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Occurrence of Pathogenic Fungi in Soil from Pre-Primary School Playgrounds in Birnin Kebbi

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Abstract
A study was conducted to determine the predominant pathogenic fungi from 5 selected pre-primary school playgrounds. The five (5) schools considered for this study were identified as PPSA, PPSB, PPSC, PPSD, and PPSE. Using stratified method, 15 soil samples were collected from each playground. Standard procedures were employed in the isolation of the fungal species; Atlas of mycology was used to identify the isolates based on their macro and micromorphologies. Pathogenic fungi isolated include *Microsporum gypseum* (16.13%), *Microsporum canis* (12.90%), *Aspergillus flavus* (6.45%), *Aspergillus niger (9.68%), Aspergillus fumigatus* (6.45%), *Rhizopus stolonifer* (6.45%), *Fusarium sp*. (6.45%), *Trichophyton rubrum* (9.68%), *Trichophyton mentagrophytes* (9.68%), *Penicillium sp*. (9.68%), and *Alternaria sp*. (9.68%). *Microsporum gypseum* was isolated from all the five schools. The presence of these pathogenic fungi from all the study sites heightens public health concern.
Key words: Pathogenic fungi, *Aspergillus*, soil

INTRODUCTION

Fungi are an important component of the soil *microbiota* typically constituting more of the soil biomass than bacteria (Mukerji *et al.,* 2006). The saprobic fungi represent the largest proportion of fungal species in soil and they perform a crucial role in the decomposition of plant structural polymers, such as, cellulose, semicellulose, and lignin, thus contributing to the maintenance of the global carbon cycle (Karesh and Sharma, 2014). Many species of fungi have harmful consequences in both agriculture and medicine (Mukerji *et al.,* 2006). Out of approximately 1.5 million species of fungi, about 300 are identified as aetiological agents for *Aspergillosis, candidiasis, mucormycosis, blastmomycosis, coccidioidomycosis, histoplasmosis* and eye infections (CDC, 2012). School play grounds, public parks are often occupied by animals and humans there by leaving organic residues such as nails and hair which may contaminate the soil with keratinous debris. Generally, *Dermatophytes* are anthropophilic or zoophilic in their natural habitat, whereas some occur in soil as saprophytes and are termed geophilic, for example, *Microsporum gypseum* and *Trichophyton terrestre* (Gugnani, 2000). Non-dermatophytic keratinophilic fungi, including species of *Chrysosporium* and other genera of fungi, are known to occur as saprobes in soil; some of them are potential pathogens for humans and animals (Gugnani, 2000; DeHoog *et al.,* 2000). Most cutaneous infections are caused by homogeneous group of keratinophilic fungi known as *Dermatophytes* (Boni, 1998). *Dermatophytes* have the capacity to invade keratinized tissue of the body including skin, hair and nails (Mohamed and Ali-Shtayeh, 2000). This study was conducted to determine the occurrence of pathogenic fungi in soil samples from playgrounds of some selected pre-primary schools in Birnin Kebbi, Kebbi Sate, Nigeria.

MATERIALS AND METHODS

Samples Collection and processing

Using sterile soil augers, hand trowel and polythene bags, fifteen (15) soil samples were collected from each sampling site, making a total number of 75 soil samples (Akinyanju and Fadayomi, 1989). Serial dilution was carried out (James and Natalie, 2011).

Isolation of fungi

Using a sterile pipette 1ml from 10^{-4} dilution was radially streaked onto the surface of plates containing SDA medium. The plates were then incubated for 72 hours at 28 ± 2° C for fungal growth (Kane, 1997). A distinctive fungal colony was carefully picked using a sterile wire loop and transferred onto fresh PDA medium supplemented with 1.0 mg/ml chloramphenicol.

This procedure was carried out repeatedly until pure cultures were obtained (Barnet and Hunter, 1999; Alexopoulus *et al*. 2002).

Characterization of fungi

The isolates were identified on the basis of macro morphological and micro morphological characteristics using light microscope and standard mycological manuals (Barnett and Hunter, 1999; Kane, 1997). A colony was picked and placed on a microscope slide and 2 drops of lactophenol was applied. The slide was covered with cover slip and the smear was examined using ×40 objective lens.

RESULTS

A total of 11 species belonging to 7 genera; *Microsporium, Penicillium, Aspergillus, Tricophyton, Rhizopus, Fusarium, and Alterneria* were identified from the five sampling sites. *Microsporum gypseum* (16.13%) was isolated from all the five sampling sites, *Microsporum canis* (12.90%), *Aspergillus flavus, A. fumigatus, R. stolonifer,* and *Fusarium sp.* (9.68%) each as their percentage frequency of occurrence, whereas *A. niger, T. rubrum, T. metagrophytes, Penicillim sp and Alternaria* with (6.45%) each.

Table 1 Colonial, Microscopic characteristics and Percentage frequency of the fungal isolates from five pre-primary school playground in Birnin Kebbi.

