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Original Research Article

Aeromycoflora of Indoor and Outdoor environments of Ad Darb and Shuqaiq Regions of Jizan Province, Saudi Arabia

Professor Syeda Fatima Manzelat *, Ph.D
College of Arts and Science, Ad Darb, Jizan University , Jizan.

***Corresponding Author:**
Professor Syeda Fatima Manzelat
Email: drsyedafatima@hotmail.com

Abstract: This is the first study of aeromycoflora from the two places Ad Darb and Shuqaiq in the Jizan province. The diversity of aeromycoflora of the two places was isolated to know the environmental status. A total number of sixteen outdoor and indoor environmental samples of aeromycoflora were collected and studied from the two places along with the College of Science and Arts for Girls at Ad Darb. The aeromycoflora was cultured by open petri plate method during the month of April 2017 . A qualitative and quantitative assessment of the indoor and outdoor mycoflora was carried out by exposing the Potato Dextrose Agar petri plates in the different environments each for a period of two minutes.The fungal cultures on the petri plates and the slides were identified by microscopic and macroscopic characteristics. The outdoor aeromycoflora was represented by *Aspergillus niger, Aspergillus flavus, Aureobasidium, Curvularia, Dresclera, Epicoccum, Fusarium, Penicillium and Yeast*. The indoor aeromycoflora was represented by *Aspergillus, Colletotrichum , Penicillium, Chrysosporium and Ulocladium.* The most predominant genus was *Aspergillus*. The colony forming unit $(CFU)^3$ of each of the represented mycoflora was calculated to find out the level of contamination at the different sites. The $(CFU)^3$ values indicated that the outdoor mycoflora was higher than the indoor mycoflora .The fungal genera isolated are pathogenic. Hence a polluted aerial environment is harmful not only to human health but also to the animals and plant life .Therefore a constant monitoring for a clean and germ free environment is needed at all times**.**

Keywords: Aeromycoflora, indoor , outdoor, Ad Darb, Shuqaiq, Jizan.

INTRODUCTION:

Environmental quality is a major problem all over the world.The study of aeromycoflora is an important tool in the effective monitoring of the quality and quantity of fungal pollutants in the air. The qualitative and quantitative analysis of the aeromycoflora helps in the biodiversity of the region which shows the amount and types diverse groups of fungi of inhabiting the region. Aeromycoflora is the study of the microscopic harmful fungi present in the air around us which we breath. Hence aerial environment around us must be clean and germ free always.There are records of research on aeromycoflora of different places of Saudi Arabia like Riyadh, Taif, Dammam, Al Khobar, Abu Arish in Jizan etc.This is the first study of aeromycoflora from the two unexplored places ,Ad Darb and Shuqaiq in the Jizan province.

The diversity of aeromycoflora of the two places was isolated to know the environmental status .Fungi are ubiquitous and found in the air , water and soil environments. Aeromycoflora is present both in the outdoor as well as in the indoor environments. Outdoor mycoflora is loaded with fungal propagules which are pathogenic not only to human beings but also to animals and plant life. They are also responsible for damage to wooden articles and buildings. The fungi cause many diseases in plants like wilts, leaf spots etc. Fungi infect human beings and cause skin, nail, hair and other infections apart from aspergillosis, lung infections and cancer .animals are also similarly infected by fungi present in the air. The diversity of aeromycoflora of the two places was isolated to know the environmental status .The indoor environment is much more important as a major part of the day is spent indoor.The indoor fungi are not only responsible for causing diseases but are also responsible for the spoilage of food , clothes , books and furniture.Therefore the diversity of the aeromycoflora studied is helpful in environmental monitoring as the study recorded the quantitative and qualitative contamination of different sampling sites from the regions under study.

The quality and quantity of the aeromycoflora is effected by the parameters like temperature, humidity, wind speed and rainfall, light, sand storm and organic matter available at that place. High temperatures, sand storms and wind helps in quick

dispersal of fungal propagules along with dust particles to large distances whereas rainfall and humidity reduces the quality and quantity of fungal propagules in the air.

