UJMR, Conference Special Issue Vol. 9 No. 3. June, 2024, pp. 401 - 410

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

https://doi.org/10.47430/ujmr.2493.048

Received: 12th February 2024 **Accepted:** 14th June 2024

Surveying the Distribution and Diversity of Predominant Mycobiota in Cultivated and Uncultivated Soils of Zaria, Northwestern Nigeria

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Abstract

Soil fungi are a crucial component of terrestrial ecosystems, playing a vital role as decomposers in nutrient cycles. The diversity of soil fungi serves as an indicator of soil health (favorable/deficient). Understanding soil biodiversity and its biological functioning will help not only in crop yields but also in a healthier society as it directly or indirectly enters the food chain. This study aimed at surveying the distribution and diversity of predominant mycobiota in cultivated and uncultivated soils of Zaria, Northwestern Nigeria. Focusing on their morphological and microscopic characteristics, percentage frequency, and relationship with physicochemical properties. After removing the surface soil, soil samples were randomly collected from two locations at 0-15 cm depths. Fungi were isolated via the dilution method on PDA. Previously established criteria were used to identify eight different fungal species: Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Fusarium spp., Mucor spp., Penicillium spp., Rhizopus spp., and Trichoderma spp. Aspergillus niger was the most dominant species, with a relative frequency of 26%, followed by Fusarium spp. and Rhizopus spp. With 18% and 17%, respectively. Aspergillus fumigatus was the least abundant (2%). The highest fungal isolation frequency (32%) was observed in L1 cultivated soil, closely followed by L2 uncultivated soil (31%), with the lowest in L2 uncultivated soil (16%). Physicochemical analysis indicated that these soils are rich in mycobiota, attributed to a basic pH range of 7.12-9.15, high organic matter content, and optimal moisture levels. Soils with significant organic matter due to litter accumulation, moist deciduous vegetation, and neutral to alkaline pH levels retain considerable moisture content, fostering a rich qualitative and quantitative mycobiota for recycling dead organic matter making nutrients available for suitable utilization. Further research is recommended to evaluate the impact of these isolated fungi on soil productivity, to determine whether their presence has a positive or negative effect.

Keywords: Distribution, Diversity, Mycobiota, and Soil health.

INTRODUCTION

The soil serves as a reservoir for many microbial communities of plants and herbs, producing CO2 and nitrogen cycles (Raja *et al.*, 2017). Microorganisms are important agents that play a major role in soil ecosystems. Microbial composition and functioning play a major role in soil quality through the decomposition of organic matter, recycling of nutrients, and biological control (Stefanis *et al.*, 2013). Soil is an oligotrophic medium for the growth of fungi because fungal growths are extremely limited for most of the time and readily available, are present for short periods in a limited zone for most of the time. Fungi are either dormant or metabolized and grow very slowly utilizing a range of organic molecules (Ratna-kumar *et al.*, 2015).

Knowledge of the soil's chemical and physical properties has always interested Foresters in assessing the sites' ability and adding forest productivity (Schoenholtz et al., 2000). Forest soil (including humus, litter, and coarse Woody debris) is an important reservoir of microorganisms and soil biota that influence

carbon storage, soil structure, fertility, productivity, and plant growth.

Fungi are successful soil inhabitants because of their high plasticity and capability to adopt numerous forms in response to adverse or unfavorable conditions (Sun *et al.*, 2005). Due to their ability to produce a wide range of extracellular enzymes, they can break down almost all forms of organic matter, decomposing soil components and regulating the balance of carbon and nutrients (Zifeakova *et al.*, 2016).

Soil fungi can be classified into three functional groups, including (1) biological controllers, (2) ecosystem regulators, and (3) species participating in organic matter decomposition and compound transformations (Gardi and Jeffery, 2009).

Soil fungi play an integral role in medicine, yielding antibiotics, in agriculture by maintaining soil productivity and causing fruit and crop diseases, forming the basis of many industries' important food supply. More importantly, some fungi play a role as research tools in studying biological processes.

