

Diversity and Spatial Distribution of Fungal Spores in Northern and Southern Nigeria; Implication on Public Health and Agriculture

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Abstract

Fungal spores are innumerable dispersed in ambient environment, some are triggers of allergy and most are pathogens of our arable crops. The diversity and spatial distribution of fungal spores in northern and southern Nigeria were studied from January to June 2017, with the aim of determining the difference in spores spatio-temporal pattern in the two climatic regions and impact of weather variability on them. Spores were caught with the aid of Tauber-like pollen trap modified to achieve an average human height of 1.52 m above ground level. Recovered aerospore samples were acetolysed and absolute value of spores were determined in 100 µl of the acetolysed samples. There were no significant differences ($P < 0.05$) in fungal spores load and other spores type caught in the two locations. However there were observable infiltration of spores in southern Nigeria influenced by higher intensity of rainfall. Similar aeromycoflora content with varying prevalence was observed in both regions. Similar trend relationship between spores and rainfall was also found. Spores of *Curvularia*, *Puccinia* and *Nigrospora* were dominant in the two regions. Asthmatic and other hypersensitive individuals in southern region could be more exacerbated in the rainy season than those in northern Nigeria due to higher rainfall intensity which favoured more sporulation.

Keywords: Fungal spores; Allergic diseases, climatic region, atmosphere, Nigeria

INTRODUCTION

Fungal spores are microscopic reproductive structures which are produced from fungi, their functions mimic that of the seed in higher plant. (Ezikanyi, 2017). Spores of fungi vary in type, sizes, colour and could be produced by either sexual or asexual methods with majority having the propensity to remain airborne (Andersen and Paterno, 2009). They make up a greater component of the atmospheric bioaerosol, as they are innumerable discharged into ambient air and are therefore ubiquitous in both outdoors and indoors environment (Zukiewicz-sobaczak, 2013). Their less susceptibility to adverse environmental conditions among other factors predisposed them to be ubiquitous in almost every environment except in environment covered with ice and snow (Horner *et al.*, 2000). Major contributors of aeromycobiota are fungi growing on living plants and plant debris in the soil (Andersen and Paterno, 2009). Outdoors fungal spores are inadvertently introduced into the indoors environment where they sometimes constitute sick building syndrome. Sick building syndromes are diseases which are building related due to fungi colonization (Joshi, 2008).

Some fungal spores are allergenic, most are human or plant pathogens, which are implicated in economic crop diseases, leading to huge loss in economy (Andersen and Paterno, 2009). Allergenic fungal spores possess protein allergen which cause hypersensitivity in predisposed individuals and mainly manifested on respiratory system and on skin (Ezikanyi *et al.*, 2016; Twaroch *et al.*, 2015). The aetiology of fungal spores in different allergic diseases such as allergic rhinitis, asthma, nasobronchial allergy, conjunctivitis, dermatitis, allergic alveolitis etc have been documented (Topbas *et al.*, 2005; Twaroch *et al.*, 2015, Zukiewicz-Sobaczak, 2013). Inhalation of spores of fungi have been implicated in several ailments in human. Some fungal spores have been implicated in lower and upper respiratory tract diseases and pose a huge debilitating effect on the immune-compromised individuals especially those living with human immunodeficiency virus (Khan *et al.*, 2013).

Research has established a correlation between asthma exacerbations and fungal spores concentration and also on fungi sensitization and severity of asthma (Pringle, 2013; Agarwal and Gupta 2011; Knutsen *et al.*, 2011). Knowledge on fungal spores spatial distribution in different climatic regions in Nigeria is important in predicting their occurrences and taking prophylactic approaches.

MATERIALS AND METHODS

The study was conducted in two climatic regions in Nigeria; equatorial, southern (Enugu state) and arid, northern (Nassarawa state). Modified Tauber- like pollen

traps were used for the collection of the aero samples. Traps were placed at the height of 1.52 m above the ground level. Solutions made of glycerol (50 ml), formaldehyde (10 ml) and phenol (5 ml) were prepared and poured into each trap. The recipient solutions were collected monthly for the period of six months. Samples were sieved through 100 µm mesh wire gauze to filter off large organic particles. The liquid with suspended palynomorphs were centrifuged at 2500 revolution per minute for 5 minutes to recover the palynomorphs residues. The residues were washed three times with water and acetolyzed according to Ezike *et al.*, 2016 procedures. Palynomorphs were prepared and examined using light Olympus CH Trinocular microscope (LM), equipped with an MVV3000 Future WinJoe digital camera (Future Optics Science and Technology, Hangzhou, China) at x 400 and x 100 magnifications. Identification was made using books and journals. Spores obtained were counted monthly and expressed in frequency per 100 µL of acetolyzed samples. The data obtained were analyzed using the SPSS statistical package version 20 (SPSS Inc. Chicago, Illinois USA). Correlation coefficients were generated to examine the relationship between fungal spores frequency and meteorological data. Meteorological data were obtained from Meteorological Centres in studied regions.

