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Isolation and Identification of Soil Mycoflora in the Upland and Lowland Soils of Usmanu Danfodiyo University, Sokoto, Sokoto State

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Authors' contributions

This work was carried out in collaboration between all authors. Author IYT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KS, HMM and SSN managed the analyses of the study. Authors SAY, MU and NA managed the literature searches and laboratory analyses. All authors read and approved the final manuscript.

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ABSTRACT

A total of 14 different fungal species belonging to 7 genera were isolated from the upland and lowland soils of Usmanu Danfodiyo University, Sokoto, between October to December, 2015. The mycoflora were isolated using dilution plate technique on Potato dextrose agar amended by 1% streptomycin. Identification was made microscopically using the lacto-phenol cotton blue method and macroscopically by comparing the cultural and morphological features with the help of authentic fungal manual and taxonomic key. The identified species are; *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. oryzae*, *Alternaria longifes*, *Fusarium oxysporum*, *F. solani*, *F. mangifera*,

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Rhizopus stolonifer, R. orizae, Saccharomyces cerevisae, Trichoderma harzianum and Trichophytum quallinum. The highest number of fungi (50 isolates) were obtained from lowland uncultivated soils (D), followed by the upland uncultivated soils (B) with about 34 strains, and the least (22 isolates) were obtained from upland cultivated soils (A) out of the total 132 isolates. Variations between sites were statistically analyzed.

Keywords: Upland; lowland; mycoflora; isolation; identification; Danfodiyo.

1. INTRODUCTION

The soil is a very complex environment that creates numerous barriers in the isolation, identification and quantification of soil-borne fungi, some of which are beneficial or otherwise [1]. Soil mycoflora plays a pivotal role in the evaluation of soil conditions and stimulating the wellbeing of plants [2]. Soil is characterized as the most precious natural resources and contained the most diverse assemblage of living organisms [3].

Fungi are very important in the soil ecosystem, as they play a vital role in the decomposition of organic matter and release of plant nutrients into the ecosystem for other organisms to use [4]. Fungi formed the major group of organotrophs; responsible for the decomposition of organic compounds as they act in the biodeterioration and biodegradation of many substances in the soil [5]. Some of the fungi formed mycorrhizal associations with plant roots, where the plant provides the fungi with food (Sugars and other roots exudates) while the fungi provide the plants with enhanced availability of plants' nutrients such as phosphorus, zinc, calcium, magnesium, manganese, iron and also confer resistance against diseases and help in drought tolerance [6]. However, some soil-borne fungi are plant pathogenic that creates major economic losses in many important crops [7]. Young tissues of plants are infected and affected much more severely by these pathogens [8].

2. MATERIALS AND METHODS

2.1 Study Site and Locations

The study was conducted at Usmanu Danfodiyo University, Sokoto, a main campus which is located at longitude 5º11" 30' E and 5º14" 30' E and latitudes 13º8" 30' N and 13º7" 0' N [9]. The research area (upland and lowland) are located at about ten kilometers north of Sokoto metropolis. Sokoto State lies on an altitude of 308 m above sea level, within the Sudan savanna belt [10,11]. The climate is a hot semi-arid type and characterized by long dry season from October to May and short rainy season from June to September, with an annual mean rainfall of 724mm for a period of six years [11,12]. Mean annual temperature fluctuates roughly between 45ºC maximum and 15ºC minimum [13].

2.2 Method of Soil Sample Collection

The soil mycoflora were isolated from soil samples that were randomly collected using sterile soil auger at a depth of 0-15 cm from twenty-four (24) different locations, twelve (12) each from the lowland and upland areas (i.e, six from cultivated and uncultivated of each site respectively). The soil samples were placed into polythene bags, properly labelled and transported to Mycology laboratory, Biological Sciences Department, Usmanu Danfodiyo University, Sokoto within one hour of collection for fungal analysis following modified techniques of Gaddeyya et al. [2] and Durowade et al. [14] Table 1.

2.3 Isolation and Sub-culturing of Soil Mycoflora

Soil mycoflora were isolated using soil dilution plate method as described by Sajid and Bihar [7] and [15,16] with slight modifications. Exactly, 1g of the soil sample was weighted and suspended in 10 ml of sterile distilled water, which was used also in making a serial dilution of (10^{-1} to 10^{-3}), and diluent of 10^{-3} was used for the fungal isolation. One millilitre (1 ml) of the serially diluted suspension (10^{-3}) concentration was plated onto sterile Petri dishes (in triplicate) each of which contained 15ml of sterile Potato Dextrose Agar (PDA) amended with 1% Streptomycin to prevent bacterial growth. The inoculum was then incubated at 28±2ºC for 5 days. However, all the fungal isolates were continuously sub-cultured until after the pure cultures were obtained.