Fungi strongly influence the ecosystem's structure and functioning, therefore playing a significant role in many ecological services (Orgiazzi *et al.*, 2012). Soil fungi perform crucial roles within the soil concerning the cycling of nutrients, disease suppression and water dynamics, all of which help plants become healthier and more vigorous (Suhang, 2016). Using fungi to recover nutrient content is currently a favourable method for many agricultural practices over chemical fertilizers since using chemical fertilizers and pesticides has created the problem of environmental pollution (Suhang, 2016; Aziz and Zainol., 2018).

The use of fungi in agricultural practices seems to be a promising technique to farmers as this technique is environmentally friendly and cost-effective in addition to the application of chemical fertilizers, resulting in reduced colonization of plant roots with symbiotic fungi, thus reducing the beneficial effect of soil fungi (Aziz and Zainol, 2018).

Some fungi, such as *Penicillium* spp, *Aspergillus* spp, *Mucor spp,* and many other species, are notable soil microbes whose presence might inhibit the growth and establishment of mushroom mycelium and are prominent and major fungi isolated from mushrooms (In-young *et al.*, 2010, Dimkpa and Orikoha, 2021).

Continued use of chemical fertilizers over a long period may cause an imbalance in soil microflora and indirectly affect soil's biological properties, leading to soil degradation (Gaddeyya *et al.*, 2012). Pesticides may affect soil microbial populations, stimulating the growth of certain microorganisms, exerting toxic effects, and inhibiting the growth of others. Therefore, identifying and characterizing these microbial species is important to study their potential candidates in bioremediation (Rohilla and Solar, 2012).

Nigeria is endowed with a vast variation of soils determined by differences in age, present materials, and physiographic and climatic conditions (Ojeniyi, 2018). These soils differ in their inherent fertility, with the soil most suitable for crop production covering a large proportion of the land area (Ojanuga, 2006). Soil is a highly complex system with many components playing diverse functions, primarily due to the activity of soil organisms (Lavelle et al., 2006). Therefore, understanding soil biodiversity and its biological functioning will enhance crop yield and contribute to a healthier society, as it will directly or indirectly enter the food chain (Wall et al., 2012). The assessment of fungal biodiversity as a quality indicator cannot be limited to determining biodiversity indices but should also include a structural analysis of the fungal population to determine their functions in affecting soil quality and plant health (Gams & Samson, 2013). The study aimed at isolating and identifying soil fungi from cultivated and uncultivated soils of NAPRI and Samuru Zaria, Kaduna state.

MATERIALS AND METHODS

Sample collection and preparation

Soil samples were collected from different cultivated and uncultivated fields to isolate and identify fungal organisms. The soil samples were collected from a depth of 15cm with the help of a sterilized cork borer pushed horizontally into the ground. The soil caught was emptied into sterilized polyethylene bags. The place, time, and date of the collection of samples were appropriately labelled on each sampling bag. The samples were then taken to the laboratory for further processing.

Soil samples were dried, grounded, and sieved before analysis. Soil samples were air-dried at an appropriate temperature in wooden trays. Each tray was numbered, and sample identification followed each set of numbered

racks throughout the analysis. The dried soil was grounded in a mortar and then sieved through a 2mm sieve for a smoother result. (Saravanakumar et al., 2010)

Media used

Various media are used to isolate different groups of fungi that influence vegetative growth and colony morphology. The media used were potato dextrose agar (PDA) and cornmeal agar (CMA)

Media preparation

Potato Dextrose Agar: Two hundred grams of Irish potato was peeled and chopped, then cooked using 2.0 litres of distilled water. The suspension was filtered, and 20g of agar-agar was added. Dextrose sugar (20g) was also added to the filtrate and stirred to obtain Potato Dextrose Agar (PDA), which was sterilized using an autoclave under standard conditions and allowed to cool at room temperature (Choi et al., 1999).

Cornmeal Agar: Fifty grams of corn was added to 1 litre of distilled water and cooked. The suspension was filtered, and 20g of agar-agar was added to the filtrate and stirred to obtain Cornmeal Agar, which was sterilized using an autoclave under standard conditions and allowed to cool at room temperature (Andrews & Pitt, 1986).

