

Prevalence of airborne *Aspergillus flavus* in Khartoum (Sudan) Airspora with reference to dusty weather and inoculum survival in simulated summer conditions

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Abstract

Khartoum air was scanned for airborne *Aspergillus flavus* for 12 months using the horizontal gravitational settling method. Frequency of occurrence was related to total fungal catch and dusty weather. The Aspergilli were prevalent (68% of total isolated/plate/month) and *A. flavus* constituted 31% of the total Aspergilli. In June (hot, dry & dusty) Aspergilli constituted 79% of the total isolates, whilst *A. flavus* represented 30% from amongst the other Aspergilli. *A. flavus*, *A. niger*, *A. nidulans* (conidial & ascospore states), *A. terreus*, *Eurotium amstelodami* and *A. fumigatus*, in descending order of prevalence were isolated in June. Other pathogenic or potentially pathogenic forms, isolated, were *Cladosporium*, *Curvularia* and *Penicillium*. Amongst winter isolations *A. flavus* was sporadic to absent in occurrence.

A. flavus spore inocula that underwent hourly intermitted exposure to 45 °C, showed a decrease in spore germinability as well as reduced germ length.

Introduction

Aerobiology has become of paramount importance in many countries especially where deforestation is becoming a major problem. The Sudan, one of the largest African countries (1 000 000 square miles in area), suffers from this problem resulting in the dusty weather 'haboob' or sand-storms especially during the hot months of the year (May to September). This results from the lack of vegetational cover and wind breaks such as the green belts. The frequency of such sand-storms and their duration of several hours during each episode may pose a health hazard to man and domestic animals. For example, the implication of airborne fungal spora, particularly the Aspergilli, in the induction of various diseases such as pulmonary aspergillosis, allergic asthma, hypersensitivity pneumonitis, nosocomial infections have been reported [8, 15, 16, 18]. These diseases are provoked by soil-borne or dust-carried

Aspergillus spp. of which *A. fumigatus* Fresenius and *A. flavus* Link are the most common causative agents [22, 3, 9, 12, 4]. To these pathogens Mahgoub added *A. nidulans* (Eidam) Winter [13] and *A. flavus* [14] as causal organisms of maduromycetoma in the Sudan. Moreover, other airborne solid particles may induce allergies, particularly in house-dust-sensitive patients. Dust particles are also reported to affect microbial viability and their infectivity [8]. *A. flavus* is also famous for the production of the carcinogenic toxin called aflatoxin that induces liver cancer in man and poultry [25].

It is note-worthy that the distribution of *A. flavus* in the cultivated relatively humid Gezira area of the Sudan ranged from sporadic to moderate [9], whilst the sandy soils of the Sudan had higher *A. flavus* populations, based on the fungi isolated from the geocarps of groundnuts [2]. In the Sudan the drastic reduction in soil-borne fungi in uncropped or fallow soils [20, 21] may be attributed to drought. In the ab-

sence of irrigational water, temperature increase is important in determining the viability of spores. A rise in temperature as occurs in tropical and sub-tropical climates, may be altering spore tolerance and germinative capacity by interference with the spore or fungal propagule enzymatic system(s) or cellular structures [19]. The survival of most fungi is prolonged at low temperatures whereas members of the genus *Aspergillus* Micheli ex Link tend to have relatively higher temperature optima for survival [6]. Some *A. flavus* strains tolerate temperatures at or slightly higher than body temperatures, which could justify placing them within the thermotolerant or rather thermophilic category [1].

During observations on aflatoxin production by *A. flavus* laboratory isolates associated with paranasal contaminations from Sudanese patients [unpubl. work, 18], the prevalence of dusty weather conditions and the high frequency of occurrence of *A. flavus* in geocarps of groundnut from sandy soils of hot areas [2] stimulated this investigation on *A. flavus* in Khartoum airspora. Survival and germ tube elongation as influenced by conditions simulating summer weather that prevail in the greatest area of the Sudan were also examined.

Materials and methods

Sampling of airborne mycoflora

Khartoum air was scanned for airborne fungal contents over 12 months using horizontal gravitational settling method by exposing potato dextrose agar (PDA) plates to the air for 2 min at a distance of 1.75 m below the air source. This simple method simulates the condition under which the human body and respiratory tract contaminants gain access to infection sites [24]. Sampling was conducted when dusty weather prevailed and between 11.00 and 12.00 hours when air temperatures start to rise. After exposure, each replication of 5 plates was incubated at 30 °C for 7 days at which time fungal colonies were identified and counted. The frequency was calculated as a percent of total number of isolates.

Survival of A. flavus at high temperatures

Clay soil (20 g) in glass Petri plates was oven-sterilized at 150 °C for 12 hrs. From a 9-day-old *A. flavus* culture (from a dusty summer mid-day sampling) 10 mycelial plugs (3 mm diameter) were randomly buried in the sterile soil. The plates were incubated at 45 °C between 0 and 5 hrs to simulate the average air temperatures that prevail during summer in Khartoum. At the end of the exposure duration the mycelial discs were recovered, one from each plate. The discs were immersed in 20 ml aliquots of sterile distilled water, in a 50 ml erlenmeyer flask for 5–10 mins. The discs were mechanically agitated (200 revs/min) to dislodge conidia. After 7 hr incubation at 30 °C the percentage germination and average germ tube length were determined using a haemocytometer, based on examination of 10 microscope fields. Results were compared to non-exposed controls.

