Biodiversity and Distribution of Airborne *Cladosporium* Species in Riyadh city

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Abstract: Species of the genus Cladosporium are among the most common fungi to be isolated from the environment almost anywhere in the world, in indoors as well as outdoors air. Many species are known to be plant pathogens, while others are regularly encountered as contaminants and spoilage agents in food or industrial products. Cladosporium spp. are pathogenic for humans, causing localized infections, more severe respiratory diseases, or systemic mycoses. This study is a first step towards the identification of Cladosporium spp. in the atmosphere of Riyadh, Saudi Arabia. In order to investigate the geographical distribution of Cladosporium spp. air was sampled from forty sites on north east, North West, south east, south west and middle of Riyadh. A total of 870 fungal colonies were isolated, 108 (12.4%) of them were Cladosporium spp. The genus Cladosporium spp. was represented in all studied sites. Nineteen isolates belong to five Cladosporium species were identified. In all sampling sites, the most prevalent Cladosporium species were *Cladosporium cladosporioides* (Fresenius) de Vries and *Cladosporium sphaerospermum* Penzig, followed by *Cladosporium herbarum* (Persoon) Link, *Cladosporium macrocarpum* Preuss, and *Cladosporium chlamydosporis* Matsushima. Density of Cladosporium spp. during the investigation of Seasonal variation was affected by month and site. The two main effects of ANOVA (month and site) were all very highly significant sources of variation in density of Cladosporium spp. isolated from Riyadh city. Also, the two-way interaction for month × site was a very highly significant source of variation in the case of density of Cladosporium spp. ($P = 0.0000$).

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1. Introduction

The genus *Cladosporium* Link is one of the largest genera of hyphomycetes. This genus was established in 1816 by Link (Cited from Schubert, 2005), who described it as follows: '*Thallus e floccis caespitosis, erectis simplicibus aut subramosis, apicibus* in sporidia secedentibus. A recent published checklist contains data for 772 *Cladosporium* names, i.e., valid, invalid, legitimate and illegitimate species, varieties and formae as well as herbarium names (Dugan *et al.*, 2004). The most common species of the genus *Cladosporium* include *C. herbarum*, *C. sphaerospermum*, *C. cladosporioides* and *C. elatum*, (Qiu-Xia *et al.* , 2007).

Cladosporium spp. are cause of fungal allergy (Yano *et al.*, 2003). Some of the allergenic fungi are pathogenic for humans, causing localized infections, more severe respiratory diseases, or systemic mycoses (Mari *et al.*, 2003; Yano *et al.*, 2003; Qiu-Xia *et al.*, 2007). Infection with subcutaneous phaeohyphomycosis due to the genus *Cladosporium* have been described (Romano *et al.*, 2002). Some species of *Cladosporium* were isolated from brain and skin lesions and rarely from lung lesions (Yano *et al.*, 2003). *Cladosporium* also was involved in cases of Hypersensitivity Pneumonitis (Jacobs *et al.*, 1986). Chromomycosi is a chronic fungal infection of the skin and subcutaneous tissues caused by nearly 30 different fungal species including *Cladosporium* spp (Lortholary *et al.*, 1999; Ezughah *et al.*, 2003). In addition, many airborne fungi can cause human diseases, particularly in individuals that are immunocompromised or otherwise sensitive to a broad range of allergenic and toxigenic biological material (Burge and Rogers, 2000; Ross *et al.*, 2000; Fierer *et al.*, 2008). With the prevalence of asthma increasing worldwide in recent decades, there is a growing need to better understand the diversity and spatiotemporal dynamics of airborne microbes (Isolauri *et al.*, 2004; Fierer *et al.*, 2008).

The seasonal occurrence of airborne fungal spores has been studied in tropical, temperate, and arctic regions, predominantly by allergologists and plant pathologists. *Cladosporium*, one of the most cosmopolitan airborne fungi, is able to colonize a large array of substrates (Fernandez *et al.*, 1998;

Molina, *et al.*, 1998; Hollins *et al.*, 2004), a fact that will considerably facilitate its presence in the atmosphere (Fernandez *et al.*, 1998; Molina *et al.*, 1998). It is therefore often referred to, in aeromycological calendars, as one of the most common and abundant airborne fungi (Mitakakis *et al.*, 1997; Molina *et al.*, 1998).

Fungi usually enter a building through outdoor air intakes of the heating, ventilation, and air conditioning system, through doors and windows, and as contaminants on building materials and contents. If elevated moisture conditions exist for a sufficient time in a building, fungal growth and sporulation may occur. Outdoor air often is the dominant source of indoor fungi, so an understanding of the outdoor fungal populations in different seasons and in different regions necessarily underlies interpretation of the results of indoor fungal sampling (Jones and Cookson, 1983; Ren *et al.*, 1999; Pei-Chih *et al.*, 2000; Shelton *et al.*, 2002).