Isolation of fungi from the soil samples

The soil dilution method on media such as potato dextrose agar and corn meal agar was used as an isolation techniques (Waksman, 1922).

Soil dilution

Soil dilutions were made by adding 1g of soil from each sample to 10 ml of sterile distilled water. 10-1 and 10-3 dilutions were used to isolate fungi to avoid overcrowding the fungal colonies. One millilitre of the suspension of each concentration was added to a sterile petri dish in triplicate of each dilution, containing a sterile PDA medium. One percent streptomycin solution was added to the medium to prevent bacterial growth before pouring into petri plates. The plates were then incubated at 28°C for 3-5 days (Hemanth *et al.*, 2015).

Organisms were easily isolated because they formed well-sp dispersed surface colonies, particularly at higher dilutions. One isolate of fungal colonies was selected at random for further subculturing. The subcultures were maintained in PDA slants. Another pure culture was done in which soil dilutions (10^{-3}) were transferred into a petri dish containing a warm molten cornmeal agar, covered using sterilized polythene bag, and incubated for 5-8 days at a temperature of 30°C. The growth observed was subculture using PDA containing streptomycin (Rajendra *et al.*, 2016).

Inoculating techniques

The working benches in the laboratory were thoroughly swapped with a methylated spirit soaked in cotton wool, and a burning blue flame was allowed to sterilize the surrounding air before the inoculation. The conical flasks were corked tightly with cotton wool, and the petri dishes were fully autoclaved.

Identification

Morphological characteristics such as (colour, shape, and growth pattern) were microscopically used to observe the growth obtained, and the organisms were also identified and characterized based on them by staining with Lacto Phenol Cotton blue and observed under a compound microscope and by using taxonomic guides and standard processes. (Aziz et al., *2018*).

Fungi Staining techniques

Inoculating needles were flamed over the burning Bunsen flame, and then, using the needle, a small portion of the growth on the culture plate was transferred into the drop of lactophenol in cotton blue on the slide. The specimen was picked carefully using inoculating wire loops to avoid squashing and overcrowding of the mycelium. The specimen was observed under the microscope for identification. (Waksman, 1927)

Physicochemical analysis of the soil

The physicochemical parameters of the soil samples were analysed at the soil and water management laboratory (SWML) Institute of Agricultural Research, Ahmadu Bello University, Zaria. Standard methods were adopted to

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

measure and characterize the physicochemical properties of the soil collected.

Percentage Organic Carbon Determination

A prepared soil sample (1g) was weighed into a 500ml conical flask. To the flask, 10 milliliters of 0.1667 M potassium dichromate (K2Cr2O7) solution and 20 milliliters of concentrated sulfuric acid (H2SO4) containing silver sulfate (Ag_2SO_4) were added and mixed thoroughly. The reaction mixture was then diluted with 200 milliliters of water and 10 milliliters of phosphoric acid (H_3PO_4). Subsequently, 10 milliliters of sodium fluoride (NaF) solution and 2 milliliters of diphenylamine indicator were added. The solution was titrated with a standard 0.5 M ferrous sulfate (FeSO4) to a brilliant green color. A blank titration was carried out simultaneously without the soil sample (Aziz & Zainol, 2018).

The percentage of organic carbon (% OC) was calculated using the formula:

The percentage of organic carbon (% OC) was calculated using the formula: $\%OC=\frac{(B-T)\times 0.003\times N\times CF\times 100}{\text{wt}}$

Where:

- B = Blank reading
- T = Sample titre value
- N = Normality of ferrous sulfate used in titration
- CF = Concentration factor, which is 1.33
- wt = Weight of the soil sample (1g)

Percentage Organic Matter Determination

The percentage of organic matter (% OM) was determined using the calculated percentage of organic carbon (% OC). The formula gives the relationship:

% OM = % OC x 1.724

This factor (1.724) converts organic carbon to organic matter, as organic matter is approximately 58% carbon (Ratna-Kumar et al., 2015).

