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DIVERSITY AND DISTRIBUTION PATTERN OF AIRBORNE FUNGI IN GREATER TUNB, ABU-MUSA, AND SIRRI ISLANDS, PERSIAN GULF, IRAN

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Abstract

Airborne fungi play an important role in the cause of different adverse effects on humans and animals health because of their pathogenicity, allergic reactions and toxicity. The Persian Gulf Islands have a warm and humid climate and there is no information regarding airborne fungi in these regions. Therefore, this study aimed to determine the mycoflora of environments of Iranian islands, namely Greater Tunb, Abu Musa, and Sirri. In this study, a total of 90 air samples were prepared by settle plate method using Sabouraud dextrose agar with Chloramphenicol. The fungal species were identified the bases of morphology, molecular and sequencing techniques. A total of 231 fungal isolates, including 16 genera were detected. *Cladosporium* was determined as the predominant genus (32.46%); *Aspergillus* (20.34%), sterile mycelia (9.08%), *Alternaria* (8.23%) and *Penicillium* (4.75%) were revealed as the common fungal

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spores. In our study, dematiaceous fungi had a high incidence with the frequency of ~56% of total isolated fungi that reason may be due to the resistance of spores in colored fungus against UV rays of the sun. Although different airborne fungi isolated in this study does not necessarily imply a cause and effect relationship with illness, but the results provide a better perception of the diversity and distribution pattern of airborne fungi, which may be beneficial for physicians, allergists, as well as epidemiologists.

Introduction

Fungi as a heterogeneous group of microorganisms have a high distribution in the environment including soil, plants, animals, water, and air (GONÇALVES et al. 2017). There are many biological contaminants in the air including fungi, pollens, viruses, and bacteria (AYANBIMPE et al. 2012). Fungi are the most important and most abundant group of organisms in the air because they are present in all habitats and can originate from various sources such as plants, animals and soil (SINDT et al. 2016). Among different airborne biological contaminants in the environment, fungi play an important role in the cause of different adverse effects on humans and animals health (SHAMS-GHAHFAROKHI et al. 2014, SHARMA et al. 2015). World concerns about the air quality and its health become more prominent due to the vital need of air for the survival of life on earth (GHIASIAN et al. 2016, LEUNG 2015). Airborne fungal spores can act as a cause of allergies and asthma, respiratory infections, primarily irritations and toxic effects. Many of airborne fungi can also act as etiologic agents of keratomycosis, otomycosis, chronic bronchitis, emphysema, and onychomycosis. Besides, air-transmitting diseases caused by airborne fungi such as histoplasmosis, invasive aspergillosis, acute allergic alveolitis are life--threatening to immunocompromised hosts (GHIASIAN et al. 2016, IRGA and TORPY 2016, KHAN and KARUPPAYIL 2012, RUDERT and PORTNOY 2017). Fungal spores are almost always present in the air, but their quality and quantity vary according to the geographical situation, climate, time of day and the presence of spore sources in the environment (NICHOLAS et al. 2015, RUZER and HARLEY 2012). Knowledge of airborne fungi in each region is important for the ecological diagnosis and specific treatment of allergic manifestations induced by inhaled allergens. Up to now, numerous studies have been conducted on the presence and diversity of airborne fungal spores in different occupational and residential areas of the world (AKGÜL et al. 2016, GONÇALVES et al. 2017, ILOND and NWEKE 2016, MORENO-SARMIENTO et al. 2016, TONG et al. 2017). There are also several studies from various parts of Iran and so on demonstrated a wide array of fungi in air samples from environments (GHIASIAN et al. 2016, HEDAYATI

et al. 2005, PAKSHIR et al. 2015, Sepahvand et al. 2013, SHAMS-GHAHFAROKHI et al. 2014, Soleimani et al. 2013). The Persian Gulf Islands have a warm and humid climate and there is no information regarding airborne fungi in these regions. With this background in mind, the current study was conducted with the aim of determination of the diversity and distribution patterns of airborne fungi in Greater Tunb, Abu-Musa, and Sirri islands using morphological and molecular (DNA sequence analysis) methods.