An aerobiological study to identify and quantify allergenic fungi and their seasonal fluctuations was conducted at two different sites, (Al-Batha, and Al-Ulia) in Riyadh city. The seasonal variation of the total airborne fungi and for major generic categories (*Cladosporium*, *Penicillium* and *Ulocladium*) appear to show a higher concentration in the cold months of winter (November) and a low concentration in hot summer months(Al-Suwaine *et al.*, 1999a, b).

Airborne mould may originate from the outdoor air and enter through the ventilation system or from humid niches in the environment (Kure *et al.*, 2008). In new buildings, indoor levels of spores are lower than outdoor levels, even with natural ventilation. Buildings that foster fungal growth may generate spore levels higher than those outdoors. Cases of such problem buildings may require special treatment, including elimination of moisture sources and water-damaged materials (Kowalski, 2000). During parts of the year when windows are open, indoor fungi are comparable to outdoor species (*Cladosporium*, *Alternaria*, and *Aureobasidium*) (Sneller and Roby, 1979; Kuhn and Ghannoum, 2003). Consequently, the aim of this study was investigation of the Geographical distribution, describe the species and frequencies of *Cladosporium* spp. in outdoor air, as well as Comparing the relative frequencies of outdoor airborne *Cladosporium* species with other fungi for 12 consecutive months using samples obtained from different sites of Riyadh, Saudi Arabia, in different seasons of the year.

2.Material and Methods

1. Site description

Riyadh is the capital of Saudi Arabia. It is situated in the center of the Arabian Peninsula on a large plateau, and is home to over 4,260,000 people. (The Saudi Arabian Information Resource; http://en.wikipedia.org/wiki/Riyadh#cite_note-0). It is 1600 square kilometers in area. The city is 606 meter above the sea level. (Al-Suwaine *et al.*, 1999a).

2. Climate

There is relatively little wind in Riyadh during the winter, but it sometimes becomes windy and dusty during the summer (Al-Suwaine *et al.*, 1999b). Summer temperatures are very hot, frequently exceeding 45°C. Winters are mild with cool nights. Although the city is located in a highly arid area, it receives some rainfall. Hail occasionally falls in Riyadh during winters .

3. Geographical distribution

3.1 Air samples

Air was sampled at 40 sites on Riyadh . Samplers were placed at each site in a line vertical with the sampling surface 1 m above the ground. The samples were collected with Microbiological air sampler SAS HiVAC PETRI Cat. 17407, which are viable impaction samplers, were analyzed, using three for each site of Petri plate that contain about 15 ml of Malt Extract Agar (MEA) (MERCK). Operation of air sampler was according manufacture instruction as follow; the cover of the sampler was removed using the plastic cup with avoiding touching the inside or outside of the drilled area. Closed filled "Petri Plate" were inserted into retaining slot and then the lid were removed with avoiding contamination from droplets and aerosol infection. The cover was then replaced on sampler. The main switch was Turned ON. Required volume of air was selected (200 liter). Start button was pressed to start sampling. At the end of the time cycle the sampler cover was unscrewed, Petri plate lid were replaced and covered plate were removed from unit. The brass collecting arms were cleaned with ethanol. Cultures were incubated in the laboratory at Sanyo Microbiological Incubator (Sanyo Incubator MIR-152 SANYO Electric Co., Ltd. Japan) at 25°C. Plates were inspected after 4 days and periodically up to 14 days after primary exposure. Purification of colonies was onto malt extract agar.

3.2. Fungal concentrations

Outdoor samples were analyzed separately using descriptive statistics. The analytical criterion was to identify *Cladosporium* species if many species were present. All fungal concentrations were expressed as colony forming unit per cubic meter (CFU m^{-3}) of air. Actual plate counts, not estimated counts, were used to calculate the CFU m^{-3}, and analyzed Using Microsoft Office Excel 2007 software. Regions were defined as North East, North

West, South East, South West and Middle. The samples of each Region were defined as follow: North East Region was included samples 1-8. North West Region was included 9-14. Middle Region was included 15-22. South East Region was included 23-32. South West Region was included 33-40.

3.3. Fungal purification

The purification procedure of the fungal isolate under investigation was carried out by the agar streak plate method. All colonies of *Cladosporium* forms on the growth medium were picked up and re-streaked onto the agar surface of plates containing the same medium. At the end of incubation period, only the growth which appeared as a single separate colony was picked up and re-streaked again for several consecutive times onto the surface of agar plate of the isolation medium to ensure its purity which was checked up microscopically and morphologically. Pure colony of *Cladosporium* isolates only were subcultured and stored on slants of Malt Extract Agar (MEA) and Potato Dextrose Agar (PDA) media at 5°C and kept for further investigation (Marshall, 1997).