Percentage Nitrogen Determination

The percentage of Nitrogen (% N) in the soil was estimated using the percentage of organic carbon (% OC). The formula used is:

$\%N = \frac{\%OC}{7}$

This calculation assumes a typical carbon-to-nitrogen ratio in organic matter (Seifert, 1992).

Determination of exchangeable bases (K, Na, Ca, and Mg)

An air-dried soil sample of 1g was weighed into a 60ml plastic bottle, and 20ml of ammonium acetate was added. The bottle was covered tightly and shaken for 20 minutes using a mechanical shaker. The mixture was then transferred into another clean bottle through a funnel fitted with filter paper to obtain a clear solution. Sodium (Na) and Potassium (K) were determined using a flame photometer, while Calcium (Ca) and Magnesium (Mg) were determined by atomic absorption spectrophotometer or by the intrametric method (Dimkpa & Orikoha, 2021; Stefanis et al., 2013).

pH determination

Ten Grams of the soil sample were shaken with 10ml of distilled water for 5 minutes, and the pH of the supernatant liquid was determined using a benchtop pH meter.

Moisture content determination

A soil sample weighing 100g was poured into an aluminum moisture box, and the box lid was removed. The sample was then placed in an oven set at 105°C until it reached a constant weight, typically requiring approximately 24-36 hours. After reaching a constant weight, the sample was allowed to cool first in the switched-off oven and then in a desiccator to prevent moisture absorption from the surrounding environment. Once cooled, the sample was weighed again. The loss in Weight represented the moisture content of the 100g soil sample (Waksman, 1922).

The percentage of moisture is calculated as follows;

$$\text{Moisture \%} = \frac{\text{Loss in weight}}{\text{Oven – Dry Weight of soil}} \times 100$$

Phosphorus determination

Seven millilitres of the phosphorus extracting solution and 1.0N NH4F + 0.5N HCl were added to a 100ml conical flask containing 1g of soil sample. The mixture was shaken for 1 minute to ensure thorough mixing and then placed in a centrifuge for 5 minutes to obtain a clear solution. Subsequently, 5 ml of the filtered soil solution was transferred into a 25 ml measuring flask using a bulb pipette. To this, 5 millilitres of the developed reagent were added, and the volume was diluted to approximately 20ml with distilled water, filling the flask up to the 25ml mark. The solution was shaken thoroughly, and the blue colour was allowed to develop for 15 minutes. The absorbance of the solution was then measured at 600nm using a spectrophotometer after setting the instrument to zero with a blank prepared similarly but without the soil sample (Saravanakumar & Kaviyarasan, 2010).

$$\text{The calculation of (Mg/Kg)} = \frac{Df \times Ev \times G \times R}{Wt}$$

Data analysis

Data on soil samples were analysed using descriptive statistics and presented in tables and figures.

RESULTS

Table 1 provides the physicochemical analysis of soil samples, indicating that the pH in Location 1 cultivated soil (7.12) and Location 2 cultivated soil (7.42) was neutral. In contrast, Location 1 uncultivated soil (9.15) and Location 2 uncultivated soil (9.12) exhibited alkaline pH levels. The analysis revealed optimal moisture content and organic matter in Location 1 cultivated soil (10.96% and 0.68%), Location 2 cultivated soil (15.56% and 1.64%), and Location 2 uncultivated soil (10.86% and 0.90%), which are favorable for fungal growth. Location 1 uncultivated soil had the lowest moisture content (6.27%) and organic matter (0.95%). Despite these variations, all soil samples were rich in macronutrients (N, O, C, Na, P, and K) and micronutrients (Ca and Mg), essential for fungal growth.

Figure 1 demonstrates that *Aspergillus* species were the most prevalent, comprising 39% of the isolated fungal species. *Aspergillus niger* was the most abundant (26%), followed by A. flavus (11%) and *A. fumigatus* (2%). *Fusarium* spp. represented 18% of the isolated fungi.

