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Original Research Article

ISOLATION AND CHARACTERIZATION OF FUNGI FLORA FROM THE SOIL SAMPLES OF ADEKUNLE AJASIN UNIVERSITY, AKUNGBA-AKOKO STAFF SCHOOL PLAYING GROUND.

***Olajubu, F. A. and Folorunso, V. T.**

Department of Microbiology, AdekunleAjasin University,
Akungba-Akoko, Nigeria

Abstract:
Soil is an unconsolidated or loose combination of organic and inorganic materials that support the survival and multiplication of various pathogenic and non-pathogenic microorganisms including fungi. The school playing ground is often the delight of school children as they enjoy playing with little or no supervision. This study was then designed to examining the soil samples of the playing ground to ascertain the possibility or otherwise of the children picking harmful fungi from the soil of their playing ground while recreating. The playing ground was mapped out into five regions namely West (Ws), East (Es), North (Ns), South (Ss) and Central (Cs) for sample collection. The soil plate method and hair baiting technique were used for the isolation of the fungi while the identification of the isolates was done through colonial morphology (macroscopy) and microscopy. The soil sample label Cs, collected from the Central part of the playing ground yielded more fungi species(7). The most frequently isolated fungus was *Aspergillus niger*. Other saprophytes isolated includes *Penicillium*sp, *Fusarium*sp, *Rhizopusstolonifer and Alternariaalternata* Somekeratinophiles isolated include *Chrysosporium indicum, Trichophytonmenta grophytes, Fusarimsolani* and *Microscoporium gypseum*. Since some of these fungi species have been incriminated in some disease conditions and there is need to maintain the soil's microbiota, it is suggested that the students be well guided as regard developing and maintaining simple hygienic culture and not in support of any form of soil treatment.

Key words: Playing ground; fungi; keratinophiles; AAUA; Staff school.

For Correspondence:
olajubufaATyahoo.com
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Introduction

The incidence of fungi infections is increasing at an alarming rate, presenting an enormous challenge to healthcare professionals. This increase is directly related to the growing population of immunocompromised individuals including children and the use of intensive

chemotherapy and immunosuppressive drugs (Akansha *et al*., 2010). Fungi are chemoheterotrophic eukaryotes that are ubiquitous inhabiting among other things the soil where they serve as decomposers (Gadd, 2007). During growth, fungi produce bioactive compounds which could be useful as vital biocontrol substances in agricultural sector or as antimicrobials in the pharmaceutical sector (Duart *et al*., 2006; Fapohunda *et al*., 2007). Of great importance among soil fungi are the keratinophiles. Keratinophilic fungi are responsible for the breakdown of Keratinaceous substrates and are present in soil environment worldwide as reported bySharma and Sharma (2010),Gugnani *et al*., (2012) and Agu *et al*., (2013). Among commonly isolated Keratinophiles are *Trichophytonmenta grophites, Trichophyton terrestre, Microsporum gypseum, Chrysosporium keratinophilium, Tinea corporis, Malassezia furfur, Fusarium solani* and *Geotrichum* sp. (Gugnani *et al*., 2012; Maruthi *et al*., 2012) The occurrence of these organisms in nature, man-made habitats and their affinity for keratin has been reported (Sharma and Sharma, 2010; Agu *et al*., 2013) Among the keratinophilic fungi are the dermatophytes which cause various mycotic diseases in both man and animal, thus of great medical importance (Yuen *et al*., 1999; Mahmaudabodi and Zarrin,2008). Keratinophilic fungi can be considered as a potential pathogen, though majority of dermatophytes can live saprophytically. Medically important saprophytes include *Coccidoidesimmitis, Histoplasma capsulatum, Aspergillus* sp. and *Candida* sp. School playing grounds are often invaded by animals such as birds, dogs, cats and rats that usually contaminate the soil with propagules of these pathogenic fungi. Unfortunately, this is usually the part of the school environment where students play unrestricted and unsupervised coupled with the fact that children have strong affinity forplaying with soil. These activities often results in soil contaminated hands, legs, faces, heads and sometimes bruising of these body parts.

There has been, however, no record of study of fungal diversity of soils of AdekunleAjasin University (AAU) staff school playground. In view of its importance, this study was designed to examine the fungal diversity of the soil of AAU staff school playground with specific reference to the prevalence and distribution of geophilickeratinophiles and related dermatophytes in the soil samples of the playground.

Materials And Methodologies

Study Sites

AdekunleAjasin University Staff school is located at the North-Eastern part of University which is situated at Akungba-Akoko in Akoko South West Local government area of Ondo state with latitude 7^0 31'N and 5^0 45'E longitude.

