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Original Research

A study of airborne fungal allergens in sandstorm dust in Al-Zulfi, central region of Saudi Arabia

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ABSTRACT

Background: The impact of sandstorm dust events on local air quality and public health are becoming a greater concern in the Kingdom of Saudi Arabia. Among sandstorm dust particles, airborne fungal spores cause serious respiratory ailments to those who are exposed to the dust. Although the study of dust storm material has attracted research interest, little work has been carried out in Saudi Arabia and no major study has been conducted in the Al-Zulfi, Riyadh province region. Hence, the aim of the study was to investigate airborne fungal allergen concentrations in sandstorm dust in the Al-Zulfi city, Saudi Arabia. **Materials and Methods:** During the study period, 12 sandstorm dust samples and 3 control samples were collected from various locations (educational campus, people gathering, and recreational places) by gravitational plate exposure method using Sabouraud dextrose agar. Following incubation, the fungal colonies were identified by microscopic and morphological identification. **Results:** A total of 2590 fungal isolates were identified among 36 exposures of sandstorm dust samples. From the samples dematiaceous fungi and hyaline fungi were observed, divided 56.2%, 43.8%, respectively. The incidences of predominant fungal genera were *Fusarium* (21%), *Cladosporium* (15.8%), *Ulocladium* (11.1%), *Aspergillus* (10.9%), and *Alternaria* (8.6%). **Conclusion:** Our observations infer that some of the most important allergenic fungal spores are predominantly observed in sandstorm dust samples and incidences of dematiaceous fungi are higher than hyaline fungi. This study highlights the need for precautionary safety measures to protect the public against sandstorm dust exposures.

KEY WORDS: *Alternaria*, air sampling, *Cladosporium*, dust, fungal allergens, *Fusarium*, sandstorm, settle plate

INTRODUCTION

Sandstorms, also called dust storms, occur frequently and periodically under the strong winds which blow dust across the dry deserts around the globe, especially in the Middle East. The Kingdom of Saudi Arabia (KSA) is the largest country in the region and it constitutes the vast majority of the Arabian Peninsula. Most the land surface is made up of deserts and semi-arid lands. Based on its topography and condition of drought, light textured topsoil, and scanty vegetative cover much of the region and thus the KSA is highly susceptible to sandstorm dust. Sandstorm episodes in the deserts of KSA are seasonal, with the majority of atmospheric transport occurring within the February to May months [1]. Notwithstanding this seasonal pattern, a significant increase in frequency and the intensity of sand and dust storms has been observed in the Middle East over the past 15 years [2]. In addition to the KSA and Middle East countries, all sandstorms facilitate the long-distance dispersal of dust-associated biological particles includes bacteria, fungi, and viruses, and it is not uncommon for these organisms to be carried across several continents [3]. According to the WHO, dust storms contribute to poor air quality and this is attributed to the death of around 7 million people every year [4]. A proportion of these deaths will relate to fungi; there are several thousand of types of fungal spores found in both indoor and outdoor environments including sand dust; moreover, as well as species diversity, a significantly high number (around 10^6) of spores are found in sandstorm dust [1,5].

The medical risk to human health arises from the fungi associated with sandstorm dust producing metabolites (mycotoxins) that can initiate a toxic response to humans. Here repeated exposure causes respiratory irritation or allergic sensitization [1,6]. Inhalation exposure of fungal spore and hyphal fragments can cause allergic, infectious, and toxic diseases to humans. Infants, children, and elderly people with

respiratory conditions are more vulnerable compared with the general population [7,8]. Prolonged exposure to airborne dust fungi can lead to chronic breathing and lung problems, and possibly heart disease.

As medical understanding advances, the impact of severe sandstorm dust events on local air quality and public health have become of greater concern in the KSA in recent years. Although the study of dust storm material has attracted many researchers globally, little work has been undertaken in the KSA [7,9]. Furthermore, there has been no published work performed in relation to the central region of the Arabian Desert. Hence, the aim of this work was to isolate fungal allergens in sandstorm dust and analyze the incidence of allergens in the central region of KSA.

