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Isolation and Identification of Air Borne Fungal Spores and Fragments in Buildings within Usmanu Danfodiyo University Sokoto, Nigeria

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Abstract - Indoor air contains a complex mixture of microorganisms, microorganism fragments, and by products such as molds, bacteria, endotoxins, mycotoxins, and volatile microbial organic compounds. Airborne fungi and bacteria can be toxic, allergenic and/or infectious. A research was conducted to determine the number and types of airborne fungal spores in Buildings of Usmanu Danfodiyo University Sokoto, Nigeria. Five (5) areas were chosen within the University for the Survey, these were student Hostel, Staff Quarters, Botanical garden, Microbiology laboratory and city campus of Usmanu Danfodiyo University. . A total number of fifteen (15) petri dishes containing potato dextrose agar each were vertically placed in each sampler and exposed at end of each height and site for 10 and 20 minutes respectively. A total of thirteen (13) different fungal specie were identified namely; *Aspergillus niger, A. flavus, A fumigates, A. ustus, A. terreus, Fusarium solani, F. oxysporum, Alterneria altenata, Rhizopus oryzae, R. stolonifer, Helminthosporum* sp., *Penicillum candidum* and *Absedia corymbifera. Aspergillus niger* had the highest frequency of occurrence of (14.9%), *Helminthosporus* species had the least frequency of occurrence of (1.5%). Conclusively it was observed that the concentration of fungal spores was high in the upper surface than the ground level at the time of the survey.
Keywords: Isolation; Identification, Airborne Fungal spores; Fragments', Buildings

Introduction

Fungi are the plural of the word fungus which is derived from the Latin word fungour which means to flourish. The word was primarily used with reference to mushrooms which develop overnight. Fungi include nucleated spore bearing *achlorophylous* organisms that generally reproduce sexually and filamentous branched somatic structures are typically surrounded by cell wall, containing cellulose or chitin or both (Alexopoulos, 2000). Fungi are chlorophyll-less non-vascular plants, theyinclude nucleated spore bearing achlorophylous organism that generally reproduce sexually and filamentous branched somatic structure typically surrounded by cell wall, containing cellulose or chitin or broth (Alexopoulos, 2000).

According to Deacon (2008) fungi are heterotrophic organism possessing a chitinous cell wall: The majority of species grow as multicellular filament called hyphae forming a mycelium; some fungal species also grow as single cells, sexual and a sexual reproduction of fungi is commonly via spores, often produced on specialized structures or in fruiting bodies.

Fungi are an important component of the ecosystem, they are essential for the recycling of mineral and carbon by the decomposition of organic debris and waste. It has been estimated that fungi recycle millions of tons of organic waste annually (Davis *et al.,* 2002). Life for other types of organisms would come to a virtual halt without the activity of fungi. In addition, mushroom and other fleshy fungi are a source of nutrition for many animals, including human. However many plants and animals disease are fungal (Moor-Landeeker, 2005). Fungi reproduce both sexually and asexually and both methods result in the production of spore. The life cycles of fungi involved both sexual and asexual stages. The asexual spores are referred to as the anamorph, while the sexual spores and their associated reproductive structure are called the teleomorph. The spores from these two stages of life cycle can be morphologically dissimilar. The teleomorph spores (sexual produced) are classified according to the sexual structure in which the spores develop. Many complex fungi produce multicellular reproductive bodies a sporocarp. A familiar example of a sporocarp is the gilled mushroom, which is specialized to produce and discharge the spores resulting from sexual reproduction (Davis *et al.,* 2002). The anamorph spores are grouped together into the fungi imperfecti group, also referred to as the form, division Deuteromycota (Moore-Landecker, 2005).

Fungal spores occur in a great number in outdoor air and as estimated by Kendrick (2000) that, there are over 100,000 fungi whose spores may become airborne, many parameters influence airborne fungal spore concentrations. These include geographic, meteorological and human factors. Generally, these are lower airborne concentration in large towns and cities. When compared to rural and semi rural sites (Davis *et al.,* 2002).