3.4. Identification of *Cladosporium* isolates

Cladosporium colonies present were identified by macroscopic and microscopic analysis. Fungal isolates were primarily identified according to Ellis (1971, 1976), and based on culture characterization, macroscopic and microscopic properties of 68 single spore isolates, then *Cladosporium* isolates were divided into 19 groups.

3.5. Morphological identification

Random isolate from every group was selected and sent to Assiut University, Mycological Centre (AUMC) to confirm morphological identification.

3.6. Microscopic standard methods

Collections were examined using a stereomicroscope to detect the areas where the fungus was growing. Small amounts of pure colony was excised and mounted in distilled water on a slide. Stains were not used, as the fungal hyphae, conidiophores and conidia are pigmented and thus clearly visible. Morphological descriptions are based on observations with standard light microscopy under oil immersion using a Nikon Eclipse E600 microscope. Where possible, twenty conidiophores, conidiogenous cells, conidia and conidiogenous loci and hila were measured in each collection, and a representative range was depicted. Digital photographs were taken using a Nikon digital camera DXM1200 (Schubert, 2005).

4. Seasonal variation

4.1. Air samples

All samples during Seasonal variation studies were in Riyadh, Saudi Arabia, from March 2007 to February 2008. Operation of air sampling was performed during last five days of each month. A total of 756 outdoor samples, from 21 site were analyzed.

4.2. Sampling and analysis

Air was sampled at 21 sites on Riyadh . Operation of air sampling was performed as described before. Fungal air samples collected, using Petri plate that contain about 15 ml of Malt Extract Agar (MEA) (MERCK), with Microbiological air sampler SAS HiVAC PETRI Cat. 17407, as described previously. Cultures were incubated as described before at 25°C. Plates were inspected after 4 days and periodically up to 14 days. The concentration of fungi per cubic meter of air was calculated, and *Cladosporium* colonies present were identified by macroscopic and microscopic analysis, purified using Streak Plate. The analytical criterion was to identify *Cladosporium* genus. All fungal concentrations were expressed as CFU per cubic meter of air. Actual plate counts were used to calculate the CFU per cubic meter. The volume of air collected during sampling was 200 liter.

Seasons of investigated period were winter (December–February), spring (March–May), summer (June–August), and autumn -fall-(September–November). Regions were defined as north east, north west, middle, south east and south west . The samples of each Region were defined as follow: North East Region was included samples 1-6. North West Region was included 7-10. Middle Region was included 11-14. South East Region was included15-18. South West Region was included 19-21.

3. Results

1- Geographical distribution and Screening of *Cladosporium*

A total of 870 fungal colonies were isolated from 40 sites, 108 (12.4%) of them were *Cladosporium*. The genus *Cladosporium* was isolated from all studied sites (Figure 1).

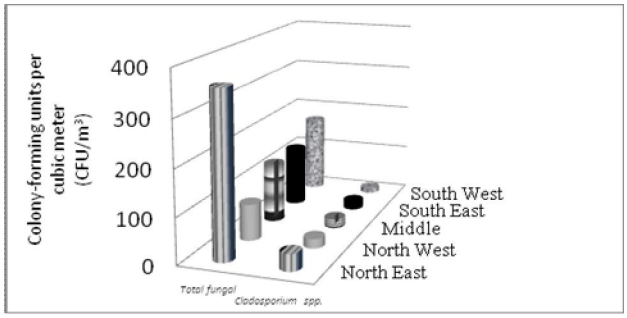

Fig.1. Average values of total count of fungi and *Cladosporium spp.* in the geographical distribution scanning

In all sampling sites, the most prevalent *Cladosporium* species were *Cladosporium cladosporioides* (Fresenius) de Vries and *Cladosporium sphaerospermum* Penzig, followed by *Cladosporium herbarum* (Persoon) Link, *Cladosporium macrocarpum* Preuss, and *Cladosporium chlamydosporis* Matsushima (Figure 2).

During Geographical distribution scanning, Nineteen isolates belonging to five *Cladosporium* species were identified (Table 1). Five isolates, one of each species, were used in the biological studies, and all of the nineteen isolates were used in the genetic studies.

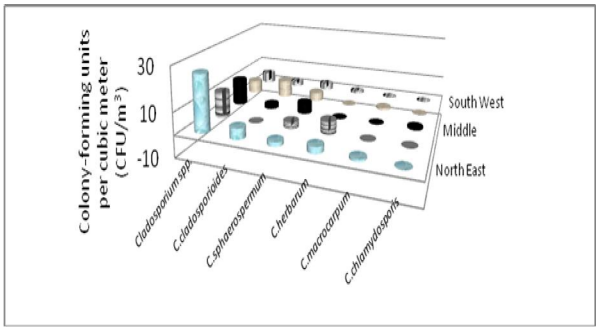

Fig.2. Variation of colonies and geographic distribution of different airborne *Cladosporium* spp.