Materials and Methods

Geographical characteristics of the studied islands

Greater Tunb, Abu Musa, and Sirri islands are located at the Persian Gulf in the most southern part of Iran (Figure 1). These three islands are considered as part of Hormozgan province. The Greater Tunb (10.3 km^2 wide) has a longitude and latitude of 55° 28–55° 34 and 26° 34–26° 30 respectively. Abu Musa Island (12 km^2 wide) has a longitude and latitude of 54° 26–55° 19 and 25° 51–26° 19, respectively. Furthermore, Sirri Island has situated 76 km from Bandar-e Lengeh and 50 km west of Abu Musa Island. This island is almost 5.6 km long with a width of about 3 km. It covers an area of 17.3 km^2. All three islands have a warm and humid climate (AFSHARI et al. 2017).

Fig. 1. Map of Persian Gulf, showing relative positions of the three islands of Greater Tunb, Abu Musa, and Sirri

Sample collection and cultivation

This descriptive study was conducted in the second half of 2011 in three Iranian islands of Greater Tunb, Abu-Musa, and Sirri. A total of 90 air samples (i.e., 30 samples from each island) were prepared using settle plate method according to HOEKSTRA et al. 2000 and ANDON et al 2006. In this method, the plates to diameter of 9 cm containing Sabouraud's dextrose agar medium (Merck, Germany) plus chloramphenicol (0.1 mg mL^{-1}; Sigma-Aldrich, USA) were placed at the height of about one meter from the floor, for 30 minutes. After collection of samples, the plates were taken to the laboratory of mycology for isolation and identification of fungi. The plates were incubated at 28°C in the dark for 3–4 weeks and control and evaluated daily for fungal growth (RIPPON 1988). Different types of colonies were subculture on Sabouraud's dextrose agar and tested by slide Riddle method. Fungal isolates were identified according to their microscopic and macroscopic morphological criteria according to standard procedures (RIPPON 1988).

Molecular identification of unknown isolates

The unknown fungal isolates were identified through performing DNA sequence analysis. DNA was extracted according to LEE 1990. The quality and quantity of the extracted DNA were evaluated using electrophoresis and NanoDrop, respectively. The ITS1-5.8S-ITS2 rDNA was amplified using ITS1 and ITS4 as forward and reverse primers (WHITE et al. 1990).

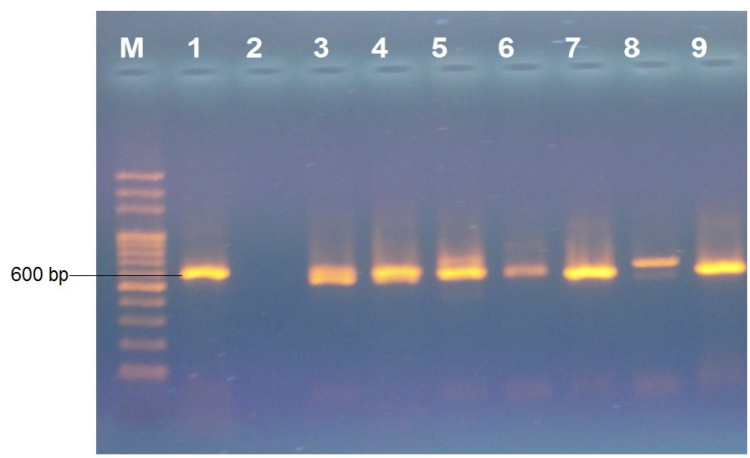

Fig. 2. Agarose gel electrophoresis and polymerase chain reaction products from number of isolates: M – 100 bp DNA ladder; lane 1 – positive control (*A. flavus*); lane 2 – negative control; lane 3–5 – *Neocamarosporium chichastianum*; lane 6 – *Stemphylium solani*; lane 7 – *Chaetomium subaffine;* lane 8 – *Embellisia phragmospora*; lane 9 – *Phoma tropica*