Table 1. Soil samples collected from different locations of Usmanu Danfodiyo University, Sokoto

Samples no.	Nature of field	Place of collection
1	Upland cultivated	Agric. dry land farm
2	Upland non-cultivated	Agric. dry land
3	Lowland cultivated	Kwalkwalawa
4	Lowland non-cultivated	Kwalkwalawa

2.4 Identification of the Soil-borne Fungi

All the fungal pure culture were studied macroscopically (morphologically) by observing their colony features (colour and texture) and then microscopically by staining with lacto-phenol cotton blue after inoculating the hyphal fragment onto a grease free sterile glass-slide and covered with a coverslip. The conidia, conidiophores and arrangement of spores of each, were observed through the microscope (X40 and X100 magnification) comparing with mycological atlas and taxonomic key for their identification [7,17,18,19,20] and 21].

2.5 Determination of Physico-chemical Parameters of the Soil Samples

The physicochemical parameters of the collected soil analyzed include; moisture contents, organic carbon, nitrogen, pH, available phosphorus, exchangeable potassium, iron, copper and zinc were analyzed. Macro nutrients such as, nitrogen, phosphorus, potassium and carbon were analyzed using the procedures adopted from [22]. Temperatures of the soil sample were determined using mercury dry-thermometer at the field as described by [16,23]. As shown in Table 2.

2.6 Statistical Analysis

Number of colonies per plate in a single gram (1 g) of soil was calculated to have the percent contribution of each isolate using the following formula (a), also the frequencies of occurrences of each individual isolate as well as the genus were also calculated using the formula (b) as expressed below:

$$\%\ \text{contribution} = \frac{\text{Total no.of CFU of an individual species}}{\text{Total no.of CFU of all species}} \times 100 \quad \text{(a)}$$

CFU- Colony Forming Unit

$$\text{Frequency of occurrence} = \frac{\text{Number of individual species isolated}}{\text{Total number of all the species isolated}} \times 100 \quad \text{(b)}$$

3. RESULTS AND DISCUSSION

3.1 Physico-chemical Properties of the Soil

The texture of the soils varied from sand to sandy loam. Table 2 below showed that soil pH and Mg were significantly higher ($P< 0.05$) in lowland cultivated soil sample (C) (pH= 8.3, Mg=1.33 cmol/kg) when compared with upland cultivated (A), upland uncultivated (B) and lowland uncultivated (D). No significant difference was observed in soil pH and Mg among the Upland cultivated (A), Upland uncultivated (B) and lowland uncultivated soil samples. Organic carbon (OC), Nitrogen (N), potassium (K) and CEC values differ significantly ($P<0.05$) among the treatments with lowland uncultivated (D) having higher values (OC= 1.24%, N= 0.11%, K= 0.95 cmol/kg, and CEC= 6.68 cmol/kg) than, upland cultivated (A), upland non-cultivated (B) and lowland cultivated (C) soil samples. Soil pH, moisture, organic content, percentage nitrogen, among other physico-chemical properties of the soil were the main factors affecting the soil fungal population and diversity which corresponds to the findings of [24,25,26,27] whom did their research in different part of Xiaolongshan, Tianshui city and also [5,23] that did theirs in Tamil-Nadu Southern India found out that physico-chemical parameters are having a lot of effects on the distribution of soil microorganisms especially the bacteria and fungi.

3.2 Percentage Contribution and Frequency of Occurrence of the Isolated Fungi

Table 3 shows the percent contribution as well as the number of fungal isolates. The highest number of fungi (50 isolates) were obtained from lowland uncultivated soils (D), followed by the upland uncultivated soils (B) with about 34 isolates, and the least (22 isolates) were obtained from upland cultivated soils (A) out of the total 132 isolates. However, *Fusarium*

Table 2. Physico-chemical properties of the soil collected in UDUS Dry land farm and lowland farm between October to December

Treatment	pH	Moisture (%)	OC (%)	N (%)	PMg/Kg	Ca (Cmol/Kg)	Mg (Cmol/Kg)	K (Cmol/Kg)	Na (Cmol/Kg)	CEC (Cmol/Kg)
A	7.4±0.10^{b}	1.0±0.02^{c}	0.32±0.02^{d}	0.06±0.00^{d}	0.79±0.12^{c}	1.25±0.07^{b}	0.60±0.07^{b}	0.46±0.02^{d}	0.43±0.05^{c}	5.26±0.05^{d}
B	7.5±0.20^{b}	1.0±0.02^{c}	0.56±0.02^{c}	0.07±0.00^{c}	0.94±0.04^{b}	1.35±0.06^{a}	0.60±0.03^{b}	0.56±0.04^{c}	0.48±0.02^{c}	5.42±0.09^{c}
C	8.3±0.20^{a}	1.5±0.03^{b}	1.08±0.02^{b}	0.10±0.00^{b}	1.12±0.06^{a}	1.05±0.03^{d}	1.33±0.55^{a}	0.87±0.04^{b}	0.74±0.03^{b}	6.52±0.07^{b}
D	7.2±0.26^{b}	2.0±0.06^{a}	1.24±0.05^{a}	0.11±0.00^{a}	1.16±0.03^{a}	1.15±0.02^{c}	0.70±0.06^{b}	0.95±0.04^{a}	0.91±0.03^{a}	6.68±0.03^{a}
(SE)	0.14	0.05	0.11	0.00	0.05	0.36	0.12	0.06	0.06	0.07
Sig.	*	*	*	*	*	*	*	*	*	*