This is the first study of aeromycoflora from the two places Ad Darb and Shuqaiq in the Jizan province. The diversity of aeromycoflora of the two places was isolated to know the environmental status. A total number of sixteen outdoor and indoor environmental samples of aeromycoflora were collected and studied from the two places along with the College of Science and Arts for Girls at Ad Darb. The aeromycoflora was cultured by open petri plate method during the month of April 2017 . A qualitative and quantitative assessment of the indoor and outdoor mycoflora was carried out by exposing the Potato Dextrose Agar petri plates in the different environments each for a period of two minutes.The fungal cultures on the petri plates and the slides were identified by microscopic and macroscopic characteristics. The outdoor aeromycoflora was represented by *Aspergillus niger , Aspergillus flavus, Aureobasidium, Curvularia, Dresclera , Epicoccum, Fusarium, Penicillium and Yeast*. These common genera of fungi which were also isolated from indoor environment of Riyadh are *Alternaria, Aspergillus, Cercospora, Chaetomium, Cladosporium, Curvularia, Drechslera, Fusarium, Penicillium &Ulocladium* [1]. *Aspergillus sp. and Cladosporium sp.* were the two dominant fungal genera indoors and outdoors. *Alternaria, Ulocladium and Drechslera* were the dominant types in the air of Alkhobar, Hofuf and Abha.[2]. *Cladosporium spp., Penicillium spp., Aspergillus spp., Alternaria spp. and Ulocladium spp.* were included as major components. Minor components included *Drechslera spp., Rhizopus spp., Fusarium spp. and Stachybotryis spp.*

Alternaria, Cladosporium, Aspergillus, Penicillium Epicoccum and Fusarium from Al Khobar schools [3].The indoor aeromycoflora was represented by *Aspergillus ,Colletotrichum , Penicillium, Chrysosporium and Ulocladium.* The most predominant genera was *Aspergillus. Aspergillus niger and Cladosporium sphaerospermum* were the most dominant indoor airborne fungi obtained from Saudi Arabia [4]. *Asperillus niger , Aspergillus flavus and Penicillium* were reported in the indoor air of Abu Arish[5]. Due to the high temperature of the places during the summer xerophilic fungi was also obtained. Chrysosporium a xerophilic genera was isolated from the waiting hall of the college [6]. It is keratinophilic fungus causing hair, nail and skin infection. *C. keratinophilum* was recorded as a widespread mold in house dust from homes in Saudi Arabia [7].The most prevalent species of the *Chrysosporium were C. indicum, C. tropicum* in the hair samples of sheep in Saudi Arabia, [8]. The total fungal count in indoors was less than that of the outdoors [4].

Although many of the fungal genera are represented in this study but still a lot remains yet to be explored. Hence this study opens the door for exploring different indoor and outdoor environments of the two places for finding more and more of new fungal genera of this region.

The level of their contamination in the air was measured by the colony forming unit CFU/m^3 and the results were interpreted in tables. Statistical analysis of the data was done and bar diagrams were used to depict the results for both the indoor and outdoor mycoflora. The colony forming unit (CFU) ^{3} of each of the represented mycoflora was calculated to find out the level of contamination at the different sites. The (CFU) ^{3} values indicated that the outdoor mycoflora was higher than the indoor mycoflora. The fungal genera isolated are pathogenic .This can help in environmental monitoring and controlling the fungal pathogenic diseases of humans , animals and plants. Findings and results of this study can also help in disease forecasting of plant pathogenic aeromycoflora. Hence field crops growing in the region can be protected in advance .The study can also help in the diagnosis of human, plant and animal diseases. Hence a polluted aerial environment is harmful not only to human health but also to the animals and plant life .Therefore a constant monitoring for a clean and germ free environment is needed at all times.

MATERIALS AND METHODS:

List of Equipment:

Hot Air Oven, Autoclave, Heaters, Microscope, Bunsen burner, Inoculation Chamber, Refrigerator.

Minor instruments: Needles and spatulas.

Glassware

Petridishes , test tubes and other glassware thoroughly washed after rinsing with k2Cr2O7 and the plates other glassware were then sterilized in hot air oven at 160˚c for 24 hours. Other glassware includes slides and cover slips.

Consumables : Agar powder, distilled water , lactophenol , alcohol, potatoes, sucroseCotton plugs and alcohol swabs were also used.

Media Employed:

The following media was used:

a) Potato Dextrose Agar (PDA)

Potato	: 200 gms
Dextrose	: 20 gms
Agar	: 20 gms
Water	: 1000 ml

200 gms of potatoes were peeled and boiled in 500ml of water for one hour and the extract was filtered and to that 20 gms of sucrose and 20 gms of agar was added ,boiled and made upto 1000 ml and sterilized . This medium was employed to estimate the aeromycoflora of

Ad Darb and Suqaiq in the Jizan province of Saudi Arabia.