Many spores are difficult to identify to a species level, so they are often grouped together according to morphological similarities. Many fungal spores have been shown to be allergenic (i.e. to induce allergic responses in susceptible individuals) and allergens from fungal spores can be potent inducers of asthma and seasonal allergic rhinitis (SAR) (Woolcock *et al.,* 2006). Three groups of fungi which are of primary concern to aerobiologist and allergist are: fungi imperfecti (including the asexual spores of many types of fungi including mould); ascomycota (including cup fungi and mildews) and basidio-mycota (including mushrooms, puff balls, jelly fungi, rust and smuts) (Smith, 2005).

Unicellular or multicellular, reproductive or distributional cell developing into a number of different phases of the complex life cycles of the fungi. Fungi spores can be readily classified by the saccardian system, which relies on the number, shape and placement of spore cell to classify the fungi imperfecti. Most fungal spores in pollen preparation probably are phaeospores (dark spores) of the fungi imperfecti, rather than ascospores, basidiospores, or spores of the lower fungi. However repeating (sexual) spores of the basidiomycetes are very common in some site (Wolf, 2002). Dark fungal spores are more resistance to acetolysis than in palynology include the forms Helmintho- sporium and Altenaria that are common in aeroallergy studies. The dung fungus sporermiella is an indicator of herbivore density and has been shown to increase in historic times after the introduction of grazing animals and before 11,000 year ago prior to megafaunals extinction (Deacon, 2008).

Generally dark, thick-walled fungal spore of the fungi imperfecti are common in soil samples, such as those often studied in archaeological palynology. In these same forms occur in abundance equal to that of terrestrial pollen when soil is washed into aquatic basin by watershed erosion, particularly after fires of intense human disturbance (Davis *et al.,* 2002).

The study was carried out in order to assess the air quality of the selected areas, and also to determine the concentration of fungal spores present as well as their economic importance to

the people living in the areas. The research is also important because food consume by the people of the selected areas is mostly kept and expose to these height and there is higher tendency of different spores to be deposited there in so knowing the type and nature of the spores present will enable us design a preventive measures.

Materials and Methods

Sample collection

A total of five areas were chosen for the research, these areas where fairly away from each other. The areas include botanical garden, male student hostel, staff quarters, and city campus of the university and microbiology laboratory. The indoor air was sampled by using gravitational petri plate method for monitoring of airborne microorganisms in indoor (Sen and Asan, 2009). External variations like wind, rainfall, vegetation did not affect indoor air monitoring. For each area, three heights where considered, these include the ground floors in which represents 0. 0 meter, the first floor represents 5.0 meters, and the second floor represent 10 meters of height. For monitoring of indoor contaminants, a set of sterilized petridishes treated with potato dextrose agar were exposed at the time to capture and cultivate the available fungi in the area. This petridishes treated with PDA. A total number of 15 petri dishes containing potato dextrose agar were vertically placed in each sampler and exposed at the end of each heights and sites for 10 and 20 minutes respectively. The plates were incubated at room temperature (29 ^{0}C) for 7 days, and after which adhesive tape where observed for the growth of fungal colonies. The growing fungi were sub-cultured aseptically into sterile potato dextrose agar plates to obtain pure cultures (Cosentiano *et al*., 2008).

Identification of air-borne fungal spores

Spore concentration were determined by mounting the transparent tape, on a glass slide, in a drop of lactiphenol, examined under an optical microscope at x10 and x40 magnification. Fungal species composition was identified by cutting the trapped fungal colonies on freshly prepared potato dextrose agar plates. Fungal spore identified were based on cultural and morphological features with reference to mycological atlas. The nature of mycelium, the type of fruiting body, spores and structural series as criteria for the identification of fungal were isolated. Features such as colony colour, growth pattern, nature of the spore e.t.c were also observed macroscopically (Kendrick, 2000).

Results and Discussion

The results show that thirteen different species of fungi were isolated from five areas within Usmanu Danfodiyo University Sokoto. Table 1 indicates the fungi isolated from the surveyed areas and Table 2 indicates the percentage of occurrence of the isolated fungi. *Aspergillus niger* found to have the highest percentage frequency of occurrence of 14.9%. *Heminthosporium* species is the least percentage frequency of occurrence of 1.5%. The students' hostel has the highest percentage of fungal spores of 22%.