RESULT AND DISCUSSION

A total of 53 and 48 spores type were caught in southern and northern Nigeria respectively (Table 1 and Table 2). Fungi which belong to phylum Ascomycota were more preponderant. Fungal spores caught in southern Nigeria were more in morphotypes and quantity in a ratio of 3:1 than those recovered from northern Nigeria.. In southern Nigeria, dominant spores were *Bispora*, *Curvularia*, *Nigrospora*, *Helminthosporium*, diatom and *Puccinia* in decreasing order of dominance. There were no significant differences ($P < 0.05$) in fungal spores load and other spores type caught in the two locations. Dominant fungal spores in both locations; *Nigrospora*, *Puccinia* and *Curvularia* were not significantly different (Fig 1). In southern Nigeria, most spores present during the drier months were evoked to sporulate with the record of 1mm rainfall in the month of March. The first rain favoured sporulation of fungi leading to their increased preponderant both qualitatively and quantitatively as the rainfall increases in intensity. At higher rainfall record of 204 mm, the atmosphere became highly infiltrated with innumerable spores of *Curvularia*, *Bispora* and *Ovularia*. (Plates 1a & 1b). Most fungal spores recorded at the peak of rainfall have previously been confirmed as potent asthma triggers in previous works.

Spores of *Curvularia*, *Puccinia* and *Nigrospora* were persistently present in both locations from January to June and were associated with higher rainfall intensity. These fungal spores have been reported as component of the aerosol in various parts

of the world (Lim *et al.*, 2009; Meredith, 1961; Barnes *et al.*, 2005). *Curvularia* spores infiltrated in southern region, they are excitant of respiratory allergic disorder (Gupta *et al.*, 2002). Its implication in allergic bronchopulmonary mycosis characterized by severe asthma is also documented (Knutsen *et al.*, 2011). It is not clear whether *Puccinia* possess allergenic potential however all species in *Puccinia* are obligate plant pathogens known as rusts. The spores of *Puccinia graminis* cause devastating stem rust on wheat, oats, barley and rye (Fetch *et al.*, 2018). Spores of *Puccinia* were high in northern region in January, perhaps due to a higher wind speed which prevails during the harmattan which could have facilitated their spores dispersal. Higher rainfall intensity also favoured their sporulation in both locations in June. Northern Nigeria is known for huge wheat production, the high prevalence of *Puccinia* spores especially during the rainy season fortunately does not correspond to the planting period of wheat in Nigeria. Wheat is usually grown during November – March in Nigeria when the night temperature range from 10-15 °C (Olugbemi, 1990).

Nigrospora spores are widespread dermaticeous fungi which belong to Ascomycota. They were frequent component of the atmosphere of the studied regions, which also increases with higher rainfall intensity. Fan *et al.*, (2009) reported the implication of *Curvularia* in Onychomycosis, there have also been report on *Nigrospora* induced human eye and skin infection and also implicated in corneal ulcer in immunocompetent (Ananya *et al.*, 2014). *Nigrospora* species are also endophytic and saprobic plant pathogens (Wang *et al.*, 2017) and are implicated in maize ear rot disease which result to yield loss and risks of health in humans and animals (Gxasheka *et al.*, 2015).

High spores load was recovered from both locations in June which was associated with higher monthly rainfall. In both regions, total fungal spores correlated positively with monthly rainfall and humidity but negatively with temperature. Among the dominant fungal spores in southern Nigeria, *Ovularia* and *Curvularia* spores correlated positively with rainfall and relative humidity, others such as *Nigrospora* and *Helminthosporium* correlated negatively with rainfall (Table 3). All dominant fungal spores, except *Bispora*, correlated positively with relative humidity. In northern Nigeria, all dominant fungal spores with the exception *Puccinia* correlated positively with rainfall and wind (Table 4). The 100 % negative correlation of dominant fungal spores with temperature shows the negative effect of temperature on most fungi sporulation. The influence of rainfall on fungal spores dispersal had previously been studied. Ezike *et al.* (2016) , Njokuocha *et al.*, (2016) and Ezikanyi (2017), found similar direct relationship between fungal spores and rainfall in the atmosphere of north-central and southern Nigeria.