Slide Preparation: Lactophenol and cotton blue in lactophenol were used as mounting and staining media for preparing semi permanent slides which were sealed with DPX mountant.

Microscopic and Macroscopic observation: Research microscope with adequate high power has been used through out the study.Identification was carried out by using standard manuals and keys to the identification. Colony characters on media. Morphological characters include like nature of mycelium, its colour ,sexual and asexual structures and their characters , conidiophore nature and spore nature [9].Photographs of all the fungal cultures on the petriplates along with the photomicrographs of the slides were taken with a camera (Figs. 4 to 14 and 17 to 21).

Weather Report of Ad Darb and Shuqaiq:

Environmental factors such as humidity and temperature plays an important role in dispersing fungi spores in air for short and long distances and when spores deposited a solid or liquid surface and if conditions of moisture and food are appropriate, they germinate [10, 11]. Normal indoor conditions such as humidity and temperature provide a suitable environment for the growth of fungal spores [12, 13].Hence the following data was recorded from the two places at the time of sampling. The quality and quantity of the mycoflora is effected by the parameters like temperature , humidity , wind speed , rainfall, light and organic matter available at that place.

Table 1. Temperature, humidity and rainfall of Ad Darb and Shuqaiq on different sampling dates.

Name of the sampling Site	Date of Sampling	Temperature of the Place In in C		Humidity %		Wind Speed In MPH	Rainfall
		Maximum	Minimum	Maximum	Minimum		
Ad Darb	21 April 2017	36	27	66	42	1.51	Nil
Shuqaiq	28 April 2017	35	26	69	53	1.71	Nil

Fig.1 Laboratory equipment utilized during the project. (Hot air Oven , Heater , Autoclave and Refrigerator)

Sampling sites for sample collections:

Samples were collected both from the indoor and outdoor environments of the College of Arts and Science , Ad Darb and other places of Ad Darb , Shuqaiq and Huraidha valley. The sites for sample collection for outdoor environments were gardens from both the places , ground of the college, Huraidha valley, air near the residential trash , boat at the Shuqaiq sea shore and the busy Ad Darb Triangle. The indoor sampling sites were student's waiting hall of the college, both ground and first floor corridors of administration building, laboratory ,cafeteria, student's corridor ground floor, mosque, garments store and restaurant.

Fig.2 Map of Ad Darb and Shuqaiq , Jizan

Fig. 3 Pictures of Indoor and Outdoor sampling sites from the two places

RESULTS AND DISCUSSION:

The aeromycoflora was studied from sixteen different sites from the two places ad Darb and Shuqaiq in the Jizan province . Outdoor aeromycoflora was collected from seven different sites and indoor aeromycoflora was collected from nine different sites from these places.A total of eleven fungal colonies were obtained from the seven sampling sites of the outdoor environment (Table 2). The fungal cultures on the petriplates and the slides were identified by microscopic and macroscopic characteristics. A total of 11 fungal isolates were obtained from the outdoor environment which are represented by eight fungal genera with two species of *Aspergillus.* . *Curvularia* (Fig.4) was isolated from Garden (Shuqaiq) ,*Epicoccum*(Fig.5) from Garden (Ad Darb) ,*Aspergillus flavus* Fig. 6 and Yeast (Fig.7) from the College Ground. *Aspergillus niger* (Fig. 8) and *Fusarium* (Fig.9 were isolated from the Huraidha Valley. *Aspergillus flavus*(Fig.10), *Dresclera*(Fig.11)and *Aureobasidium* (Fig.12) from Ad Darb air near residential trash. *Cladosporium*(Fig.13) was isolated from the boat near the sea shore of the Red sea beach at Shuqaiq . *Aspergillus* ,*Cladosporium* ,*Drechslera* ,*Penicillium and Ulocladium* were common in the air of Taif [14]. . In general, higher concentrations of fungi were found at the developed (Al-Batha) site than at the less developed (Al-Ulia) site, both in the morning and in the afternoon. Al-Suwaine, A.S., Hasnain, S.M. & Bahkali*, A.H. Aerobiologia [7]. *Aspergillus niger* (Fig.14) was isolated from Ad Darb triangle which is the most busy and central region of place. The most predominant genera was *Aspergillus. Cladosporium and Penicillium* were reported in the indoor and outdoor mycoflora of Saudi Arabia [15].