MATERIALS AND METHODS

Study Site

Al-Zulfi is a city in Riyadh province in the central desert region of the KSA, about 260 kilometers northwest of Riyadh. Al-Zulfi lies in the northern-central region of the Najd and to the south of the Samnan Valley. The city surrounded by sand dunes to its north and west, which are known locally as the Al-Thoyr Sands.

This study was conducted at the various places of Al-Zulfi, including the educational campus (College of Science, Al-Zulfi), an area with a high population density (Zulfi Dates market) and a recreational place (Zulfi West – City view). Samples were taken during sandstorms and during periods when no sandstorms were occurring.

Sampling Methods

Sampling was undertaken by a passive air sampling method (settle plates, where fungal spores are capture by gravitational deposition). This was performed using agar-filled Petri dishes exposed at a 1-m height from the surface [10]. Each Petri dish contained (90 mm diameter) Sabouraud dextrose agar (SDA) (sometimes described as Sabouraud glucose agar) (Himedia, India). SDA is an agar especially formulated, through the inclusion of peptones, for the isolation and growth of dermatophytes and other types of fungi [11].

A total of 12 sandstorm episodes occurred during the study period, from the beginning of February 2016 to the end of May 2016. During each sandstorm, one of the identified locales was sampled and for each sampling location triplicate SDA plates were exposed (in total 36 samples were taken across the course of the study). The metrological parameters such as temperature and wind speed were recorded (obtained from an internet source) [12]. After sampling, the plates were incubated at 20-25°C for 6-8 days. The total fungal colonies were counted and recorded on the 2nd or 3rd day of incubation, and then, at the end of incubation. For further species identification, fungal colonies were subcultured to a second SDA plate and then incubated for 5-7 days. Fungal identification performed based on macroscopic and microscopic morphology following the keys and description given by Samson *et al.*, Lalitha *et al.*, and Vijayakumar *et al.*, [13-15]. To compare the fungal counts with a non-sandstorm episode (to act as a control), the same procedure was followed and samples collected for one period under non-sandstorm conditions.

The significance of the incidences of fungal allergens, among the various sampling locations, was analyzed using Student's *t*-test, a $P < 0.05$ was considered as statistically significant.

RESULTS

During the study period, 12 sandstorm dust sample sets were collected. These consisted of six sets from the educational campus; three sets from the people gathering area (Zulfi dates market); three sets from Zulfi city – west view location [Figure 1]. Triplicate samples were taken for each sample set and a total of 2590 fungal colonies were observed from the 36 exposed plates. In addition, from the educational campus during non-sandstorm conditions a total 3 samples (9 exposures) were collected (during the daytime). All samples taken are listed in Table 1.

The total fungal colonies observed from the educational campus ranged from 12 to 87 CFU/plate. In the people gathering area significantly higher levels of fungi were recorded compared with the educational campus ($P = 0.032$). With the three sets (9 exposures) performed in the Zulfi dates market area, all samples were collected during busy periods such as 4-6 pm. With these samples, the range of mold colonies was 87-131 CFU/plate. A further 9 exposures were performed in the Zulfi city west view

Figure 1: The impact of sandstorm over the Al-Zulfi city, (a) Clear atmospheric condition on March 22, 2016, and (b) sandstorm episode on March 27, 2016

area and these showed a range of 34-110 CFU/plate. Control samples (9 exposures) were collected from the next day after a sandstorm had occurred (that is non-sandstorm conditions with an observable clear sky); here all plates showed less than 15 CFU/plate [Table 1].

The total of 2590 fungi isolated from the 36 exposures of sandstorm dust samples is listed in Table 2. Based on a microscopic and macroscopic morphological identification of the fungi, 13 different genera of fungi were identified from the isolates. The predominant colonies were species of *Fusarium* (21%) followed by *Cladosporium*, *Ulocladium*, *Aspergillus*, *Alternaria*, and *Penicillium* (15.8%, 11.1%, 10.9%, 8.6%, and 7.3%, respectively). The totality of dematiaceous fungi isolated (56.2%) was greater than hyaline fungi (43.8%) ($P < 0.0001$). A proportion of the fungi (9.3%) could not be identified due to a lack of sporulation. These have been reported as "unidentified dematiaceous" and "hyaline fungi," respectively. The ranking of incidence of fungi and their pathogenicity to cause allergic diseases (based on comparable with worldwide reports) are listed in Table 3.