Table 1. Isolate Code, Isolate date, AUMC number and Geographic origin of *Cladosporium* spp.

Isolate Code	Isolate date	*Cladosporium* spp.	AUMC No.	Geographic origin
Clad#1	27/9/2006	*Cladosporium cladosporioides* (Fresenius) de Vries	4432	South East
Clad#2	12/9/2006	*Cladosporium sphaerospermum* Penzig	4433	South East
Clad#3	12/9/2006	*Cladosporium herbarum* (Persoon) Link	4434	North West
Clad#4	4/10/2006	*Cladosporium sphaerospermum* Penzig	4435	Middle
Clad#5	12/9/2006	*Cladosporium herbarum* (Persoon) Link	4436	South West
Clad#6	4/10/2006	*Cladosporium herbarum* (Persoon) Link	4437	North East
Clad#7	4/10/2006	*Cladosporium herbarum (Persoon) Link*	4438	North East
Clad#8	4/10/2006	*Cladosporium cladosporioides* (Fresenius) de Vries	4439	Middle
Clad#9	27/9/2006	*Cladosporium sphaerospermum* Penzig	4440	South East
Clad#10	4/10/2006	*Cladosporium sphaerospermum* Penzig	4441	North West
Clad#11	4/10/2006	*Cladosporium herbarum (Persoon) Link*	4442	Middle
Clad#12	4/10/2006	*Cladosporium macrocarpum* Preuss	4443	North East
Clad#13	4/10/2006	*Cladosporium sphaerospermum* Penzig	4444	North East
Clad#14	4/10/2006	*Cladosporium cladosporioides* (Fresenius) de Vries	4445	North East
Clad#15	12/9/2006	*Cladosporium macrocarpum* Preuss	4446	South East
Clad#16	4/10/2006	*Cladosporium chlamydosporis* Matsushima	4447	Middle
Clad#17	4/10/2006	*Cladosporium sphaerospermum* Penzig	4448	Middle
Clad#18	12/9/2006	*Cladosporium sphaerospermum* Penzig	4449	South West
Clad#19	27/9/2006	*Cladosporium cladosporioides* (Fresenius) de Vries	4450	South West

(AUMC)* Assiut University, Mycological Centre

2- Morphological identification of *Cladosporium* Link ex Fries

a- *Cladosporium sphaerospermum* Penz., 1882, Michelia, 2: 473

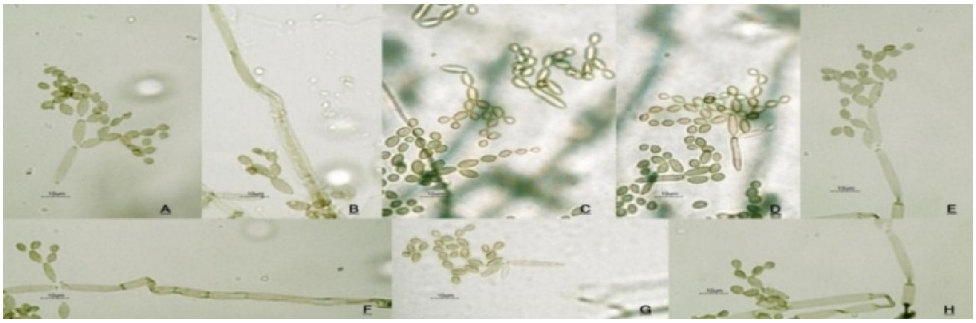

Figure 3. *Cladosporium sphaerospermum* Penzig. A-H Micromorphology of conidiophores, showing acropetal conidial chains, ramoconidia, and conidia. Scale bars = 10µm.

b. *Cladosporium herbarum* (Pers.) Link ex S. F. Gray, 1821, Nat. Arr. Br. Pl., 1: 556.

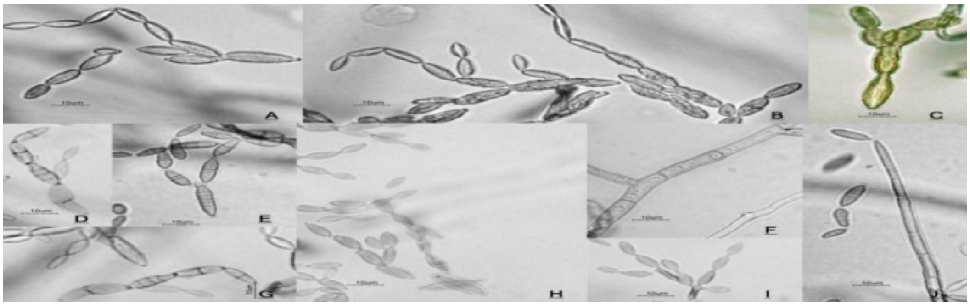

Figure 4. *Cladosporium herbarum* (Persoon) Link. A-J Micromorphology of conidiophores, showing acropetal conidial chains, ramoconidia, and conidia. Scale bars = 10µm.