A total of six fungal colonies were obtained from the nine sampling sites of the indoor environment (Table 4). The fungal cultures on the petriplates and the slides were identified by microscopic and macroscopic characteristics. A total of six fungal isolates were obtained from the indoor environment which are represented by five fungal genera with two isolates of *Colletotrichum. Ulocladium* (Fig.17) was isolated from the Corridor (2nd Floor) of the College of Arts and Science , Ad Darb. *Penicillium* (Fig. 18) and *Aspergillus*(Fig.19) from garments store , *Colletotrichum*(Fig.20) was isolated both from the mosque and garments store and *Chrysosporium* (Fig.21) from the waiting hall of the college.The most predominant genera was *Colletotrichum.*

Many of the fungal genera isolated are human pathogenic causing aspergillosis , allergy, skin . nail and other respiratory diseases as well as cancer . Hence a polluted aerial environment is harmful not only to human health but also to the animals and plant life. Therefore a constant monitoring for a clean and germ free environment is needed at all times.

Calculation of Colony Forming Unit (CFU/m^3) and it's Statistical Analysis:

The total number of aeromycoflora in the air samples collected from different sites was determined. The total number of colony forming unit (CFU/m^3) was calculated. Then it is converted to organisms per cubic meter air using the standard equation given below:

(CFU/m^3) = (no. of colonies on the petriplate) x 10000/(petriplate surface)
(petriplate surface)x (petriplate exposure time)x 0.2

Petriplate surface = 10 cm

The colony forming unit $(CFU)^3$ of each of the represented mycoflora was calculated to find out the level of contamination of different sites. . The normal standard range of CFU/m^3 is 61-460 set up according to the guidelines of World Health Organization. The values of CFU depend on many factors like the temperature , humidity , the time of exposure of the petri plates etc.

CFU/m^3 of the outdoor aeromycoflora :

The highest CFU of 1000 was recorded from Garden at Shuqaiq with approximately 160 colonies of Curvularia with an exposure time of two minutes which is very high as compared to the normal standard range (Table 4) .750 CFU/m3 was recorded from three fungal colonies isolated from the air near the residential trash at Ad Darb with an exposure time of two minutes.The college ground at Ad Darb and Huraidha valley each recorded 500 CFU/m^3 with an exposure time of two minutes each. The garden at Ad Darb, the boat near the seacoast of Shuqaiq and the Ad Darb triangle recorded 250 CFU/m^3 with an exposure time of two minutes each which within the normal standard values.There was no sampling site from the outdoor environment which was free from contamination (Fig.15).

CFU/m^3 of the indoor aeromycoflora :

The highest CFU of 750 was recorded from the sample collected from the garments store at Ad Darb with three fungal colonies isolated with an exposure time of two minutes which is very high as compared to the normal standard range (Table 5) . The waiting hall at Ad Darb college was sampled twice initially with an exposure time of 2 minutes with no aeromycoflora . On second exposure seven fungal colonies with a value of 700 CFU/m^3 was recorded with an exposure time of five minutes from the waiting hall.

Some of the indoor sampling sites which showed no contamination with a 0 CFU/m^3 were ground floor corridor of administration building, laboratory ,cafeteria, student's corridor ground floor of the college and also the restaurant at Ad Darb. (Fig. 16)

The (CFU)^3 values indicated that the outdoor mycoflora was higher than the indoor mycoflora both qualitatively and quantitatively.

The highest CFU of 1000 was recorded from Garden at Shuqaiq which is very high as compared to the normal standard range of 61-460 CFU/m3 according to the guidelines of World Health Organization.

Table 2. Total count of fungal colony from outdoor environment

Sl.No.	Name of the sampling site	Total Number of Colonies (Type)	*Aspergillus*	*Aureobasidium*	*Drescleria*	*Epicoccum*	*Fusarium*	*Curvularia*	*Cladosporium*	*Yeast*
1	Garden (Shuqaiq)	1						+		
2	Garden (Ad Darb)	1				+				
3.	Ground (College)	2	+							+
4.	Huraidha Valley	2	+				+			
5.	Near the Trash	3	+	+	+					
6.	On the Boat (Shuqaiq)	1							+	
7.	Triangle (Ad Darb)	1	+							
Total fungi isolated (11)		11	4	1	1	1	1	1	1	1

(+) = Present.

