause of spoilage in the *garri* at a faster rate when compared to the other fungi. *Aspergillusniger* was also noted to appear first on the *garri* before the other fungi. Also, the presence of these fungi pathogens in theses Pawpaw *garri*could pose a serious threat to the health of its consumers.

The occurrence of fungal spoilage of tomato fruit 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 (Helgason, *et al.*,2014,James, 2015 and Mensah, *et al.*,2019).

CONCLUSION

This study detected the profile of fungi in soil samples in Uli campus. It also showed that fungi were involved in the spoilage of many foods and causative agents of infection. Food spoilage however can be controlled by the following practices: Washing of harvested crops with clean or pure water; proper cleaning and sanitation of warehouses and disinfection of packaging and transit containers; 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 process or sells the tomato fruit into bags for transportation, the marketers and consumers take necessary precaution in preventing contamination and eating of contaminated tomato fruit. 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.

Recommendations

This study revealed high fungal presence in the different soil samples investigated. This tends to reflect the level of bio security and hygienic practices in the processing handling and storing of the

tomato fruit. These findings emphasize the need for constant quality assessment of these tomato fruit on sales.

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