Figure 2 indicates that the percentage frequency of fungi in Location 1 cultivated soil was 32%, and in Location 2 uncultivated soil, it was 31%. This slight variation is attributable to the similar moisture content (10.96% and 10.86%, respectively) and organic matter (0.90% and 0.68%, respectively) in these soils. Conversely, Location 1 uncultivated soil exhibited the lowest percentage frequency of fungi (16%) due to its low moisture content (6.27%) and alkaline pH (9.15).

Table 1: Physicochemical Analysis of Soil Samples

Sample	Cultivated Soil		Uncultivated Soil	
	L1	L2	L1	L2
Moisture Content (%)	10.86	6.27	15.56	10.96
pH	9.12	9.15	7.42	7.12
Organic Carbon (%)	0.52	0.56	0.95	0.4
Total Nitrogen (%)	0.074	0.08	0.136	0.056
P (mg/kg)	17.25	18.52	16.81	12.52
OM (%)	0.9	0.95	1.64	0.68
K (CMol/kg)	0.64	0.66	0.56	0.17
Na (CMol/kg)	1.02	1.22	1.57	0.7
Ca (CMol/kg)	12.8	13.4	10	6
Mg (CMol/kg)	1.82	1.92	0.25	0.51

Note: L1 = Location 1; L2 = Location 2

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

Table 2: Morphological and Microscopic Characteristics of the various colony Isolated from Soil Samples Cultivated from the Locations.

AOC	Colony Morphology Observed	Colony Microscopy Observed	Fungi Identified
L1C	Greenish colony growth	Upright conidiophore glucose one-celled.	*Aspergillus niger*
	Dark Brown to Black	Conidiophore hyaline upright.	*Aspergillus flavus*
	Greenish colony growth	Ellipsoidal conidiophore	*Fusarium* sp
	White colony growth	Conidiophores are loosely unbranched.	*Mucor* sp
	Greenish colony growth	Conidiophore hyaline much-branched	*Trichoderma* sp
	Green fast-growing patches were first white and later developed to yellowish green compact.	Irregular cornidiosphore …	*Penicillium* sp
	Greenish colony growth	Glubose cornidia shape	*Rhizopus* sp
L1U:	Green fast-growing patches were first white and later developed to yellowish green compact.	Conidiospore hyaline is much branched.	*Trichoderma* sp
	Greenish colony growth	Conidiospore hyaline upright.	*Aspergillus flavus*
	Dark brown to black	Upright conidiospore glucose one-celled	*Aspergillus niger*
L2C:	Dark brown to black	Upright conidiospore glucose one-celled.	*Aspergillus niger*
	Greenish colony growth	Conidiospore hyaline upright.	*Aspergillus flavus*
	Green fast-growing patches are first white and later develop to yellowish compact.	Conidiospore hyaline is much branched.	*Trichoderma* sp
	White colony growth	Ellipsoidal conidiospore.	*Fusarium* spp
	Greenish colony growth	Conidiospore loosely unbranched.	*Mucor* sp
L2U:	Yellowish brown colony.	Upright conidiospore glucose one-celled.	*Aspergillus fumigates*
	Light green growth	Conidiospore hyaline upright.	*Aspergillus flavus*
	Dark brown to black	Upright conidiospore glucose one-celled.	*Aspergillus niger*
	Blue-green colour	Irregular conidiospore.	*Penicillium* sp
	Black in colour	Glucose conidia shape.	*Rhizopus* spp
	White colony growth	Ellipsoidal conidiospore.	*Fusarium* spp
	Greenish colony growth	Conidiospore are loosely unbranched.	*Mucor* spp

Key: AOC: Area of Collection, **L1C:** Location 1 (L1) Cultivated, L1U: Location 1 (L1) Uncultivated, L2C: Location 2 (L2) Cultivated, L2U: Location 2 (L2) Uncultivated

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

Table 3: Frequency of isolation (%) of the fungal species isolated from the soil samples