Photomicrographs of the Outdoor Fungi

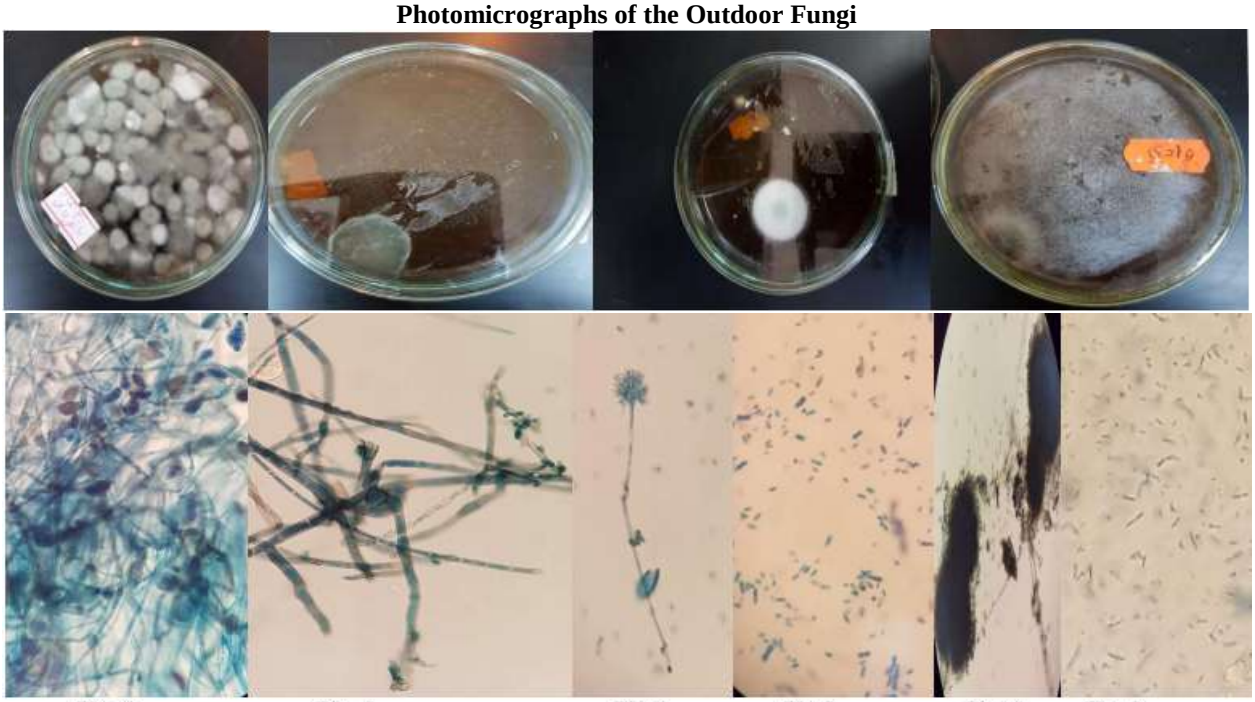

Fig. 4 Fig. 5 Fig.6 Fig.7 Fig. 8 Fig. 9

Fig. 4-*Curvularia* from Garden (Shuqaiq)
Fig. 5-*Epicoccum* from Garden (Ad Darb)
Fig. 6-*Aspergillus flavus* from College Ground
Fig. 7-*Yeast* from College Ground
Fig. 8-*Aspergillus niger* from Huraidha Valley
Fig. 9-*Fusarium* from Huraidha Valley