It was shown that *A. niger* found to have the highest percentage frequency of occurrence of 14.9%. This is because of its ubiquitous nature that make them easily to dispersed by air, followed by *Rhizopus stolonifer* and *Penicillum candium* with 11.9% then, *A. flavus* and *A. fumigates* with 10.4% follow *R. oryzae* with 9% followed by *A. ustus* and *Absedia corymbifera* with 4.5% followed by *A. terreus* and *Fusurium oxysporum* with 3% followed by *Helminthosporium* sp. having the least percentage frequency of occurrence of 1.5%.

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Table 1. Fungal isolate from the surveyed areas

Isolated organism	Collection areas of the isolated organism														
	m.l			s.h			b.g			c.c			s.q		
	0m	5m	10m	0m	5m	10m	0m	5m	10m	0m	5m	10m	0m	5m	10m
Aspergilus niger	+	+	+	+	-	-	+	-	+	-	-	+	-	+	+
A. flavus	+	+	-	+	-	-	-	+	-	+	-	-	+	-	+
A. fumigates	+	+	-	-	+	+	-	-	+	+	-	-	-	+	-
A. ustus	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+
A. terreus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Absedia corymbifera	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Alternaria altenate	+	-	-	-	-	+	-	-	-	-	-	-	+	-	-
Fusarium oxysperum	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
Helminthosporum sp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Rhizopus oryzae	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-
R. stolonifer	+	+	-	+	-	+	-	-	+	+	+	-	-	+	-
Fusarium solani	-	+	-	-	-	-	-	-	-	+	-	+	-	-	+
Penicillium candidum	+	+	-	-	-	+	-	+	+	-	-	-	-	-	-

Note: m.l = Microbiology lab, s.h=Student hostel, b.g.=Botanical garden, c.c=City campus, s.q= Staff quarters, + = Present, - = Absent

Table 2. Percentage frequency of occurrence of isolated fungi

Isolated organism	Sample area					Total No. of occurrence	Mean frequency of occurrence	Percentage of frequency occurrence (%)
	c.c	s.q	m.l	s.h	b.g			
Aspergilus niger	1	2	3	3	1	10	2	14.9
A. flavus	1	1	2	3	0	7	1.4	10.4
A. fumigates	2	1	2	1	1	7	1.4	10.4
A. ustus	0	1	0	1	1	3	0.6	4.5
A. terreus	0	0	0	1	1	2	0.4	3
Absedia corymbifera	0	1	0	1	1	3	0.6	4.5
Alternaria altenate	1	0	1	2	1	5	1	7.5
Fusarium oxysperum	0	1	0	1	0	2	0.4	3
Helminthosporum sp	0	0	0	1	0	1	0.2	1.5
Rhizopus oryzae	2	1	0	2	1	6	1.2	9
R. stolonifer	2	1	2	2	1	8	1.6	11.9
Fusarium solani	0	0	1	2	2	5	1	7.5
Penicillium candidum	1	2	2	2	1	8	1.6	11.9
Total	10	11	13	22	11	67		100

Note: c.c = City Campus, s.q = Staff Quarters , m.l = Microbiology laboratory, s.h= Student Hostels, b.g= Botanical Garden

The observation in this study shows that out of the areas selected for sample collection, student hostel was the highest number of fungal spore (22%) followed by microbiology laboratory (13%) of the number of spores. It therefore indicates that air-borne fungal spores are much in the air surface then the ground level, and higher number of fungal spores recorded in students' hostel may be as a result of human activities and other related factors that serve as it source in the environment.

According to Alexopoulos (2000) shows that air-borne fungal spores are much in the air surface then in the ground level and those spores found in air can implicate in many diseases of man. The appearance of some species at all location may be explained by the fact that these spores once liberated into the atmosphere may tend to fall due to gravity. This may account for uneven distribution of spores at different location and time of exposure.