DISCUSSION

A significant increase in the frequency and the intensity of sand and dust storms has been observed in the Middle East

Table 1: Total number of fungal colonies observed from the exposed sandstorm dust samples

Sample ID	Description	Temperature/Wind	Number of fungal colonies (CFU/plate)					
			S1	S2	S3	Arithmetic mean	Geometric mean	Total
Sandstorm samples								2590
SS 1	Educational campus	38°C; 42 km/h	76	83	56	71.67	70.69	215
SS 2	Educational campus	35°C; 35 km/h	27	48	32	35.67	34.61	107
SS 3	Educational campus	31°C; 28 km/h	36	39	28	34.33	34.00	103
SS 4	Educational campus	35°C; 19 km/h	12	21	28	20.33	19.18	61
SS 5	Educational campus	30°C; 31 km/h	62	74	87	74.33	73.63	223
SS 6	Educational campus	37°C; 28 km/h	82	69	76	75.67	75.48	227
SS 7	Zulfi Dates Market	27°C, 40 km/h	107	121	115	114.33	114.19	343
SS 8	Zulfi Dates Market	28°C, 8 km/h	116	128	131	125.00	124.83	375
SS 9	Zulfi Dates Market	31°C; 22 km/h	87	95	96	92.67	92.58	278
SS 10	Zulfi – City view (East)	38°C; 42 km/h	91	94	67	84.00	83.06	252
SS 11	Zulfi – City view (East)	30°C; 32 km/h	110	88	96	98.00	97.58	294
SS 12	Zulfi – City view (East)	32°C; 28 km/h	34	38	40	37.33	37.25	112
Control samples (non-sandstorm)								66
NS 1	Educational campus	33°C; 12 km/h	2	4	3	3.00	2.88	9
NS 2	Zulfi Dates Market	35°C; 10 km/h	6	8	11	8.33	8.08	25
NS 3	Zulfi – City view (East)	34°C; 13 km/h	6	12	14	10.67	10.03	32

S1: Exposed plate 1, S2: Exposed plate 2, S3: Exposed plate 3

Table 2: Total number and distribution of molds from the each sample

Name of the organism	SS 1	SS 2	SS 3	SS 4	SS 5	SS 6	SS 7	SS 8	SS 9	SS 10	SS 11	SS 12	Total (%)
Dematiaceous fungi													
Cladosporium spp.	35	16	16	10	34	37	52	65	40	39	47	18	409 (15.8)
Curvularia spp.	17	8	7	3	15	12	31	27	18	16	16	7	177 (6.8)
Alternaria spp.	19	10	10	7	20	21	30	31	24	19	24	9	224 (8.6)
Bipolaris spp.	8	3	4	2	7	9	12	14	11	10	12	4	96 (3.7)
Ulocladium spp.	19	10	7	6	25	28	41	40	31	28	39	14	288 (11.1)
Exserohilum spp.	8	4	3	3	11	9	14	15	11	11	11	4	104 (4.0)
UIDF	12	7	8	4	14	13	15	20	21	17	18	8	157 (6.1)
Total -Dematiaceous fungi	118	58	55	35	126	129	195	212	156	140	167	64	1455 (56.2)
Hyaline fungi													
Fusarium spp.	47	24	21	15	47	50	61	80	60	51	63	24	543 (21.0)
Aspergillus niger	8	4	6	2	9	7	15	16	11	9	12	4	103 (4.0)
Aspergillus flavus	6	3	3	2	7	7	14	12	9	8	9	3	83 (3.2)
Aspergillus fumigatus	4	2	2	1	6	3	7	8	6	6	5	2	52 (2.0)
Aspergillus spp.	4	2	1	0	4	3	7	6	5	4	6	1	43 (1.7)
Penicillium spp.	15	7	9	5	16	17	25	27	20	18	21	8	188 (7.3)
Scedosporium spp.	1	0	0	0	1	0	2	2	1	2	2	0	11 (0.4)
Mucor spp.	0	0	1	0	1	1	1	2	0	1	0	0	7 (0.3)
Rhizopus spp.	1	1	0	0	1	1	1	2	1	1	2	1	12 (0.5)
Paecilomyces spp.	1	0	0	0	1	1	2	1	1	1	1	0	9 (0.3)
UIHF	10	6	5	1	4	8	13	7	8	11	6	5	84 (3.2)
Total – hyaline fungi	97	49	48	26	97	98	148	163	122	112	127	48	1135 (43.8)
Grand total	215	107	103	61	223	227	343	375	278	252	294	112	2590 (100)