The PCR was performed in a total volume of 25 µL in each tube containing 12.5 µL Master mix (buffer, dNTP, Taq DNA polymerase, 2 mM $MgCl_2$; Amplicon III, Denmark), 1 µL of the template DNA, 1 µL of each primer (20 pmol final concentration of each the primer), and 9.5 µL distilled water. The PCR conditions were as follows. initial denaturation at 94°C for 5 min, 35 cycles with denaturation at 94°C for 30 sec, annealing at 56°C for 45 sec, extension at 72°C for 45 sec, and a final extension step of 7 min at 72°C. Amplicons were visualized by electrophoresis in 1% agarose gels using the ethidium bromide (Figure 2) and the PCR products were sent for sequencing (Macrogen, South Korea) in both directions. The sequence results were processed by using the web-based blasting program, basic local alignment search tool (BLAST), at the NCBI site (http://www.ncbi.nlm.nih.gov/BLAST), and the data were compared with the NCBI/Genebank database.

Results

In the present study, distribution and diversity of airborne fungi in three Iranian islands of Greater Tunb, Abu Musa, and Sirri were evaluated. A total of 90 air samples were prepared by settle plate method using of Sabouraud Dextrose Agar Medium with Chloramphenicol. The fungal species were identified the bases of morphology, molecular and sequencing techniques. A total of 231 fungal isolates, including at least 16 genera were detected. Overall, 90% of the samples obtained from the studied islands were contaminated with fungi; this percentage was more than 93% in some islands such as Siri and less than 90% in Abu Musa Island. The dominant species were members of *Cladosporium* species (32.46%). The frequency of these species in Great Tunb, Abu Musa and Siri were 34%, 26.79% and 33.3%, respectively. Other species by frequency include *Aspergillus* species (20.34%), sterile mycelia (9.08%), *Alternaria* species (8.23%), *Penicillium* species (4.75%), *Chaetomium* species (3.91%), yeasts (3.91%), *Fusarium* species (3.02%), *Aureobasidium pullulans* (3.02%), *Exophiala* species (1.74%), *Mucor* species (1.74%), *Stemphylium solani* (1.74%), *Neoscytalidium dimidiatum*, *Neocamarosporium chichastianum*, *Ulocladium* species and *Rhizopus* species (1.3%), *Phoma tropica* and *Embellisia phragmospora* (0.44%).The diversity and frequency of fungi isolated from Greater Tunb were higher than the other two islands. The number and frequency of fungal isolates in each island is represented in Table 1 and Table 2.

Table 1
Frequency and percentage of positive samples of airborne fungi isolated from Greater Tunb, Abu Musa, and Sirri islands, Persian Gulf, Iran

Specification	Islands			Total frequency [%]
	Greater Tunb	Abu Musa	Sirri	–
Number of samples examined	30	30	30	90
Number of positive samples	27	26	28	81
Percentage of positive samples	90	86.67	93.33	90

Table 2
Frequency and distribution of airborne fungi isolated from Greater Tunb, Abu Musa, and Sirri islands, Persian Gulf, Iran