Fungi sensitivity could be higher in southern than northern Nigeria due to infiltration

of allergenic spores during rainer period in southern than northern Nigeria. Allergenicity is dose dependent and a lot of cross reactivity occurs among fungal spores. Dominant fungal spores specific to northern Nigeria include those of *Sporomiella* whereas *Helminthosporium*, *Bispora* etc were dominantly specific to southern Nigeria.

Diatom were next to fungal spores though low in abundance. They were caught higher in southern 173 (2.87) than northern 66 (3.5 %) Nigeria. Fern spores were fewer in abundance in both regions and were mainly *Nephrolepis* type. Fungal hyphae were present, though in low abundance throughout the study period in both locations.

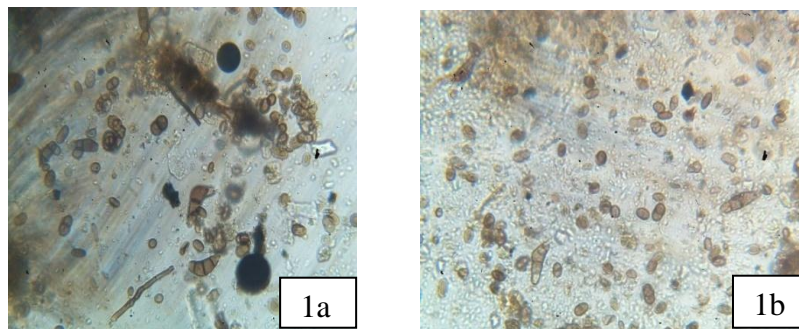


Plate 1 a and 1b: Typical component of the wet weather fungal spores
Mag 400x

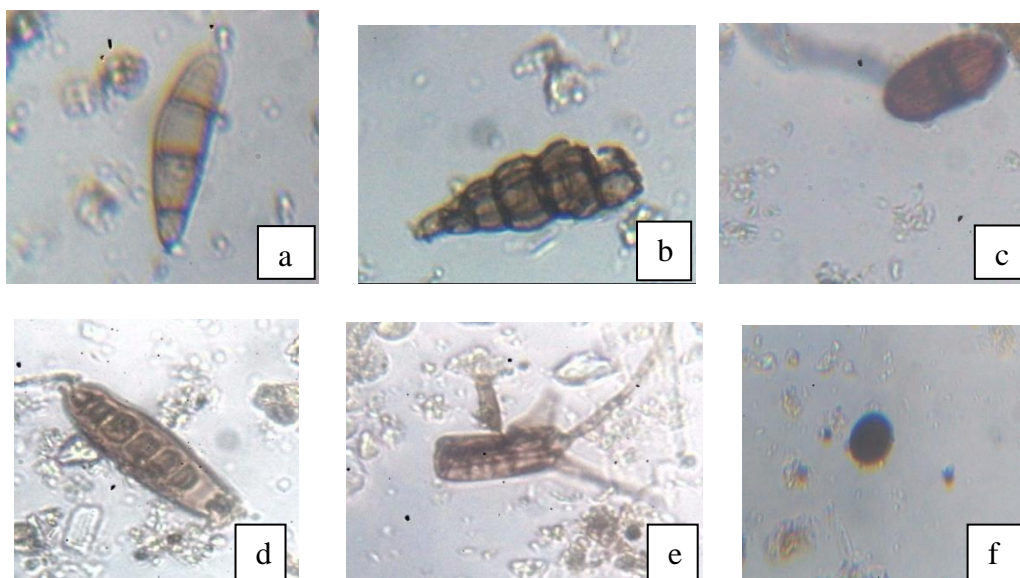


Plate 2: Photomicrographs of some fungal spores; a- *Curvularia*, b- *Alternaria*
c- *Puccinia*, d- *Helminthosporium*, e- *Tetraploa*, f- *Nigrospora*.