c. *Cladosporium cladosporioides* (Fresen.) de Vries

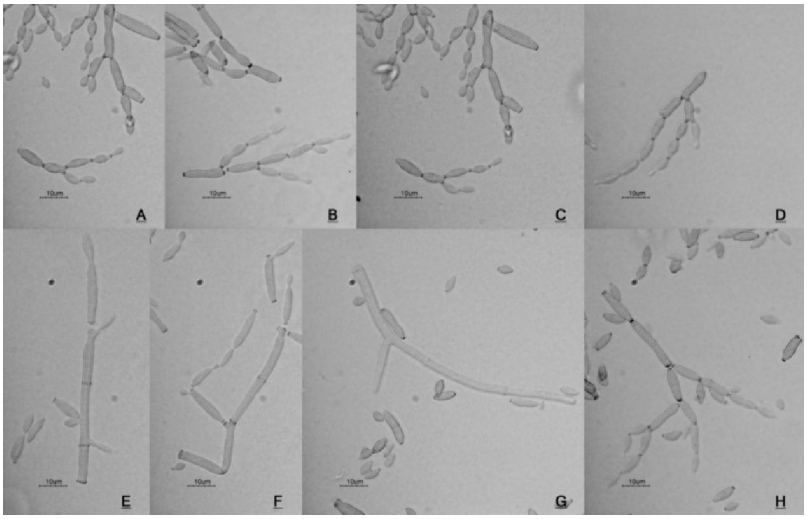

Figure 5. *Cladosporium cladosporioides* (Fresenius) de Vries. A-H Micromorphology of conidiophores, showing acropetal conidial chains, ramoconidia, and conidia. Scale bars = 10µm

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d. Cladosporium macrocarpum Preuss, 1848, in Sturm's Deut. Fl., 3: 27-28.

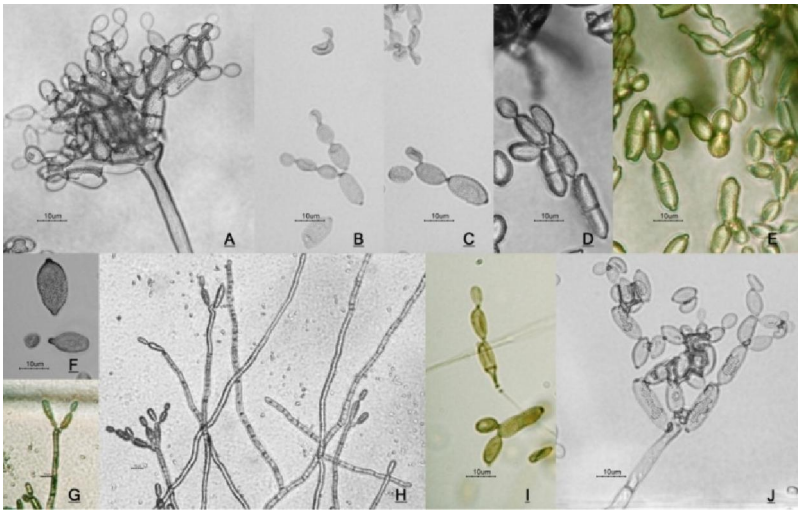

Figure 6. *Cladosporium macrocarpum* Preuss. A-J Micromorphology of conidiophores, showing acropetal conidial chains, ramoconidia, and conidia. Scale bars = 10µm.

e. *Cladosporium chlamydosporis* sp. nov., in Matsushima (1975), p. 34.

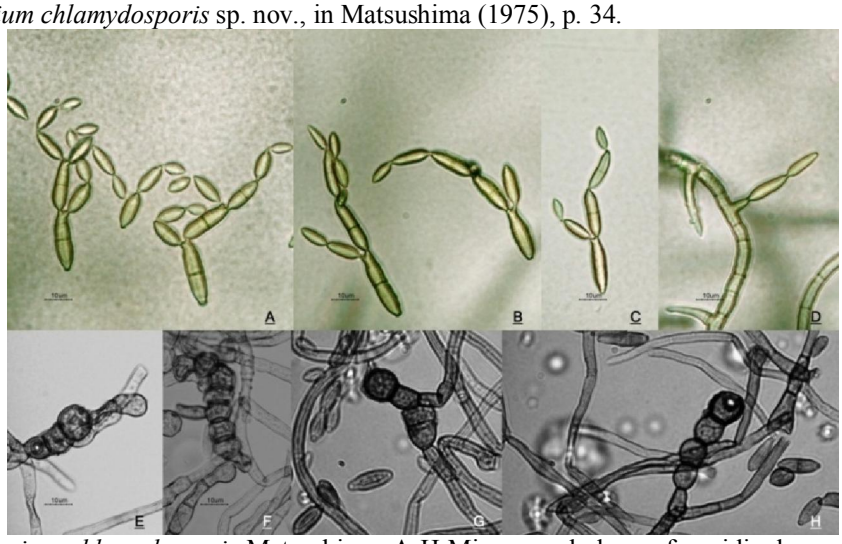

Figure 7. *Cladosporium chlamydosporis* Matsushima. A-H Micromorphology of conidiophores, showing acropetal conidial chains, ramoconidia, and conidia. Scale bars = 10µm.