UIDF: Unidentified dematiaceous fungi, UIHF: Unidentified hyaline fungi

Table 3: Incidence of commonly occurring fungal genera in this study and comparable with worldwide available reports

Rank	Fungal genera (Percentage of incidence in this study)	Predominantly reported in dust source region (References)	Reported pathogenicity to allergies
I	*Fusarium* (21%)	Turkey [30], Taiwan [35]	Allergen related disease
II	*Cladosporium* (15.8%)	Saudi Arabia [8], Egypt [29], Turkey [30], Iran [31], Taiwan [35], Africa [37]	Potent allergen related disease
III	*Ulocladium* (11.1%)	Saudi Arabia [8], Taiwan [35]	No reports
IV	*Aspergillus* (10.9%)	Saudi Arabia [8,9], Taiwan [36], Africa [37], Mali [38], Mid Atlantic [39], Israel [40]	Aspergillosis (Pulmonary allergic and colonizing), Number of different allergen related diseases in the immunocompromised person
V	*Alternaria* (8.6%)	Saudi Arabia [8], Turkey [30], Taiwan [36], Africa [37], Mali [38], Mid Atlantic [39], Israel [40]	Potent allergen (common causative agent of extrinsic asthma)
VI	*Penicillium* (7.3%)	Saudi Arabia [8], Turkey [30], Taiwan [36], Mid Atlantic [39]	Potent allergen related disease
VII	*Curvularia* (6.8%)	Taiwan [35]	Allergen-related disease

over the past 15 years. Although the focal point has been the Middle East, with Iran and Kuwait the most affected, meteorologists have recorded similar step-changes in activity in some parts of Central Asia [2]. This global rise in incidences makes the study of fungal spores in the generated dust a subject of medical importance. Human health is adversely affected by the exposure of sandstorm dust because the dust carries numerous pollutants such as allergens and microorganisms that can affect human health. Hence, there is the need for detailed studies about the microorganisms distributed the sandstorm dust, particularly fungi capable of causing allergic diseases.

Each geographical area will vary in the range and types of microorganisms recovered. Overall, there are very few research articles available about fungal genera in sandstorm dust environment and there have been none reported relating to the central region of the KSA. The location selected for study was important in terms of geography. Al-Zulfi city lies in the northern-central region of the Najd and to the south of the Samnan Valley of Saudi Arabia. It is surrounded by sand hills to its north and west and consequently Al-Zulfi city is subject to regular sandstorms because of the city surrounded by sand hills.

With this study, 36 exposures of sandstorm dust samples were collected in three different environments (an educational campus, a people gathering area, and a recreational place). These were compared to control (non-sandstorm) samples. The total numbers of fungal colonies observed from the exposed plates was 2590. Across the three locations, the numbers of fungi were higher compared with the control, indicating that airborne fungal populations are significantly elevated during sandstorms (between 3 and 10-fold higher; $P = 0.0066$). In relation to metrological factors many researchers have reported that weather patterns cause significant effects on the concentration of airborne fungal spores [1,16]; in this study, all samples collected under conditions that were metrologically assessed and it was noted that the wind velocity was >20 km/h during each sandstorm episode. This fact alone appears to yield more fungal airborne particles compared with the periods when the control samples were collected.