Table 1: Spores (in 100 μ L of Acetolyzed Samples) Frequency in Nsukka, Nigeria from January to June 2017

S/N	SPORES FUNGI	DIVISION	MONTHS							TOTAL	%
			J	F	M	AP	M	J			
1	<i>Venturia</i>	Ascomycota	1	1	18	3	1	24	48	0.80	
2	<i>Nigrospora</i>	Ascomycota	13	9	193	49	7	85	356	5.91	
3	<i>Epicoccum</i>	Ascomycota	1	1	3	7	1	2	15	0.25	
4	<i>Spadicoides</i>	Ascomycota	10	0	7	15	0	0	32	0.53	
5	<i>Sporidesmium</i>	Ascomycota	1	1	0	0	0	0	2	0.03	
6	<i>Hansfordia</i>	Ascomycota	1	2	5	1	3	0	12	0.20	
7	<i>Cordana</i>	Ascomycota	2	1	0	3	0	2	8	0.13	
8	<i>Curvularia</i>	Ascomycota	11	6	70	67	7	1285	1446	24.0	
9	<i>Helminthosporium</i>	Ascomycota	4	0	171	1	1	1	178	2.95	
10	<i>Spadicoides</i>	Ascomycota	9	1	15	19	3	18	65	1.08	
11	<i>Trichocladium</i>	Ascomycota	1	0	1	0	2	8	12	0.20	
12	<i>Cladosporium</i>	Ascomycota	2	0	2	4	0	3	11	0.18	
13	<i>Sporoschisma</i>	Ascomycota	2	1	0	0	0	0	3	0.05	
14	<i>Spilocea</i>	Ascomycota	2	1	0	0	0	3	6	0.10	
15	<i>Puccinia</i>	Basidiomycota	1	2	11	9	2	83	108	1.79	
16	<i>Pithomyces</i>	Ascomycota	1	0	16	6	0	26	49	0.81	
17	<i>Alternaria</i>	Ascomycota	0	1	1	0	0	2	11	0.18	
18	<i>Cercospora</i>	Ascomycota	0	2	2	0	0	1	5	0.08	
19	<i>Torulla</i>	Ascomycota	0	1	0	3	0	0	4	0.06	
20	<i>Tetraploa</i>	Ascomycota	0	0	0	0	8	1	9	0.15	
21	<i>Ustilago</i>	Basidiomycota	0	0	3	6	1	25	35	0.58	
22	<i>Venturia</i>	Ascomycota	0	1	5	0	0	5	11	0.18	

S/N	SPORES FUNGI	DIVISION	MONTHS						TOTAL	%
			J	F	M	AP	M	J		
23	<i>Oidium</i>	Ascomycota	0	0	2	0	0	6	8	0.13
24	<i>Alternaria alternata</i>	Ascomycota	1	1	24	6	1	4	37	0.61
25	<i>Bipolaris</i>	Ascomycota	0	0	3	0	0	0	3	0.05
26	<i>Papularia</i>	Ascomycota	0	0	1	0	0	1	2	0.03
27	<i>Dactylaria</i>	Ascomycota	0	0	1	1	0	5	7	0.12
28	<i>Helicomina</i>	Ascomycota	0	0	1	0	0	0	1	0.01
29	<i>Bispora</i>	Basidiomycota	1	2	3	0	0	1534	1540	25.6
30	<i>Apiospora</i>	Ascomycota	0	0	2	0	0	0	2	0.03
31	<i>Ulocladium</i>	Ascomycota	1	0	2	0	0	0	3	0.05
32	<i>Puccinia canadensis</i>	Basidiomycota	0	0	1	2	0	0	3	0.05
33	<i>Tetraploa</i>	Ascomycota	0	0	1	0	0	0	1	0.01
34	<i>Chalara</i>	Ascomycota	0	0	3	2	0	0	5	0.08
35	<i>Harknessia</i>	Ascomycota	1	2	7	0	3	0	13	0.22
36	<i>Dreschlera</i>	Ascomycota	0	0	2	1	0	11	14	0.23
37	<i>Blastomyces</i>	Ascomycota	0	0	0	1	0	0	1	0.01
38	<i>Balanium</i>	Ascomycota	0	0	0	1	0	0	1	0.01
39	<i>Venturia inequalis</i>	Ascomycota	0	0	0	0	1	0	1	0.0
40	<i>Pucciniopsis</i>	Ascomycota	0	0	0	0	0	13	13	0.22
41	<i>Ovulariopsis</i>	Ascomycota	0	0	0	0	0	5	5	0.08
42	<i>Beltrania</i>	Ascomycota	0	0	0	0	0	2	2	0.03
43	<i>Monilia</i>	Ascomycota	0	0	0	0	0	1	1	0.01
44	<i>Candida</i>	Ascomycota	0	0	0	0	0	1	1	0.01
45	<i>Torulla</i>	Ascomycota	0	0	1	0	0	2	3	0.05
46	<i>Phomopsis</i>	Ascomycota	0	0	0	0	0	1	1	0.01