Values are expressed as means ± SE of n=3, values with the same letter(s) along the column are statistically similar (P<0.05)

Table 3. Percent contribution of the isolated fungal species according to the sampling sites

		Number of isolates of individual species														
S/N	**Sites**	***Aspergillus***					***Alternaria***	***Fusarium***			***Rhizophus***		***Saccharom***	***Trichoder***	***Trichophyt***	**Total**
		A. n	***A. f***	***A. fl***	***A. ni***	***A. or***	***Alt***	***F. o***	***F. ma***	***F. so***	***R. st***	***R. or***	***S. cer***	***T. herz***	***T. qual***	
1	**A**	2	1	0	1	2	1	2	2	2	3	0	1	2	3	**22**
2	**B**	3	2	2	2	3	2	3	3	3	3	1	2	3	2	**34**
3	**C**	3	2	1	2	2	2	1	3	1	2	1	2	2	2	**26**
4	**D**	4	3	4	3	4	4	4	5	2	4	1	4	4	4	**50**
	Total	**12**	**08**	**07**	**08**	**11**	**09**	**10**	**13**	**08**	**12**	**03**	**09**	**11**	**11**	**132**
	%Contribution	9.09	6.06	5.30	6.06	8.33	6.82	7.58	9.85	6.06	9.09	2.27	6.82	8.33	8.33	100

*Key: **A**= Upland Cultivated Soil; **B**= Upland uncultivated; **C**= Lowland Cultivated; **D**= Lowland uncultivated*
***SE**= Standard Error, **Sig.** = Significance. *= Significant at 0.05 Confidence level*

Table 4. Frequency of occurrence of soil-borne fungi of Usmanu Danfodiyo University, Sokoto dry land farm and lowland farm

S/No	Identified fungi	Occurrence times	Frequency occurrence (%)
	***Aspergillus* species**	**46**	**34.85**
1.	*A. niger*	12	09.09
2.	*A. fumigatus*	08	06.06
3.	*A. flavus*	07	05.30
4.	*A. nidulans*	08	06.06
5.	*A. oryzae*	11	08.33
	***Alternaria* species**	**03**	**02.27**
6.	*A. longifes*	03	02.27
	***Fusarium* species**	**27**	**20.45**
7.	*F. oxysporum*	10	07.58
8.	*F. solani*	08	06.06
9.	*F. mangifera*	09	06.82
	***Rhizopus* species**	**25**	**18.94**
10.	*R. stolonifa*	12	09.09
11.	*R. oryzae*	13	09.85
	***Saccharomyces* spp.**	**09**	**06.82**
12.	*S. cerevisae*	09	06.82
	***Trichophytum* spp.**	**11**	**08.33**
13	*T. quallinum*	11	08.33
	***Trchoderma* spp.**	**11**	**08.33**
14	*T. harzianum*	11	08.33
	Total Isolates	**132**	**100.00**

magnifera show the highest percent contribution (9.85%), followed by *R. stolonifer* and *Aspergillus niger* having 9.09% each, then *Aspergillus oryzae*, *Trichoderma harzianum* and *Trichophytum quallinum* with 8.33% each. The least percent contribution was obtained in *Rhizophus oryzae* (2.27%). Meanwhile, in Table 4, *Aspergillus* species was found to be the most occurring and abundant fungi with 34.85% frequency of occurrence out of the total population of all the isolated fungal species, followed by *Fussarium* spp. (20.45%), these findings also corroborates with that of [15] who reported *Aspergillus* species as the most abundant in the soil and accounted for 71.74% and also a little higher of *Aspergillus niger* (10.46%) than what was obtained (9.09%) in this research, and also domination of the community by *Aspergillus* species is somehow similar to the findings of [23] in the same research. The domination and abundance of species might be attributed to their ubiquitous distribution in nature and also to their ability to produce toxins and mycotoxins which may prevent the growth of other fungal species [28]. Meanwhile, *Aspergillus* species's ability to withstand all sort of environmental stress ranging from temperature, humidity, salt concentration and also considering the endemic nature of their spores [8].

4. CONCLUSION

This study revealed that all the four locations were rich in fungal diversity and lowland uncultivated soils enveloped more colonies (51), followed by upland uncultivated soils (35). Moreover, among all the fungal species isolated, *Aspergillus* species and *Fusarium* species were the most dominant. It was observed from the study, that the soils of Usmanu Danfodiyo University, Sokoto are low in nutrient and slightly-higher in organic carbon which influenced the diversity of fungi. Meanwhile, farming activities too influenced the fungal diversity as a high number of species were obtained in the cultivated lands.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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