Results and discussion

Fungal spores are more drought and heat resistant than hyphal fragments. Their ability to survive is due not only to the degree of tolerance to extremes of environmental conditions, but also to the ability to survive the adverse changes that naturally occur from the time of their formation to their germination and initiation of successive generations. In the present study, monitoring air for the presence of *A. flavus* revealed that variable degrees of frequency that seem to be encouraged by relatively higher atmospheric temperatures (Fig. 1). Prevalence of this mould in Khartoum atmosphere appears to follow a seasonal pattern coinciding with the summer meteorological conditions in Central and Northern Sudan (i.e. dry, dusty & hot). *A. flavus* and other fungal propagules may be drifting with the wind and increasing in levels between May and July with a maximum level in June. Hence the extent of dustyness coupled with high atmospheric temperatures is correlated with the level of contamination of this fungus. Ultimately, health hazards such as paranasal infections [18] as well as aflatoxin toxicity may ensue following exposure to *A. flavus*-contaminated dusty weather.

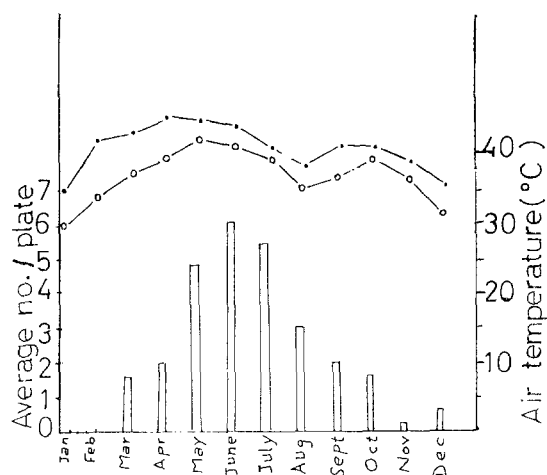


Fig. 1. The occurrence of *A. flavus* and the mean daily air temperature in the Sudan.

Moreover, these moulds are considered the causative agents of pulmonary aspergillosis [8, 16]. It has been reported that 65% of airborne *A. flavus* produced aflatoxin [12]. Aflatoxin synthesis starts simultaneously with spore emergence, and culminates with maximum spore yield [5]. Boltyanskaya [5] reported that the highest level of aflatoxin synthesis was inversely proportional to the number of *A. flavus* spore that originally infected grain. In this study, *A. flavus* isolants steadily dropped between August and November, and completely disappeared during January and February (Fig. 1), periods that coincided with the rainy and winter seasons (respectively) in the Sudan. *Aspergillus* spp. constituted 68% of the total recovered airspora (Table 1) confirming their high prevalence in tropical climates [3, 9, 22].

A. flavus alone constituted 31.1% of the mean isolates (Table 2). Summer isolations (viz. June) were dominated by pathogenic species such as *A. flavus*, *A. nidulans* and *A. terreus* Thom [13, 16, 18, 23]. Other pathogenic fungi such as *A. niger* van Tieghem, *Cladosporium herbarum* (Persoon) Link, *Penicillium* spp. [23] and *Curvularia* sp. [15] (Table 3) were also isolated.

Although the Aspergilli are reported to tolerate high temperatures and low humidities [11], exposure of *A. flavus* spores to 45 °C for 5 hrs (simulating temperature and duration of the hottest hours of the day) lowered conidial germination in a manner conforming with exposure time (Fig. 2). The percentage reductions from the controls, arranged in ascending order of exposure duration, were 17.0, 19.2, 35.1, 39.4 and 43.6% thus demonstrating *A. flavus* sensitivity to high temperatures. This observation may explain the sporadic occurrence of this mould in non-dusted weather. However, *Curvularia lunata* (Wakker) Boedijn spore inoculum, obtained from Central Sudan was noticed to disappear after 7 days at laboratory temperature [10]. On the other hand, *Eurotium rubrum* König, Speckerman & Bremer inocula tolerated 45 °C for 5 days upon re-incubation at laboratory temperature [1]. Survival at high temperature was reported to be associated with high moisture [7] or the interaction between various chemical, physical and biological attributes of a soil environment [17]. In Moat's [19] view the loss of spore viability resulting from high temperature may be due to the leakage of a variety of cellular components or the inactivation of spore enzymes and other func-

Table 1. The occurrence of *Aspergillus* and other fungi in air collected throughout the year in Sudan.