S/N	SPORES FUNGI	DIVISION	MONTHS						TOTAL	%
			J	F	M	AP	M	J		
47	<i>Diplocladiella</i>	Ascomycota	0	0	1	0	0	0	1	0.01
48	<i>Trichocladium</i>	Ascomycota	0	0	1	0	0	0	1	0.01
49	Smut fungi	Basidiomycota	0	0	1	4	0	0	5	0.08
50	<i>Paathramaya</i>	Ascomycota	0	0	3	3	0	0	6	0.10
51	Hyphae fragment		2	3	11	0	0	3	19	0.32
Total fungal spores			68	39	594	216	49	4863	5829	
Other spores										
52	Diatoms	Ochrophyta	57	45	25	26	10	9	173	2.87
53	Fern	Pteridophyta	1	1	0	1	0	0	3	0.05
54	Indeterminate		3	8	11	0	0	0	22	0.37
Total			129	93	606	222	51	4956	6,025	
Grand total; 6,025										

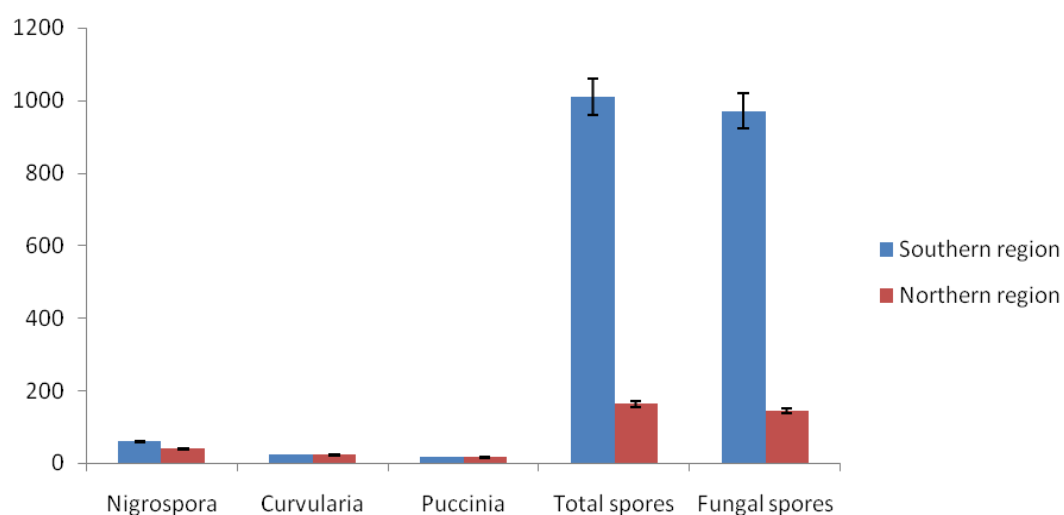


Fig 1: Mean values of dominant fungal spores, total spores and total fungal spores

Table 2: Spores Frequency in Northern Nigeria from Jan. - June, 2017

S/N	SPORES FUNGI	Mth	J	F	M	A	M	J	T	%
1	<i>Pucciniopsis</i>		0	0	0	0	4	0	4	0.5
2	Smut species		12	2	6	4	5	11	40	4.9
3	<i>Puccinia</i>		34	21	1	4	30	10	100	12.2
4	<i>Nigrospora</i>		12	15	49	49	56	50	231	28.1
5	<i>Oidium</i>		4	1	0	0	1	1	7	0.9
6	<i>Dactylaria</i>		35	2	0	1	1	0	39	4.8
7	<i>Dreschlera</i>		2	0	1	0	0	0	3	0.4
8	<i>Curvularia</i>		8	6	5	14	72	27	132	16.1
9	<i>Venturia</i>		7	4	4	2	13	9	39	4.8
10	<i>Piptocephalis</i>		2	0	0	0	0	0	2	0.24
11	<i>Venturia inequalis</i>		2	0	0	1	0	0	3	0.4
12	<i>Tetraploa</i>		1	0	0	0	0	1	2	0.24
13	<i>Spilocaea</i>		1	0	7	1	0	0	9	1.1
14	<i>Ovularia</i>		1	0	0	0	3	1	5	0.6
15	<i>Spadicoides</i>		4	1	0	3	11	1	20	2.43
16	<i>Torula</i>		1	0	13	3	0	0	17	2.1
17	<i>Helminthosporium</i>		0	1	0	2	0	0	3	0.4
18	<i>Hansfordia</i>		0	4	9	7	3	17	40	4.9
19	<i>Bispora</i>		0	5	1	0	1	0	7	0.9
20	<i>Sporomiella</i>		0	2	19	19	13	14	67	8.1
21	<i>Pithomyces</i>		0	0	2	1	15	6	24	3.0
22	<i>Epicoccum</i>		0	0	0	1	0	0	1	0.1
23	<i>Alternaria</i>		0	0	0	0	2	2	4	0.5
24	<i>Helicomina</i>		0	0	0	0	0	1	1	0.1
25	<i>Balanium</i>		0	0	0	0	0	0	0	0
26	<i>Cordana</i>		0	0	1	2	1	1	5	0.6
27	<i>Papularia</i>		0	0	0	0	0	0	0	0
28	<i>Gibellula</i>		0	0	0	0	0	0	0	0
29	<i>Cladosporium</i>		0	0	0	3	1	3	7	0.9
30	<i>Ulocladium</i>		0	0	1	0	0	0	1	0.1
31	<i>Xylaria</i>		1	0	1	1	0	0	3	0.4
32	<i>Cercospora</i>		0	0	1	0	0	0	1	0.1
33	<i>Berkleasium</i>		0	0	1	0	0	0	1	0.1
34	<i>Sporidesmium</i>		0	0	0	1	3	0	4	0.5