Soil Sample	Fungal Species	Frequency of Isolation (%)
Location 1 (L1) Cultivated		
	Aspergillus flavus	2
	Aspergillus niger	29
	Fusarium spp	10
	Mucor sp	5
	Penicillium sp	2
	Rhizopus spp	22
	Trichoderma spp	29
Location 2 (L2) Cultivated		
	Aspergillus flavus	20
	Aspergillus niger	52
	Fusarium spp	20
	Mucor spp	6
	Trichoderma spp	2
Location 1 (L1) Uncultivated		
	Aspergillus flavus	8
	Aspergillus niger	58
	Trichomonas spp	34
Location 2 (L2) Uncultivated		
	Aspergillus flavus	16
	Aspergillus fumigates	5
	Aspergillus niger	27
	Fusarium spp	27
	Mucor spp	5
	Penicillium spp	8
	Rhizopus spp	12

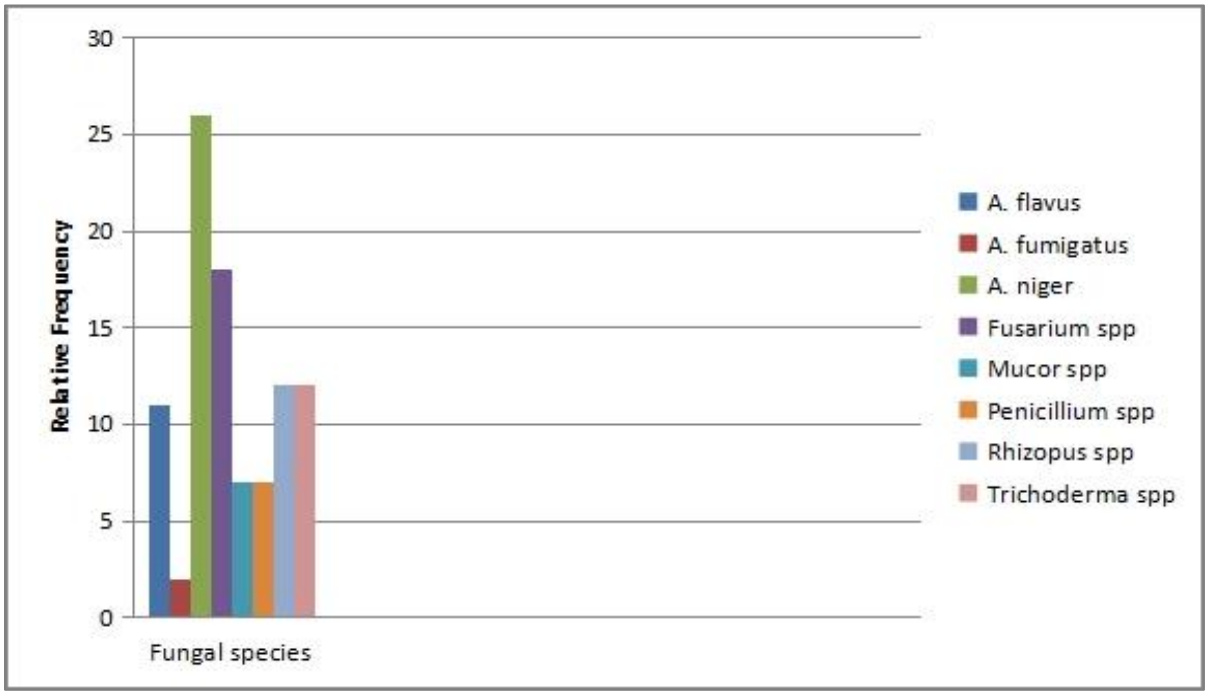

Figure 1: Relative Frequency of Isolated Fungi in all Soil Samples.