Photomicrographs of the Indoor Fungi

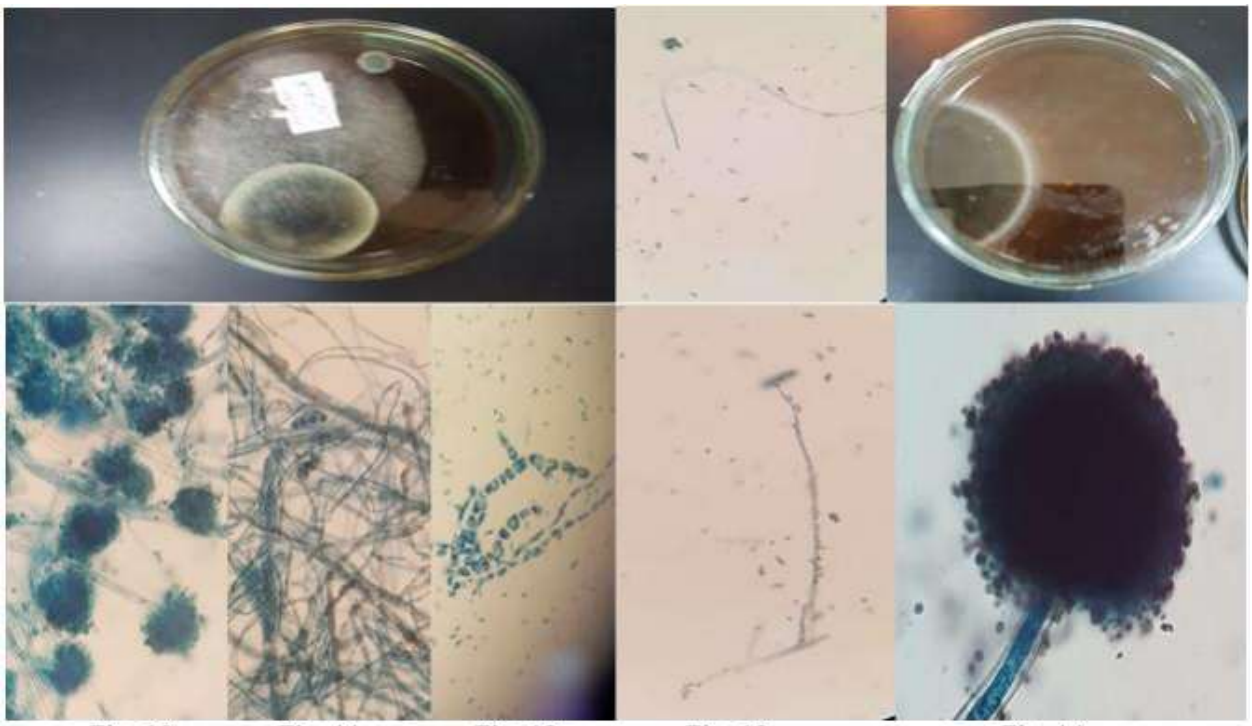

Fig. 10 Fig. 11 Fig. 12 Fig. 13 Fig. 14

Fig. 10 -*Aspergillus flavus* from Ad Darb Near Trash
Fig. 11-*Dresclera* from Ad Darb Near Trash
Fig 12-*Aureobasidium* from Ad Darb Near Trash
Fig.13- *Cladosporium* from the Boat
Fig.14-*Aspergillus niger* from Triangle (Ad Darb)

Table-3 Average number of fungal count (CFU/m^3) in outdoor air samples

Sl.No.	Name of the sampling site	Total Number(Types) of Colonies (Type)	Exposure Time in Minutes	Colony Forming Unit (CFU/m^3)
1	Garden (Shuqaiq)	Approx.160	2	1000
2	Garden(Ad Darb)	1	2	250
3.	Ground (College)	2	2	500
4.	Huraidha Valley	2	2	500
5.	Near the Trash	3	2	750
6.	On the Boat (Shuqaiq)	1	2	250
7.	Triangle (Ad Darb)	1	2	250
Total fungi isolated (11)				

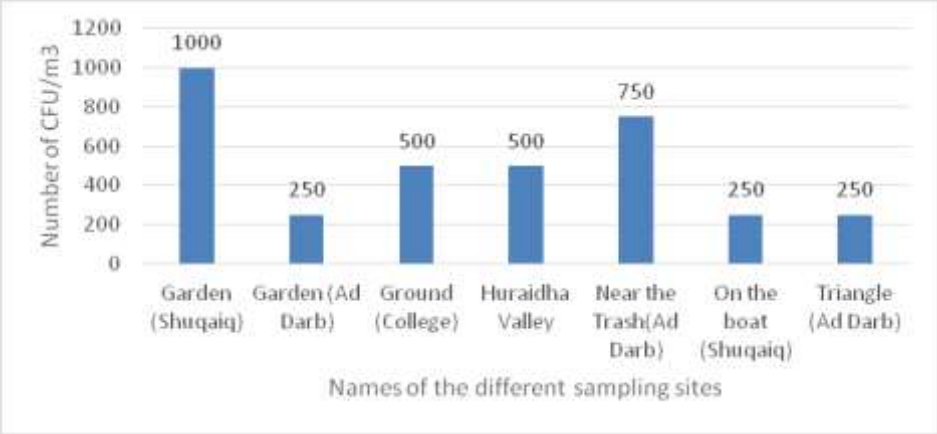

Fig. 15-Bar diagram for fungal counts from indoor sample collection sites

The highest CFU of 1000 was recorded from Garden at Shuqaiq which is very high as compared to the normal standard range of 61-460 CFU/m^3 according to the guidelines of World Health Organization followed by CFU of 750 for the sample collected from the air near the Ad Darb residential trash which is above the normal range. The College ground and the Huraidha valley each recorded CFU of 500. The Ad darb Triangle and the sample collected from the boat at Ad Darb showed a CFU of 250 each which is well within the range.