S/N	SPORES FUNGI	Mth	J	F	M	A	M	J	T	%
35	<i>Heterosporium</i>		0	0	0	0	0	0	0	0
36	<i>Bipolaris</i>		0	0	0	0	0	0	0	0
37	<i>Alternaria alternata</i>		0	0	0	0	0	0	0	0
38	<i>Circinoconis</i>		0	0	0	0	0	0	0	0
39	<i>Ovulariopsis</i>		0	0	0	0	5	1	6	0.7
40	<i>Trichocladium</i>		0	0	0	0	1	0	1	0.1
41	<i>Papularia</i>		0	0	0	0	0	4	4	0.5
42	<i>Ulocladium</i>		0	0	0	0	0	1	1	0.1
43	<i>Ovulariopsis</i>		0	0	0	0	5	1	6	0.7
44	<i>Diplocladiella</i>		0	0	0	0	0	1	1	0.1
45	<i>Indeterminate</i>		1	4	3	7	5	15	35	4.3
	Total		128	68	125	126	246	177	820	43.8
	Fungal spores grand total; 1538									
46	<i>Fern spore</i>		0	1	5	3	3	16	28	1.5
47	<i>Diatom</i>		1	21	17	4	8	15	66	3.5
48	<i>Fungal hyphae</i>		1	1	0	3	4	6	15	0.8
	Spores total;		130	91	147	136	261	214	979	52.2
	Spores grand total; 1874									

Table 3: Correlation coefficients between frequency of dominant fungal spores in southern region and weather variables

Fungal spores count	R	T	R.H
<i>Ovularia</i>	0.690	-0.139	0.308
<i>Bispora</i>	0.720	-0.181	-0.426
<i>Curvularia</i>	0.704	-0.180	0.387
<i>Nigrospora</i>	-0.100	0.407	0.227
<i>Helminthosporium</i>	-0.322	0.552	0.227
Total fungal spores	0.690	-0.139	0.308

P=0.01 level (2 tailed). R- mean monthly rainfall (mm), T-mean monthly temperature (°C), R.H- relative humidity (%)

Table 4: Correlation coefficients between frequency of dominant fungal spores in northern region and weather variables

Pollen count	R	T	R.H	W
Smut spp.	0.323	-0.691	-0.057	0.214
<i>Puccinia</i>	-0.067	-0.406	-0.584	-0.192
<i>Nigrospora</i>	0.656	-0.159	0.877*	0.792
<i>Curvularia</i>	0.660	-0.528	0.418	0.549
Total spores	0.855	-0.691	0.603	0.690

*: Correlation is significant at $P=0.01$ level (2 tailed)

R- mean monthly rainfall (mm); T- mean monthly temperature ($^{\circ}$ C); R.H.- relative humidity (%); W-wind (knot)

CONCLUSION

Fungal spores are frequent component of the atmosphere. Though the prevalence of most fungi was influenced by higher rainfall intensity which favoured more preponderance in equatorial than arid Nigeria. Hypersensitive individual should take proper proactive approaches at the onset of rainy season in other to oversmart seasonal allergies exacerbated by airborne fungal spores.

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