Specification	Islands						Total frequency	
Species isolated	Greater Tunb		Abu Musa		Sirri			
	No.	%	No.	%	No.	%	No.	%
Alternaria species	9	7.83	6	10.71	4	6.67	19	8.23
Aspergillus flavus	4	3.48	5	8.93	3	5	12	5.19
Aspergillus niger	4	3.48	4	7.14	5	8.33	13	5.63
Aspergillus species	6	5.22	8	14.29	8	13.33	22	9.52
Aureobasidium pullulans	3	2.6	1	1.79	3	5	7	3.02
Chaetomium globosum	2	1.74	0	0	0	0	2	0.87
Chaetomium subaffine	1	0.87	0	0	0	0	1	0.44
Chaetomium species	3	2.6	1	1.79	2	3.33	6	2.6
Cladosporium species	40	34.79	15	26.79	20	33.33	75	32.46
Embellisia phragmospora	1	0.87	0	0	0	0	1	0.44
Exophiala species	3	2.6	0	0	1	1.67	4	1.74
Neoscytalidium dimidiatum	2	1.74	1	1.79	0	0	3	1.3
Fusarium species	4	3.48	1	1.79	2	3.33	7	3.02
Mucor species	2	1.74	0	0	2	3.33	4	1.74
Neocamarosporium chichastianum	2	1.74	1	1.79	0	0	3	1.3
Penicillium species	6	5.22	3	5.35	2	3.33	11	4.75
Phoma tropica	1	0.87	0	0	0	0	1	0.44
Rhizopus species	2	1.74	1	1.79	0	0	3	1.3
Stemphylium solani	4	3.48	0	0	0	0	4	1.74
Sterile mycelium	10	8.7	7	12.5	4	6.67	21	9.08
Ulocladium species	2	1.74	0	0	1	1.67	3	1.3
Unknown yeasts	4	3.48	2	3.57	3	5	9	3.9
Total	115	100	56	100	60	100	231	100

Discussion

Airborne fungi can result in several adverse health effects such as asthma, rhinitis, allergic sinusitis, toxic reactions, allergic bronchopulmonary mycoses, etc. (BAXI et al. 2016). It has been reported that more than 180 genera of airborne fungi with worldwide distribution are associated with allergies and human and animal infections and the most common species are likely to belong to the genera *Cladosporium*, *Penicillium*, *Alternaria* and *Aspergillus* (HORNER et al. 2004). In the present study, the most dominant fungal genera isolated from the air samples were *Cladosporium* (32.46%), *Aspergillus* (20.34%), sterile mycelia (9.08%), *Alternaria* (8.23%) and *Penicillium* (4.75%). As mentioned, the species of *Cladosporium* and *Aspergillus* were widely distributed in the air accounting for approximately 53% of total isolated fungi. In several previous studies of airborne fungal spores in different geographic areas of the world, it has been demonstrated that the most prevalent fungi belonged to the genera of *Cladosporium* and *Aspergillus*. In the study carried out by AKGÜL et al. (2016) on the determination of airborne fungal spores of Gaziantep, Turkey, *Cladosporium* (56.48%) was determined as the predominant genus. In another study was conducted on distribution of airborne fungi in indoor and outdoor environments at a French hospital, *Cladosporium* species were the dominant genus (55%) In outdoor samples, while in the clinical units, *Penicillium* species (23 to 25%) were the most frequently recovered airborne fungi (SAUTOUR et al. 2009). In a study on mycoflora of the outdoor air environment in Abraka, Nigeria, *Aspergillus* (23.47%) was reported as the most dominant species (ILONDU et al. 2016). CHARAYA and NARUKA (2016), reported the genera of *Aspergillus*, *Alternaria*, and *Cladosporium* as the most prevalent airborne fungi in India. Several studies have been conducted in Iran and various fungi are reported as dominant air mycoflora. Airborne Fungi in Indoor and Outdoor of Asthmatic Patients' Home, of Sari in Iran was evaluated by HEDAYATI et al. (2005). They reported the genera *Cladosporium*, *Aspergillus*, *Penicillium*, and *Alternaria* as the most prevalent fungi. GHIASIAN et al. (2016), conducted a study on airborne fungi in Qazvin that the most commonly found fungi in order of frequency were *Cladosporium*, *Penicillium*, and *Aspergillus* genera. In the study carried out by Shams-GHAHFAROKH et al. (2014) on the distribution of airborne fungi in an outdoor environment in Tehran, *Aspergillus* was the most predominant fungus followed by *Cladosporium*, *Penicillium*, and *Alternaria*. These results are similar to those found in our study. The reasons why the dominant airborne fungi from these genera may be due to this fact that *Aspergillus*, *Penicillium* and *Cladosporium* species produce small and