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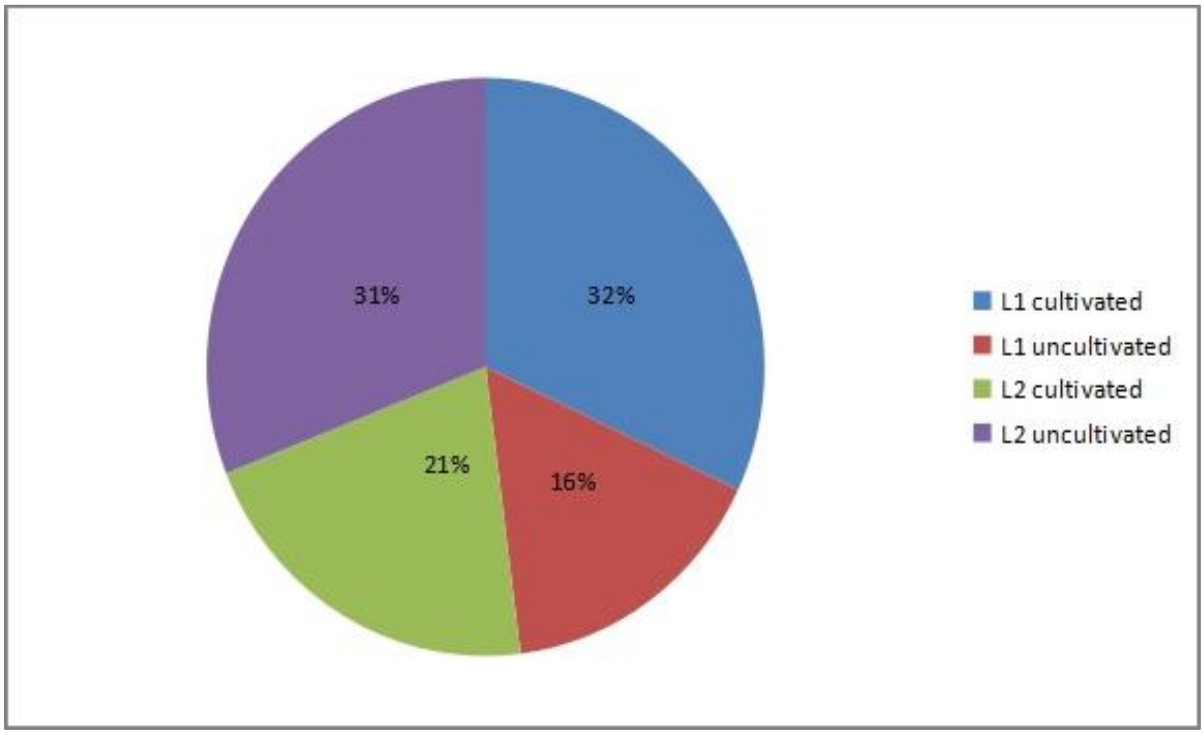

Figure 2: Percentage Frequency of Fungi per Sample

DISCUSSION

The findings of this study shed light on the fungal diversity within both cultivated and uncultivated soils, revealing the significant influence of soil pH, organic matter, and moisture content on fungal abundance and distribution. Our results are consistent with prior research by Dimkpa and Orikoha (2021), which emphasized the importance of these environmental factors in shaping fungal communities.

Our study corroborates the observations of Dong et al. (2004) and Aziz and Zainol (2018), highlighting the critical role of physicochemical parameters, including pH, organic matter, and moisture content, in governing the distribution of soil microorganisms, particularly bacteria and fungi.

The prevalence of fungal species observed in our study can be attributed to their ubiquitous nature in natural environments and their capacity to produce toxins and mycotoxins, potentially inhibiting the growth of other fungal species. These findings align with the work of Saravanakumar and Kaviyarasan (2010), who similarly identified Aspergillus species as dominant within soil samples.

Our study reveals the nuanced relationship between soil pH, moisture content, and fungal growth. We observed that alkaline soil conditions and low moisture content may not favor fungal proliferation, highlighting the intricate interplay between environmental factors and fungal community dynamics.

CONCLUSION

This study identified eight fungal species from the sampled soils: Aspergillus, Fusarium, Rhizopus and Trichoderma spp. Being the most frequently isolated. Soil pH, organic matter, and moisture content influenced the predominance and diversity of the mycoflora. These soils' diverse and abundant mycobiota are crucial in nutrient cycles and soil health. Further research is recommended to evaluate the impact of these isolated fungi on soil productivity, to determine whether their presence has a positive or negative effect.

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