Months	Jan.	Feb	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Mean monthly
Isolates													
Aspergilli													
No.	5.4	2.0	4.8	6.6	19.0	19.6	9.4	5.0	3.2	2.0	1.0	3.2	6.8
%	75	66.7	60	54.1	81.2	79.7	71.2	100	51.6	50	14.3	51.6	68
Other moulds													
No.	1.8	1.0	3.2	5.6	4.4	5.0	3.8	0	3.0	2.0	6.0	3.0	3.2
%	25	33.3	40	45.9	18.8	20.3	28.8	0	48.4	50.0	85.7	48.4	32
Total isolates per plate per month	7.2	3.0	8.0	12.2	23.4	24.6	13.2	5.0	6.2	4.0	7.0	6.2	10.0

Table 2. The occurrence of *A. flavus* and other *Aspergillus* spp. in air collected throughout the year in Sudan.

Months	Jan.	Feb	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Mean monthly
Isolates													
<i>Aspergillus flavus</i>													
No.	0	0	1.6	0.2	4.8	6.0	5.4	3.0	2.0	1.6	0.2	0.6	2.12
%	0	0	33.3	3.0	25.3	30.6	57.5	60	62.5	80	20	18.8	31.3
Other <i>Aspergilli</i>													
No.	5.4	2	3.2	6.4	14.2	13.6	4	2	1.2	0.4	0.8	2.6	4.7
%	100	100	66.7	97	74.7	69.4	42.6	40	37.5	20	80	81.3	68.7
Total isolates per plate per month	5.4	2.0	4.8	6.6	19.0	19.6	9.4	5	3.2	2	1	3.2	6.82

tional cell structures or induced lysis. These views may explain the sensitivity of *A. flavus* to high temperature (Fig. 2).

Germ tube initiation and subsequent elongation are important factors that determine incipient colonial development and ultimately spore production. High temperature drastically retarded germ tube elongation in a manner conforming with exposure duration (Fig. 2). In nature the germ tube

may attain maximal development when air or soil temperature drops. Thermotolerant *A. flavus* strains may be health hazards when their temperature optima fall within or slightly above body temperatures.

Table 3. Fungi isolated from air cultured in June in the Sudan.

Fungi	Average no. per plate	% Frequency	
<i>Aspergillus flavus</i> Link.	6.0	24.39	} (79.68)
<i>A. nidulans</i> (Eidam)			
Winter	2.0	8.13	
<i>A. niger</i> van Tieghem	5.6	22.76	
<i>A. fumigatus</i> Fresenius	0.6	2.44	
<i>A. terreus</i> Thom	3.2	13.01	
<i>Emmericella nidulans</i> (Eidam) Vuillemin	1.8	7.32	
<i>Eurotium amstelodami</i> (Mangin) Thom & Church	0.4	1.63	} (20.32)
<i>Cladosporium herbarum</i> (Persoon) Link.	0.6	2.44	
<i>Curvularia</i> sp.	1.0	4.07	
Dark sterile mycelium	0.2	0.81	
Hyaline sterile mycelium	0.2	0.81	
<i>Rhizopus stolonifer</i> (Ehrenb. ex Fr.) Lind.	1.4	5.69	
<i>Penicillium</i> spp.	1.6	6.50	
Total No. of isolates	24.6	100.00	

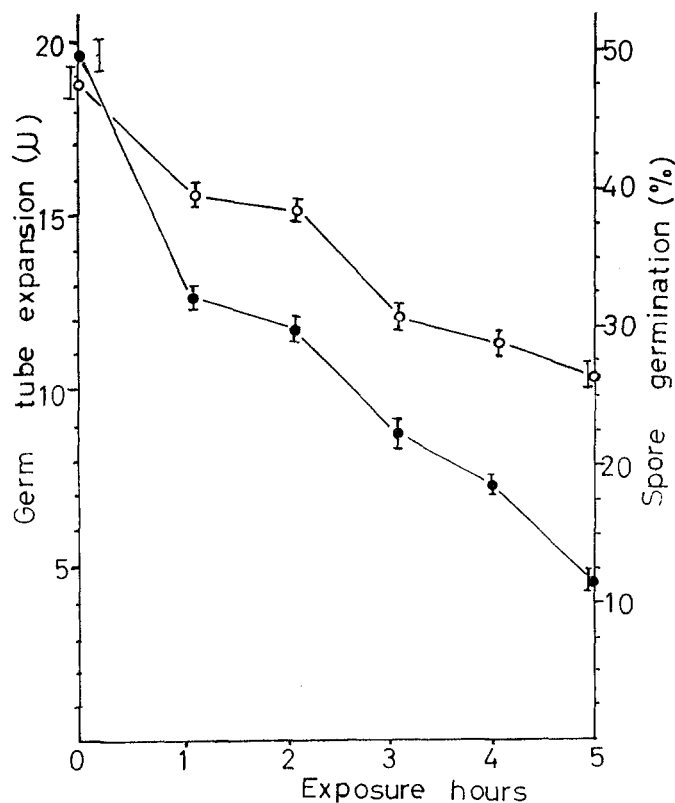


Fig. 2. Effect of high temperature upon *A. flavus* spore germinability (— o —) and germ tube expansion (— ● —). The vertical bar represents the standard error of a mean.

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