2. Seasonal variations

1. Effect of month and site on density of *Cladosporium* spp.

ANOVA (Table 2) showed that the two main effects of this study (month and site) were all very highly significant sources of variation in density of *Cladosporium* spp. collected from Riyadh city. The two-way interaction for month × site was a very highly significant source of variation in the case of density of *Cladosporium* spp. ($P = 0.0000$).

Table 2. Analysis of variance* of the effects of month, site, and their interaction on density of *Cladosporium* (CFU m^{-3}) in the air.

Source of variation [a]	D.F[b]	M.S[c]	F.value[d]	P>F
Replication	2	28.704	0.2316	
Month (M)	11	7636.541	61.6069	0.0000
Site (S)	20	4998.575	40.3254	0.0000
M × S	220	1483.037	11.9642	0.0000
Error	502	123.956		

*Analyses of variance (ANOVA). [a] Replication is random while each of month and replication is fixed. [b] D.F. – Degrees of freedom. [c] M.S. – mean square. [d] F. value used to test the hypothesis of equal population means. P-value is the area to the right of the F statistic under an F distribution with g-1 and N-g degrees of freedom.

2. Relative contribution of month, site, and their interaction to variation on density of *Cladosporium*

Relative contribution of each source of variation to variation on density of *Cladosporium* in the air is shown in Table 3-3. The interaction of month × site was the first in importance as a source of variation on density of *Cladosporium* in the air. It accounted for 63.94% of the explained (model) variation in isolation frequency from density. Site was the second in importance as a source of variation on density of *Cladosporium* in the air.

Table 3. Relative contribution of month, site, and their interaction to variation on density of *Cladosporium* in the air

Source of variation	Relative contribution [a] to variation in Density
Month (M)	16.46
Site (S)	19.59
M × S	63.94

[a] calculated as percentage of sum of squares of the explained (model) variation.

3. Effect of month, site, and their interaction on the density of *Cladosporium* Colony forming units per cubic meter (CFU m^{-3}) of air sampled

Due to the significant interaction between site and month in the case of the density of *Cladosporium* (CFU m^{-3}) in the air, least significant difference (LSD) was used to compare between sites within different months. These comparisons showed that the magnitude of the difference between the densities of *Cladosporium* was affected by isolation from different sites (Table 3-4). For example, the increase of *Cladosporium* density from site 15 and 16 caused highly significant increase in the isolation density during January. Similarly, the increase of *Cladosporium* density in the same site caused highly significant increase during different months. The most *Cladosporium* density was observed during November.

Table 4. Effect of month, site, and their interaction on the density of *Cladosporium* (CFU m^{-3}) in the air.

Site	Month												
	Jan	Feb	Mar	April	May	June	July	Aug	Sep	Oct	Nov	Dec	Mean
1	10.00	1.67	6.67	6.67	8.33	8.33	8.33	6.67	10.00	8.33	28.33	16.67	10.00
2	11.67	1.67	8.33	6.67	10.00	10.00	8.33	10.00	0.00	0.00	21.67	5.00	7.78
3	11.67	3.33	8.33	6.67	3.33	3.33	3.33	0.00	6.67	5.00	20.00	33.33	8.75
4	3.33	8.33	6.67	8.33	5.00	5.00	3.33	5.00	0.00	0.00	21.67	10.00	6.39
5	23.33	20.00	15.00	18.33	5.00	5.00	5.00	1.67	6.67	13.33	11.67	10.00	11.25
6	15.00	26.67	21.67	23.33	13.33	13.33	10.00	11.67	5.00	26.67	6.67	0.00	14.44
7	11.67	3.33	8.33	8.33	6.67	6.67	5.00	6.67	0.00	103.33	16.67	55.00	19.31
8	10.00	8.33	10.00	10.00	21.67	6.67	0.00	6.67	33.33	0.00	10.00	20.00	11.39
9	10.00	5.00	10.00	10.00	13.33	18.33	3.33	18.33	25.00	0.00	13.33	30.00	13.06
10	13.33	10.00	11.67	11.67	53.33	46.67	48.33	41.67	38.33	10.00	161.67	5.00	37.64
11	6.67	8.33	8.33	10.00	15.00	10.00	10.00	5.00	40.00	8.33	26.67	11.67	13.33
12	1.67	0.00	1.67	1.67	3.33	3.33	0.00	5.00	33.33	21.67	58.33	13.33	11.94
13	5.00	6.67	6.67	6.67	10.00	13.33	1.67	21.67	45.00	3.33	43.33	3.33	13.89
14	5.00	6.67	6.67	8.33	5.00	6.67	0.00	10.00	68.33	11.67	21.67	23.33	14.44
15	83.33	10.00	46.67	30.00	5.00	3.33	5.00	1.67	18.33	201.67	130.00	56.67	49.31
16	16.67	6.67	13.33	11.67	31.67	28.33	41.67	23.33	0.00	0.00	20.00	13.33	17.22
17	6.67	6.67	8.33	8.33	8.33	5.00	8.33	1.67	3.33	5.00	20.00	11.67	7.78
18	0.00	5.00	3.33	5.00	8.33	8.33	5.00	8.33	41.67	21.67	76.67	28.33	17.64
19	10.67	10.00	11.67	11.67	41.67	33.33	50.00	11.67	6.67	66.67	188.33	40.00	40.14
20	6.67	11.67	10.00	11.67	25.00	35.00	11.67	58.33	25.00	118.33	53.33	23.33	32.50
21	6.67	11.67	10.00	11.67	26.67	18.33	26.67	8.33	18.33	8.33	45.00	11.67	16.94
Mean	12.78	8.18	11.11	10.79	15.24	13.73	12.14	12.54	20.24	30.16	47.38	20.08	