Table 4. Total count and percentage contribution of fungal colony from indoor environment

Sl.No.	Name of the sampling site	Total Number (Types) of Colonies	*Aspergillus*	*Colletotrichum*	*Chrysosporium*	*Penicillium*	*Ulocladium*
1.	Cafeteria	0					
2.	Corridor First floor (Admin.)	1					+
3.	Corridor Ground floor (Admin.)	0					
4.	Corridor (Student)	0					
5.	Garments Store	3	+	+		+	
6.	Laboratory	0					
7.	Mosque	1		+			
8.	Restaurant	0					
9.	Waiting Hall	0					
9.	Waiting Hall (Second Exposure)	1			+		
	Total fungi isolated (6)	6	1	2		1	1

(+) = Present

Table-5 Average number of fungal count (CFU/m^3) in indoor air samples

Sl.No.	Name of the sampling site	Total Number(Types) of Colonies	Exposure Time in Minutes	Colony Forming Unit (CFU/m^3)
1.	Cafeteria	0	2	0
2.	Corridor First floor (Admin.)	1	2	250
3.	Corridor Ground floor (Admin.)	0	2	0
4.	Corridor(Student's)	0	2	0
5.	Garments Store	3	2	750
6.	Laboratory	0	2	0
7.	Mosque	1	2	250
8.	Restaurant	0	2	0
9	Waiting Hall	0	2	0
10.	Waiting Hall	7	5	700
	Total fungi isolated (6)			

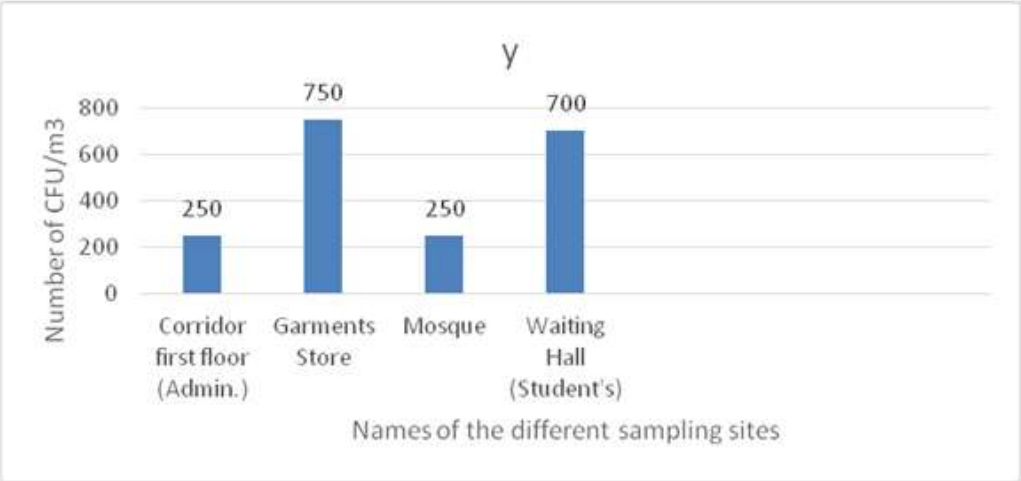

Fig. 16-Bar diagram for fungal counts from indoor sample collection sites

The highest CFU of 750 was recorded from Garments shop at Ad Darb which is very high as compared to the normal standard range of 61-460 CFU/m^3 according to the guidelines of World Health Organization followed by CFU of 700 for the sample collected from the waiting hall of the stdents at the college which is also above the normal range. The mosque and the corridor of the first floor in the administration building of the college each recorded CFU of 250 each which is well within the normal range. Some of the indoor sampling sites which showed no contamination were ground floor corridor of administration building, laboratory, cafeteria, student's corridor ground floor and restaurant

The $(CFU)^3$ values indicated that the outdoor mycoflora was higher than the indoor mycoflora both qualitatively and quantitatively.