light spores in large numbers that generally suspended in air for periods ranging from a few hours to several days, whereas other fungal genera produce fewer, larger and heavier spores which tend to have faster settling (VONBERG and GASTMEIER 2006). High temperature and humidity were shown to be suitable for *Cladosporium* species sporulation (EBNER et al. 1989). Considering the hot and humid climate of islands investigated in this study, the high frequency of *Cladosporium* species in the air of these regions may be justifiable. *Cladosporium* is of particular clinical importance because it possesses a high allergenic potential and its spores can trigger the development of symptoms such as rhinosinusitis, dry cough or bronchial asthma in susceptible persons (DENNING et al. 2014, SINDT et al. 2016). *Aspergillus* genus also is one the most common types of molds in the environment. HEDAYATI et al. (2005), stated that the high frequency of *Aspergillus* in the kitchen may be due to their thermo tolerant ability. Some members of *Aspergillus* group are the causative agents of allergy in atopic individuals and some diseases, including otomycosis, keratomycosis, onychomycosis, and mycetoma. Besides, some species are relevant as mycotoxin producers (NIELSEN 2003, PASQUALOTTO 2010). Following the *Cladosporium* and *Aspergillus* species, Mycelia sterilia (9.08%) had the highest frequency in the air of studied areas. The high frequency of Mycelia sterilia isolated in this study is in accordance with our previous study on soil-borne fungi of Greater Tunb, Abu-Musa and Sirri Islands (NOSRATABADI et al 2017). In our research, dematiaceous fungi including *Cladosporium*, *Alternaria*, *Chaetomium* and etc. had a high frequency versus hyaline hyphomycetes with the frequency of ~56% of the total isolated fungi. The high frequency of dematiaceous fungi isolated in this study is in accordance with results of another study conducted on air of Qeshm Island, that authors reported the most abundant fungi were black and colored fungi such as *Alternaria* (63.86%) and *Cladosporium* (11.81%). These authors stated that dematiaceous fungi have melanin pigment in their cell membrane structure and the role of melanin is the protection of fungus against harmful effects of the sun's ultraviolet rays (BARATI et al. 2009). In this study, fungi such as *Embellisia phragmospora*, *Neocamarosporium chichastianum*, *Chaetomium subaffine*, *C. globosum*, *Phoma tropica* and *Stemphylium solani* were identified by using of molecular and sequencing methods. All of these fungi are from black fungi and this can be a confirmation of resistance of colored fungi in the hot and humid weather of studied islands. *E. phragmospora* in classification is placed in the Ascomycota phylum, Dothideomycetes class, Pleosporales order and Pleosporaceae family. This fungus is a saprophyte and pathogenic plant that was previously known as atypical *Helminthosporium* (LAWRENCE et al. 2012). *N. chi-*

chastianum is also a new species that was introduced in 2014 (CROUS et al. 2014). This fungus was isolated from Urmia Lake for the first time in Iran. *Neocamarosporium* species detected as saprobes, and *N. chichastianum* isolated from soil and saline environments (PAPIZADEH et al. 2017).

Conclusion

In general, results of the present study showed that different groups of airborne fungi are present as real contaminants of air of Greater Tunb, Abu-Musa and Sirri Islands. These fungi especially those involved in the etiology of fungal diseases and allergies such as *Cladosporium*, *Aspergillus*, *Alternaria* and *Penicillium* species must be considered as major threats for public health. In this study, dematiaceous fungi including *Cladosporium*, *Alternaria*, *Chaetomium* and etc. had a high frequency versus hyaline hyphomycetes that reason may be due to the resistance of spores in colored fungus against UV rays of the sun. The results of this study provide a better perception of the diversity and distribution pattern of airborne fungi, which may be important for physicians, allergists, as well as epidemiologists.

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Conflicts of interest

The authors have no conflicts of interest to disclose.

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