Least significant difference (LSD) for Month ×Site interaction=17.86 ($P<0.05$) or 23.50 ($P<0.01$)..

4. Discussion

Fungi are present everywhere in indoor and outdoor environments. Many fungi are toxigenic or pathogenic that may cause various public health concerns. Rapid detection, quantification and characterization of fungi in living and working environments are essential for exposure risk assessment to safe guard public health. *Cladosporioid hyphomycetes* are common, widespread fungi. *Cladosporium* is one of the largest, most heterogeneous genera of hyphomycetes, comprising more than 772 names (Dugan *et al*., 2004), and including endophytic, fungicolous, human pathogenic, phytopathogenic and saprobic species. Species of this genus affect daily human life in various ways. The common saprobic members of *Cladosporium* occur on all kinds of senescing and dead leaves and stems of herbaceous and woody plants, as secondary invaders on necrotic leaf lesions caused by other fungi, are frequently isolated from air, soil, food stuffs, paint, textiles and other organic matters, are also known to be common endophytes (El-Morsy, 2000) as well as phylloplane fungi (Inacio *et al*., 2002; Stohr and Dighton, 2004; Levetin and Dorsey, 2006). Furthermore, some *Cladosporium* species are known to be potential agents of medical relevance. *Cladosporium herbarum* is, for instance, a common contaminant in clinical laboratories and causes allergic lung mycoses (Schubert *et al*., 2007).

Understanding the nature and concentration of in-door and out-door fungi may serve various purposes. Specifically it can provide information on which fungi sensitive individuals are exposed to seasonal changes in symptoms may be associated with seasonal variations of molds (Ren *et al*., 1999). The airspora concentrations of outdoor environments depend on numerous factor including; time of day, meteorological factors, seasonal climatic factors, and type of vegatation (Pepeljnjak and Segvic, 2003). Among the Deuteromycotina, the most representative taxon was *Cladosporium*. Meteorological conditions, such as high temperatures and low humidity during the summer and spring, as well as abundant vegetation contribute to the significant and constant presence of *Cladosporium* in the atmosphere. Researchers have found high concentrations of this genus worldwide and have classified it as a universal fungus; for example, Al-Subai (2002) showed 40.1% in Doha (Qatar). This thesis is a first step towards the identification of *Cladosporium* spp. whose spores are present, to a greater or lesser degree, in the atmosphere of Riyadh city. Most fungal categories peaked during November and December months. The density of spores of the genus *Cladosporium* from February to November was the only significant difference between in site 10. Seasonal variation of the *Cladosporium*, with the highest values in November. Spore types such as *Cladosporium* and *Alternaria*, are usually found in higher concentrations during the

warmest part of the day, dry weather conditions, with greatest wind speed and turbulence, usually referred as Middle-Day Pattern (Burch and Levetin, 2002). *Cladosporium cladosporioides* was the commonest fungal types within the genus in south and north east Riyadh city. Characterized by its smooth conidia it is cosmopolitan, a saprophyte and a plant parasite, and has been cited as the cause of lung, skin and nasal mycosis. *C. cladosporioides* and *C. herbarum* are the dominant members of the *Cladosporium* genus (Vesper *et al*., 2006). De-Vries (1952) discussed the fact that *C. cladosporioides* has often been considered as a form of *C. herbarum*, compared these two species and found sufficient morphological differences to justify the recognition of *C. cladosporioides* as a distinct species.