Photomicrographs of the Indoor Fungi

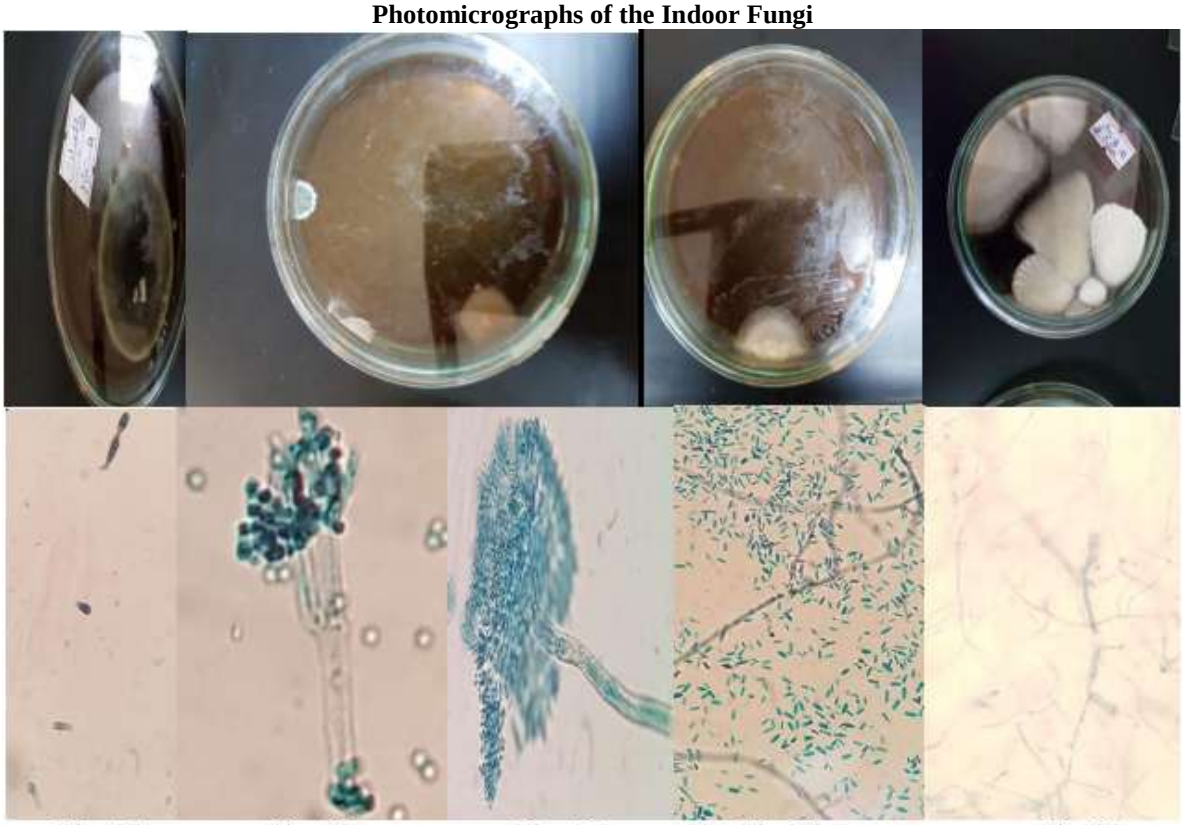

Fig. 17 *Ulocladium* from the Corridor (2nd Floor)
Fig. 18 *Penicillium* from Garments Store
Fig. 19 *Aspergillus* from Garments Store
Fig. 20 *Colletotrichum* from Mosque and Garments Store
Fig 21 *Chrysosporium* from Waiting Hall

CONCLUSION:

The present study shows that a number of harmful and pathogenic aermycoflora are present both in the indoor and outdoor environments around the two places .The quality of air which we breathe must be pure and free from any kind of harmful pathogens. The indoor and outdoor environments must be kept clean always in order to check these pathogens in the air .The indoor environment must be cleaned, properly ventilated and kept dry using dehumidifiers. Most important is that we maintain personal hygiene not only for ourselves but also for the safety of others. Fungal propagules are microscopic and produced in large quantities and disperse very easily and quickly. They are contagious and human pathogenic fungi are more dangerous and show immediate skin and lung infections upon slightest contact or inhalation.Air conditioners and other gadgets must be cleaned from time to time to check the pathogens. Harmless disinfectants vapours can be sprayed and fumigation also helps in cleaning the air around us from these pathogens.The outdoor environment must be cleaned regularly and garbage disposed properly and on time. We must always try to avoid garbage and other dirty and overcrowded places around us in order to keep ourselves safe.

RECOMMENDATIONS:

The present study opens doors to further research in other organizations like schools etc. in these unexplored places of Jizan. Although many of the fungal genera are represented in this study but still a lot remains yet to be explored . Hence this study opens the door for exploring further the different indoor and outdoor environments of the two places for finding more and more of new fungal genera of this region.

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