In the people gathering area (market), this locale showed higher recoveries than with the educational campus ($P = 0.0079$). With the market area, three sample sets (9 exposures) were collected. Each of the samples was collected during busy periods (4-6 pm) and the range of mold colonies was between 87 and 131 CFU/plate. These sampling sites will be affected by the density of people and their increased activities. The numbers of fungi recovered will be influenced by the people, for humans continually shed microorganisms (including fungi) from the outer surface of the skin [17], and this may account for the increased number of fungal counts compared with the other areas sampled.

In this study, species of *Fusarium* (21%), *Cladosporium* (15.8%), *Ulocladium* (11.1%), *Aspergillus* (10.9%), *Alternaria* (8.6%), and *Penicillium* (7.3%) are predominantly present in all of the sandstorm dust samples. This is similar to another study conducted in the KSA by Kwassi and his coworkers, which reported that the most abundant fungal genera in sandstorm dust were *Alternaria*, *Aspergillus*, and *Cladosporium* [8]. These predominant genera were comparable with other research reports relating to the Middle East countries of Kuwait and Qatar [18,19]. For example, the most prevalent fungal genera in airborne dust samples collected from the atmosphere of Taif, KSA, were (31 genera and 70 species) *Aspergillus*, *Drechslera*, *Fusarium*, *Mucor*, *Penicillium*, *Phoma*, and *Stachybotrys* [20]. In a large series study carried out by Abu Dieyeh and his coworkers in Zarqa desert region, Jordan, this reported that the highest abundance of fungi were attributable to *Cladosporium* (29.1%) followed by *Fusarium* (20%), *Alternaria* (7.7%), *Ulocladium* (6.5%), *Penicillium* (4.2%), and *Aspergillus* (3.6%) [21].

While the genera were generally similar, our study showed a higher proportion of species of *Fusarium*. This may be reflective of a niche environment since incidences of fungal genera will vary among regions based on the "true" microbial community, or it could be a product of the sampling method, agar selected, incubation time, and identification methods. For example, the most well-known human pathogens associated with desert dust storms and dust exposure originating from dried bird faces are *Coccidioides immitis* and *Histoplasma capsulatum*, respectively, yet these have been found only in the

Americas [22,23] and none of these pathogens were reported in this study's findings. This reaffirms that desert environments harbor diverse mycological communities and with this there are regional variations.

Another interesting finding relates to the incidence of dematiaceous fungi from the sandstorm dust, which was slightly higher (at 56.2%) compared to hyaline fungi (43.8%). This ratio is similar to another report [18]. The ratio is significant since the dematiaceous fungi cell wall contains a melanin pigment that contributes to the virulence of pathogens of humans as well as those of food crops. In addition, the pigment enhances fungal resistance to environmental damage. Several authors have reported that dematiaceous fungal spores are highly prevalent in hot climate atmospheres and they are more resistant to desiccation [24] and ultraviolet radiation [25], compared with hyaline fungi.

With individual predominant fungal genera, *Fusarium* was the most predominant species in this study with the total contribution of 21%. Notably, the Jordan study reported that *Fusarium* species are found at a high incidence during the month of May [21]. Given that our study's sampling duration covered the month of May, this could be the reason for the high percentage recovery of this fungus. A further reason for the prevalence relates to *Fusarium* being a well-known plant pathogen and one that is frequently isolated from the soil. Because sandstorm wind speeds, recorded in our study were typically between 30 and 40 km/h, the wind will carry sand dust materials from the agricultural surroundings of the city and this may contribute to the high incidence of *Fusarium* species in the atmosphere. Similarly, *Fusarium* species were reported as the most abundant species in the atmosphere with the Tel-Aviv, Israel study [26].

Cladosporium species was the second most predominant species in our study findings, and this is similar to several aeromycological studies from around the world [16,18,21,27,28]. Many reports from the countries nearby the KSA such as Jordan [21], Qatar [18], Egypt [29], Turkey [30], and Iran [31] have reported *Cladosporium* as the most predominant dematiaceous fungi. This coincides with our research which showed that at a percentage of 15.8%, *Cladosporium* are frequently isolated from the air and soil. Many authors have reported that the high percentage of *Cladosporium* may be attributed by the size of spores and smooth wall which favor and facilitate the transport of airborne spores in sandstorms. A further factor favoring this fungus is due to the spores being highly resistant to hot climates [18,32].