Colonial Morphology	Microscopic Characteristics	Isolates Identified	Frequency (%)
Rapid growth rate. powdery, flat, in texture. Thallus color; tan to cinnamon brown with cottony white center.	Many rough, thin walled conidia, with symmetrically rounded tip.	*M. gypseum*	5 (16.13%)
Rapid growth rate. Texture; silky to coarsely fluffy. Thallus color, white. Reverse, deep yellow. Slow growing, yellow surface, no reverse pigment, macroconidia absent.	Long rough, thick-walled macroconidia with asymmetrical knob on end. Nodular bodies, and presence of chlamydoapores.	*M. canis*	4 (12.90%)
Flat to slightly raised colonies on SDA, white to cream, suede-like to downy, with a yellow-brown to wine-red reverse.	Pyriform microconidia along unbranched hyphae.	*T. rubrum*	2 (6.45%)
Flat, white to cream in color with a powdery to granular surface with a yellow-brown to reddish-brown colour reverse pigmentation	Numerous single celled microconidia are formed. Hyaline, smooth-welled, and spherical microconidia	*T. mentagrophytes*	2 (6.45%)
Blue-green fluffy growth on plate	Blue-green conidiospores borne in multilink chains	*Penicillium sp*	2 (6.45%)
Black fluffy growth with white edges	Thick septate hyphae with conidia borne in	*Aspergillus niger*	2 (6.45%)

Table 1 continue

	chains from the sterigmata		
Grey-green fluffy growth	Septate hyphae with conidiospores borne on conidia	*A. fumigates*	3 (9.68%)
Granular, flat, yellow colonies at first but quickly bright to dark yellow to Lemon green powdery colonies	Green conidiospores with septate hyphae	*Aspergillus flavus*	3 (9.68%)
Rapid growth on SDA, woolly to cottony, flat, spreading colonies. From the front, white, cream, tan, salmon colonies. Colorless from the reverse.	Non – septate or scarcely septate broad hyphae, sporangia, and sporangiospores present; unbranched Sporangiophores brown in colour: round Sporangia with flattened bases.	*R. stolonifer*	3 (9.68%)
much septate on macroconidia with an elongated apical cell and pedicellate basal cell.	Microconidia formed on short/long simple conidiophores.	*Fusarium sp*	3 (9.68%)
Flat white cottony growth on plate	Erect conidiophores, septate hyphae with cylindrical conidia	*Alternaria sp*	2 (6.45%)

Among the sampling sites, PPSE appeared to be the most contaminated with nine (9) species of fungi isolated from the site followed by sampling site PPSA with seven (7) species. Whereas sampling site PPSB and PPSD were contaminated with fungi with six (6) fungal species respectively. Sampling site PPSC was the least contaminated with three (3) fungal species.

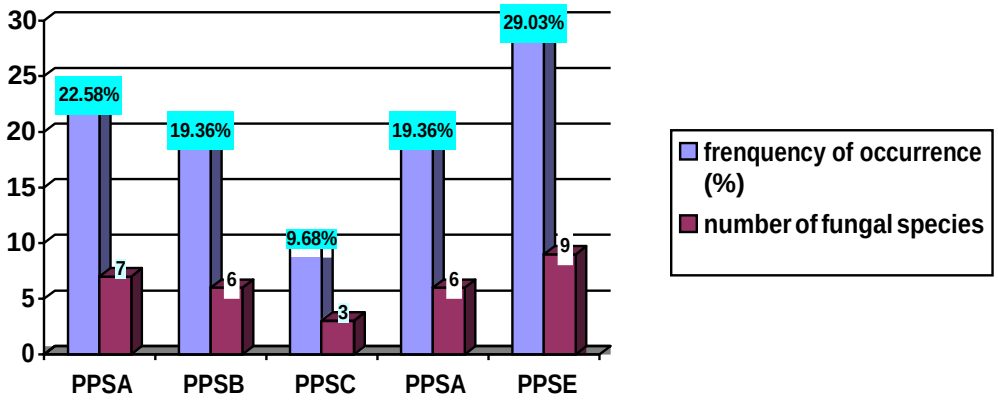

Figure 1 Frequency of occurrence of fungal species per sampling sites

KEYS:

PPSA; Pre-primary School A, PPSB; Pre-primary School B, PPSC; Pre-primary School C, PPSD; Pre-primary School D, PPSE; Pre-primary School E

DISCUSSION

The fungal isolates *Alternaria, Aspergillus*, and *Penicillium,* found in some of the schools' playgrounds, have been reported by Kashyap *et al.,* (2001) to be three of the four most common moulds that cause allergy. The isolation of *Aspergillus fumigatus*, that is known to produce Aflatoxin (that causes aspergillosis), is of medical importance. Other genera isolated include *Fusarium, Microsporium, Tricophyton, Rhizopus,* and *Fusarium,* Their common occurrence could be due to not only their high sporulating nature but their ability to grow well on laboratory media as suggested by the findings of Ekundayo (2004). From this study, it was found that no two study location had exactly same pattern of distribution of fungi. This corresponds with the findings of Anbu *et al.*, (2004) who reported that fungi are present in the environment with variable distribution patterns. The results of this study also showed that the study location PPSE was most contaminated with nine (9) fungal species isolated from the site. This can be attributed to the fact that the location is very close to a refuse dump site which can be a source of utilizable substrate for fungi, similar to findings of the Adams *et al.*, (1999) who reported that humus (organic matter) rich soils have large fungal population than soil poor in humus. Non occurrence of *Penicillium spp* in the study site PPSA, PPSC and of course PPSE could possibly be due to high alkaline condition existing in the study locations. This is similar to the findings of Ketchum, (1988) who reported that any unfavourable alkaline soil often inhibit the development of *Penicillium spp.* Overall, study site PPSC was the least contaminated.

Lower level of contamination observed from the site could possibly be due to the acidic nature of the soil from the site which is similar to results obtained by Andrew *et al.*, (2008). Finally, it is noteworthy that all the genera of fungi isolated from this study cause one form of disease or the other especially in children whose immune system is not fully developed.

CONCLUSION

It can therefore be concluded that, despite the importance of fungi in decompositions of organic matter in the soil, pathogenic fungi isolated from playgrounds of some selected schools in Birnin Kebbi is a threat to the health of pupil.

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