From this research, the total of thirteen fungal species were found in the air during the period of the survey. However it is significant to note that many identified species have been implicated in many disease of man. Many out of these diseases are virtually; found attacking skin and subcutaneous tissues, some are lead to systematic infection and probably death (Hasnain, 2011). *Aspergillus niger* is likely to cause human disease than some other *Aspergillus* species, but of large amount of spore are inhaled, a serious lung diseases *Aspergillosis* can occur. *Aspergillus niger* also causes black mold of onions. Infection of onion seedling by *A. niger* can become systematic, manifesting only when condition are conductive (Holdwaway, 2000). These fungal species are important for human use, for example human use fungi for food preparation. Many fungi are producer of antibiotics such as Penicillin (Smith, 1990). It appears therefore, that the atmosphere at the study location could be so dangerous contaminated with either spores or fragments of these medically important species. Fungi are ubiquitous in distribution and are a serious threat to public health in indoor environments. Many fungi that are reported to cause allergy belong to Ascomycota, Basidiomycota or anamorphic fungi (Haleemkhan and Muhankaruppayi, 2012).

There are many reports on fungi isolated from indoor environments. Fungi are able to grow on almost all natural and synthetic materials, especially if they are hygroscopic or wet. Inorganic materials get frequently colonized as they absorb dust and serve as good growth substrates for *A. fumigatus* and *A. versicolor* (Haleemkhan and Muhankaruppayi, 2012). Some of the buildings in the surveyed areas were made up of wood material. Wood is highly vulnerable to fungal attack. *Cladosporium* and *Penicillium* (*P. brevicompactum* and *P. expansum*) are reported to infest wooden building materials Kiln dried wood surfaces are more susceptible to fungi. Acylated wooden furnitures, wood polyethylene composites, plywood and modified wood products are susceptible to infestation by *Aspergillus*, *Trichoderma* and *Penicillium* (Haleemkhan and Muhankaruppayi, 2012)

Conclusions

From the research findings, it is apparent that air harbours microscopic organism forming a canopy that hang over our environment, as such allow these organisms not only to hang over us but to descend and surround us as they gradually settles down onto the soil from where they were initially raised as a result of mechanical disturbance. From the research work carried out it was observed that there was large concentration of fungal spores in the upper surface than the ground level during the time of the survey. There is an increase in the possibility of getting *mycotic* infection and allergic reaction at the season of the year. *Aspergillus* sp. found to evolve in almost every location. *Helminthosporium* sp. due to its complexity was not

identified to the species level. Microbial flora in indoor air residential houses may be useful both interms of ecology and allergy prevention and treatment. In the future, further studies on the dependence of indoor fungal and microbial flora on socioeconomic and hygienic factors will be of interest; such studies should clarify the relationship between indoor and outdoor fungal and microbial flora.

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References

Alexopoulos, C.J. (2000). Introductory mycology, Second edition. John Wiley and Sons Inc, New York, London.

Cosentiano, S., Fadda, M.E. and Palmas, F. (2008). Pollen and mould allergy in Southern Sardinia (Italy). Comparisons of skin-test frequencies and air sampling data. Grara, 34: 338-344.

Davis, O.K., Kolva, D.A. and Mehringer, P.J, Jr. (2002). Pollen analysis of wildcat Lake, Whitman Country, Washington: the least 1000 Years. Northwest Science, 51(1): 13-30.

Deacon, J.W. (2008). Introduction to modern mycology basic microbiology. Black Well Scientific Publication, London.

Haleemkhan, A.A. and Mohankaruppayi, S. (2012). Fungal population of indoor environments and its management. Saudi Journal of Biological Sciences, 19(4): 405-426.

Hasnain, S.M. (2011). Influence of meteorological factors on the air spora. Grana, 32: 184-189.

Holdwaway, M.D. (2000). Atopy in childhood. Gender and allergen related risk for development of hay fever and asthma. Clinical experimental allergy, 23: 991-949.

Kendrick, (2000). Fungal allergens. in sampling and identifying allergenic pollens and moulds In: E.G. Smith (ed). Blewstone Press, San Antonio, USA.

Moore-landecker, E. (2005). Fundamentals of the fungi, fourth edition Prentice-Hal International (UK) Limited, London.

Smith, E.G. (2005). Sampling and identifying allergenic pollens and moulds, Seconds Editions. Blewstone Press, San Antonio.

Wolf, F.A. (2002). Disintegration of fungus spores. Journal of Elisha Mitchell Scientific Society, 85: 92-94.

Woolcock, A.J., Peat, J.K. and Trevillion, L.M. (2006). Is the increased in asthma prevalence linked to increased in allengen load?. Allergy, 50: 935-940.