Collection of Soil Samples

Surface soil samples of a depth not more than 1-2cm were collected in sterile tightly closed polythene bags from five (5) major sites (East, West, North, South and Central) of the AAU Staff school playground. Each sample was properly labelled and processed in the laboratory within the same day of collection. When this is not possible, the samples were stored at 4^0C until processed usually within two or three days. Soil samples were collected during the raining season (July – September, 2012) and the dry season (December – January, 2013).

Moisture Content and pH Determination

The pH and the moisture contents of the soil samples were analysed using the methods of Jones (2001) and Topp (1993) respectively.

Mycological Analysis

The soil dilution technique of James and Sutherland (1939) was employed in the determination of soil fungal flora. Ten grammes (10g) of each soil sample was dissolved in 90mls of Peptone water and serially diluted. One millilitre (1ml) of the dilutions were asceptically introduced into Petri dishes containing 19mls of molten Potato Dextrose Agar (Lab M, UK) fortified with 0.05mg/ml of chloramphenicol, mixed, allowed to set and incubated at 28^0C for 3 – 7 days. Average colony counts were taken at the 3rd day of incubation for fast growing fungi and day 6 for the slow growing ones. The isolates were

purified by sub-culturing on fresh PDA plates and the pure isolates were stored on PDA slants.

Identification of the isolates

This was done by macroscopic and microscopic observations using standard mycological textbooks and atlas for the description, illustrations and pictures of common fungi (Bulmer, 1978;Paul and Yu, 2008; Sharma and Sharma, 2010)

Baiting of the Soil Samples

Hair baiting technique described by Vanbreuseghen (1952) was adopted. Each soil sample was thoroughly homogenised and 10mg portion of the soil samples were placed in different Petri dishes. Bits of sterilized human hair and nail were used as baits. These were scattered uniformly on the moistened soil samples. Each of the Petri dishes were separately labelled indicating the date, site of collection and time of incubation. These Petri dishes were then incubated at room temperature (28^0C) for 30 days but with regular checking at every 3 days interval, checking for fungal growth on the baited hair and nails. Samples with visible fungal growth were subcultured to a fresh plate of SDA for purification and identification.

Results and Discussion

The phisico-chemical properties of the soil samples are as shown in Table 1. The soil texture reveals sandy environment with little combination of clay toward the Western and Central parts of the field and loamy soil towards the East – South axis. This texture is very suitable for a playground as it allows the children to play all year round because they are neither waterlogged during raining seasons nor too dusty during the dry season. The pH of the soil samples ranged between 6.00 in the West and 8.75 in the Central during raining season and between 6.8 in the West and 8.11 in the Central during dry season. However, there is no significant difference in the seasonal variation of the pH. Similar pH levels have been reported by Ezekiel *et al.,* (2009) and Neetu and Sharma (2011). The highest percentage of moisture content (18.2%) was observed in the soil sample from the West during raining season while the least (0.8%) was observed in the Northern part of the playground. This variation is expected as rain moistened the soil and not all the water gets evaporated unlike during the dry season. However, the moisture content favours the growth of both the keratinophiles and the saprophiles. No effect of seasonal variation was observed in the diversity ofsaprophitic fungal species isolated from the different soil samples, hence Table 2 shows the different species of fungi isolated, the colony count in relation to sites of sample collection. *Aspergillus niger* (a saprophyte) was isolated from all the soil samples with average colony count ranging between 3 and 5 per milligram (mg) of soil sample. *A. niger* which has been incriminated in wound infections and respiratory problems (Thomas *et al*., 2002; Akansha *et al*., 2010) has also been isolated from soils of school playing grounds and hostel environments by many researchers (Agu *et al*., 2013; Rizwana *et al*., 2012; Ezekiel *et al*., 2009). Other saprophytes isolated include *Aspergillus flavus, Penicillium* sp, *Rhizopus stolonifer* and *Mucor*sp.which have equally been reported from other studies on soil (Ezekiel *et al*., 2009; Sharma and Sharma, 2010; Maruthi *et al*., 2012) with worldwide distribution. The importance of *Penicillium*sp in the production of antibiotic has been documented (Yuen *et al*., 1999) and the decomposing role of these saprophytes is of importance to the maintenance of the ecosystem. However, fungi species like *Aspergillusflavus* and *Fusarium* sp have been reported for mycotoxin production (Adejumo*etal*., 2009). Mycotoxins are extracellular substances produced by fungi that cause neurological disorders and cancer. Hence, the isolation of these fungi is equally of medical importance. *Microsporum gypseum*, *Trichophytonmenta grophytes*, *Fusarium solani* and *Chrysosporium indicum* are the Keratinophilic fungi isolated from soil samples. Except for *M. gypseum* which was isolated from the Central site of the playing ground during raining season, all other Keratinophiles were isolated during the dry season as shown in Table 3. It is possible that the supposed luxuriant growth of the saprophytes during raining season might make the recovery of the Keratinophiles difficult. The Keratinophiles especially the dermatophytes are known to