Culture-based examinations are time consuming and laborious and not all airborne spores can be cultivated due to variations in viability. Thus, quantification of airborne fungi based on cultivation may not accurately reflect the true concentrations (Borneman and Hartin, 2000; Polzehl *et al*., 2005; Wu *et al*., 2002). Thus, accurate detection and quantification methods are needed to better clarify the distribution of *Cladosporium* in working and living environments. Although it is known that *Cladosporium* spores are important aeroallergens, the majority of *Cladosporium* species are still not characterized. Development of species-specific detection and quantification systems for each *Cladosporium* species is not feasible and would be time consuming in applications.

Because *Cladosporium* species may differ in minor morphological features, identification can be a difficult task for those not familiar with these fungi. Generic group-specific detection and quantification methods are desirable and would facilitate the environmental monitoring of *Cladosporium* for exposure risk assessment. Cultural characteristics used in the key to distinguish the species treated in this study were determined after 14 d growth at 25 ºC on five types of media. All isolates grew on all five media tested; however, Sabouraud Dextrose Agar and Potato Dextrose Agar were most favorable for rapid radial growth of mycelium of all *Cladosporium* species. The hyphae of *Cladosporium* species are consistently septate, mostly branched, smooth and lightly pigmented. The conidiophores in species of the genus *Cladosporium* are mostly cylindrical, subcylindrical or filiform, but further differentiations are often due to sympodial proliferations causing geniculations with conidiogenous loci often situated on small lateral shoulders or intercalary swellings.

C. herbarum colonies on PDA reaching 19–37 mm diam after 14 d at 25 ºC, grey-olivaceous to olivaceous-grey, whitish to smoke-grey or pale olivaceous-grey due to abundant aerial mycelium. Colonies on MEA reaching 17–36 mm diam. after 14 d at 25 ºC, smoke-grey to pale olivaceous-grey towards margin, olivaceous-grey to iron-grey reverse, velvety, margin white, entire edge to slightly undulate, aerial mycelium abundant, and dense. *Cladosporium herbarum* (Pers.: Fr.) Link, type species of the genus *Cladosporium* Link, is one of the most common environmental fungi to be isolated worldwide. It abundantly occurs on fading or dead leaves of herbaceous and woody plants, as secondary invader on necrotic leaf spots, and has frequently been isolated from air (Samson *et al*., 2000). *Cladosporium herbarum* has very wide hyphae on the agar surface, which gave rise to conidiophores as lateral branches. The elongation of secondary ramoconidia varies among the different species. *Cladosporium macrocarpum* has broadly ellipsoid to cylindrical secondary ramoconidia usually with broadly rounded ends. Zalar *et al*. (2007) considered *C. sphaerospermum* as halo- or osmotolerant. Although *C. sphaerospermum* has commonly been isolated from osmotically stressed environments. The isolate broadens the morphological limits of *C. sphaerospermum* by production of obclavate, occasionally transversely septate conidia with subrostrate conidiogenous apices (‘alternarioid’ conidia), and by production of conidia larger than those in prior standard descriptions (Dugan *et al*., 2008). All species belonging to the *C. herbarum* complex are characterized by possessing conidia which are ornamentated, the ornamentation ranging from minutely verruculose to verrucose, echinulate or spiny whereas in the *C. sphaerospermum* complex species with both smooth-walled as well as ornamented conidia are included (Zalar *et al*., 2007).

C. macrocarpum colonies on PDA reaching 38–40 mm in diam after 14 d at 25 ºC, dark dull green to olivaceous-grey, olivaceous-grey, dark olivaceous- to iron-grey reverse, pulvinate, velvety, paler zones towards the margin, margin regular, aerial mycelium sparse to more abundant in the colony centre or covering large areas of the colony. While. colonies on MEA reaching 46–47 mm in diam after 14 d at 25 ºC, grey-olivaceous to olivaceous-grey or iron-grey, sometimes pale olivaceous-grey to whitish due to abundant aerial mycelium. *C. macrocarpum* Preuss, a second component within the herbarum complex, has hitherto been known and treated as an allied, but morphologically distinct species on the basis of its wider and somewhat larger, frequently 2–3-septate, more regularly verrucose conidia, shorter conidial chains and more pronounced prolongations of the conidiophores. Dugan and Roberts (1994) carried out examinations of morphological and reproductive aspects of both species, and in so doing demonstrated

a morphological continuum between *C. macrocarpum* and *C. herbarum*, concluding that the name herbarum should have preference. Density of *Cladosporium* spp. during the investigation of Seasonal variation was affected by month and site. The two main effects of ANOVA (month and site) were all very highly significant sources of variation in density of *Cladosporium* spp. isolated from Riyadh city. Also, the two-way interaction for month × site was a very highly significant source of variation in the case of density of *Cladosporium* spp. (P = 0.0000).

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