Another predominant dematiaceous airborne fungus is *Alternaria* species. With our study, the incidence of this fungus was 8.6%. Species of *Alternaria* are commonly reported as predominant fungal contaminants from outdoor environments [33]. A large sandstorm study conducted by Kwaasi *et al*., (1998) at Riyadh, Saudi Arabia reported that the predominant fungal genera were *Alternaria*, *Aspergillus*, and *Cladosporium* [8]. These findings reaffirm what this study reports.

According to the available reports from the various desert regions worldwide, the fungal genera of *Aspergillus*, *Alternaria*, *Cladosporium*, *Curvularia*, *Fusarium*, and *Penicillium* isolated from the sandstorm dust sources are mild to potent allergens [1,34-40]. During dust storms, a variety of airborne microbial particles remain suspended in the fine dust and some get blown over long distances and they can be forcibly introduced into human orifices such as eyes, nostrils, and ears [1,4,34]. Those particles which are <10 μm in size can penetrate into the lungs and those of <2.5 μm can penetrate into the subepithelial environment and can cause an allergic response. The spore sizes of the predominant fungal general reported in this study are less than 10 μm and thus they could cause an allergic reaction. In addition, this study has indicated that some of the most important allergenic fungal spores were isolated from the sandstorm dust in high numbers. These were *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, *Paecilomyces*, and *Penicillium*. Thus, based on size and morphology, the fungi contained in sandstorm dust can potentially cause allergenic respiratory problems with those who are exposed to sandstorm dust. These tallies with a recent study from the KSA, which reported that people exposed to a sandstorm for duration of 24 min developed various respiratory complaints including asthma [7]. The report from KSA reaffirms that sandstorms carry numerous allergens through the air and with 70% of Saudi Arabian asthmatics already suffering from allergies, the risk of respiratory complications due to sandstorms is extremely high. In one single sandstorm in 2015 over 400 Saudi Arabian citizens were hospitalized due to respiratory problems, of this one quarter of the admissions was pediatric [34].

Based on the findings of this study that sandstorm dust carries fungal spores which cause an allergenic response in humans, we recommend that precautionary measures be taken to minimize exposure to the sandstorm dust. In particular, preventative recommendations should be made to outside workers and for returning residents. Such measures should be based on the use of appropriate personal protective equipment (dust mask) as well as avoiding unnecessary exposure to the dust. Various types of facemasks available to the general public can protect against inhalation of dust, pollutants, allergens, and pathogenic organisms. In addition, airtight goggles can be worn. It is important for children with chronic respiratory problems to avoid all outdoor activity during a sandstorm. Another important recommendation is for people who wear contact lenses. Here contact lens wearers should not expose their contact lenses to dust and people should ideally wear eyeglasses (or goggles) during a sandstorm. Where contact lenses are worn these should always be kept clean otherwise the dust exposure may lead to a corneal ulcer developing.

Limitations of the Study

The main limitation of the study was with the control samples. These were collected for only one sample (3 exposures) per location. This was because the control samples were used only for comparisons and the main aim of the work was to analyze

the fungal allergens in sandstorm dust samples. A baseline for non-sandstorm conditions can be developed through further study.

CONCLUSION

This study concludes that sandstorm dust from the central region of the KSA carries high numbers of fungal spores. The predominant fungal spores are *Fusarium*, *Cladosporium*, *Ulocladium*, *Aspergillus*, and *Alternaria*. The concentrations of these fungi are high as compared with non-sandstorm periods. Of the different types of fungi, dematiaceous fungi incidences are higher than hyaline fungi. The predominant fungal spores characterized can act as potent allergens and cause respiratory allergic diseases. To the best of our knowledge, this study is the first to highlight the distribution of fungal genera in sandstorm dust from the Al-Zulfi region, the central region of KSA. This study recommends that protective measures such as wearing a mask to minimize the respiratory asthmatic diseases and airtight goggles to protect the eyes. Finally, further studies of aeromycological levels are needed to further understand the microbial ecology, biogeography, and spread of fungal allergens in sandstorm dust.

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