cause various mycoses in both adult and children but more among immunocompromised children (Akansha *et al*., 2010). The Keratinophiles have affinity for the keratin present in hair, nails and skin of individuals which they use as substrate for growth, thereby becoming pathogenic to man and animal. Many studies have demonstrated the presence of these Keratinophileic fungi from the soil of playgrounds. Rizwana *et al* (2012) reported 86% prevalence rate of keratinophilic fungi from the soils of public parks and playgrounds of Riyadh, Saudi Arabia, Gugna *et al*., (2012) reported a prevalence of 45% and 69% from St Kitts and Nevis, all in the West Indies, Agu *et al*., 2013 reported a prevalence rate of 45% from schools playing grounds in Sagamu, Ogun State of Nigeria, 43.75% prevalence was reported by Maruthi *et al*., (2012) in India. However, in the present study, the prevalence rate of the keratinophiles is 15.4% which is relatively low when compared with other studies. However their presence calls for close monitoring. T. mentagrophytes was the most frequently isolated keratinophile as it was isolated from three (3) of the five (5) soil samples. Since there had not been any documentation about the diversity of fungi flora and the presence of Keratinophiles in the soils of AAU StaffSchool playing ground, this study provides a reference for close monitoring of the soil environment. The young and innocent children should also be taught about simple hygiene like hand washing and bathing each time they return from school as these measures will go a long way in protecting them from infections. The researchers are not recommending any form of soil treatment because of the adverse effect it will have on the micro-environment.

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Table 1.Some physico-chemical properties of the soil samples by location and season.

S/No	Location	Texture	pH		Moisture content (%)	
			Wet	Dry	Wet	Dry
1.	East	Sandy-loam	6.25	7.02	11.5	9.3
2.	West	Sandy-clay	6.00	6.81	18.2	12.2
3.	North	Sandy	7.25	7.53	1.5	0.8
4.	South	Sandy-loam	6.45	7.02	15.1	10.1
5.	Central	Sandy-clay	8.75	8.11	12.4	10.2

Table 2.Diversity of fungal species in the different sites of the playing ground.

Location	Growth code	Fungi species	No of colonies/mg
East	Es1	*Aspergillus niger*	5
	Es2	*Aspergillus flavus*	3
	Es3	*Penicillium* sp	1
	Es4	*Microsporium gypseum*	1
	Es5	*Chrysosporium indicum*	1
	Es6	*Trichophyton mentagrophytes*	1
	Total	**6 Species**	**12 colonies**
West	Ws1	*Alternaria alternate*	2
	Ws2	*Rhizopus stolonifer*	2
	Ws3	*Aspergillus niger*	3
	Ws4	*Mucor* sp	2
	Ws5	*Geotrichum* sp	1
	Total	**5 Species**	**10 colonies**
North	Ns1	*Aspergillus niger*	3
	Ns2	*Trichophyton mentagrophytes*	1
	Ns3	*Aspergillus flavus*	2
	Ns4	*Penicillium* sp	1
	Ns5	Unidentified	2
	Total	**5 Species**	**9 colonies**
South	Ss1	*Aspergillus niger*	4
	Ss2	*Penicillium* sp	2
	Ss3	*Mucor* sp	2
	Ss4	*Fusarium solani*	1
	Ss5	Unidentified	1
	Total	**5 Species**	**10 colonies**
Central	Cs1	*Microsporium gypsium*	1
	Cs2	*Aspergillus niger*	3
	Cs3	*Rhizopus stolonifer*	2
	Cs4	*Penicillium* sp	1
	Cs5	*Trichophyton mentagrophytes*	1
	Cs6	*Mucor* sp	2
	Cs7	Unidentified	1
	Total	**7 Species**	**11 colonies**

Table 3. Distribution of the keratinophilic fungi in the soil samples

Location	Seasons	No and types of keratinophiles				
		M. gypseum	*C. indicum*	*T. mentagro*	*F. solani*	*Geotrichum*sp
East	Raining	0	0	0	0	0
	Dry	1	1	1	0	0
West	Raining	0	0	0	0	0
	Dry	0	0	0	0	1
North	Raining	0	0	0	0	0
	Dry	0	0	1	0	0
South	Raining	0	0	0	0	0
	Dry	0	0	0	1	0
Central	Raining	1	0	0	0	0
	Dry	0	0	1	0	0