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Occurrence of Entomopathogenic and Human Potentially Pathogenic Fungi in Carpet Dust from Mosques and Residential Houses

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Abstract: A limited work is currently available on the incidence of entomopathogenic and other potentially opportunistic pathogens in carpet dust in residential houses and mosques. The aim of the study was to isolate fungi from carpet dust using modified medium based on oatmeal agar (OTA) amended with cetyltrimethyl ammonium bromide (CTAB) and cyclohexamide. Identification of the species was based on the cultural and micro-morphological characteristics of their reproductive structures. The study revealed the isolation and identification of 30 species from carpet dust in houses and 24 species from carpet dusts in mosques in addition to yeasts and non-sporulating mycelia. Three entomopathogenic species assigned to *Beauveria bassiana*, *Metarhizium anisopliae* and *Purpureocillium lilacinum* were detected. Dermatophytes and related keratinophilic fungi were represented by species in the genera *Chrysosporium*, *Gymnoascus* and *Microsporum*. Several species of opportunistic pathogens that can tolerate a high level of cyclohexamide including species in the genera *Acaulium*, *Alternaria*, *Arthrographis*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Cunninghamella*, *Fusarium*, *Geomyces*, *Geotrichum*, *Microascus*, *Myceliophthora*, *Penicillium*, *Phaeoacremonium*, *Rhizopus*, *Scopulariopsis*, *Sordaria*, *Stachybotrys*, *Syncephalastrum* and *Trichoderna* were also detected.

Keywords: Carpet dust, Entomopathogenic fungi, Dermatophytes, Opportunistic fungi.

Introduction

Floor dust is a complex of both biological and chemical components. Chemical components is a highly heterogeneous mixture of inorganic and organic particles that incorporated in the products used by consumers (Santillo et al., 2003) as well as toxic substances that are produced by bacteria and fungi such as volatile fungal metabolites, 1, 3-B-glucan, ergosterols, mycotoxins and bacterial toxins (Wu et al., 2012; Skora et al., 2017). Biological contaminants included mites, bacteria, fungi, insect parts, pollens, protozoans and viruses. These biological agents can cause diseases only to people who are sensitive to them (Nevalainen & Seuri, 2005).

Floor dust can cause a variety of health problems such as the increase of risk of asthma (Jaakkola et al., 2002), respiratory allergies (De Ana et al., 2006), development of allergic rhinitis (Stark et al., 2005), pulmonary diseases (Kawel et al., 2011), increase in the hypersensivity syndrome (Jacobs & Andrew, 2003) and mycoses (Weitzman & Summerbell, 1995).

Several studies have shown that the floor dust accumulated on fitted carpets are a suitable niche for the growth of several dermatophytes, related keratinophilic fungi, potentially pathogenic fungi and mycotoxin producing fungi (Beguin & Nolard, 1996; Bahkali & Parvez,

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1999; Abdullah & Al-Musa, 2000; Al-Musa & Abdullah, 2001; Engelhart et al., 2002; Singh et al., 2009; Al-Humiany, 2010; Abdullah & Al-Musa, 2011).

The present study aimed to isolate and identify the entomopathogenic, potentially pathogenic for human and cyclohexamide resistant fungi from carpet dust using a modified medium.

Materials and Methods

Collection of Dust Samples

A total of 100 dusts samples (50 samples, for each of residential houses and mosques), were collected from different sites in Duhok province included Duhok center, Amadi, Summel, Zakho and Chira cities, during September, 2014 to May, 2015. Dust samples were taken from the surface of fitted carpets by the help of home vacuum cleaner. The samples were stored in sterilized collecting bags at 5°C and were processed within 1-2 weeks after collection.

Isolation Method

A selective medium used for isolation of entomopathogenic fungi as described by Pasudos et al. (2012) was modified to include Oatmeal Agar Medium (OTA) (30 g Quiker oat, 15 g agar, l L distilled water), 0.6 g/ l cetyltrimethyl ammonium bromide (CTAB), 0.5 g/ l cyclohexamide and 50 mg/ l chloramphenicol. Initial dilution was made by mixing 1 g of floor dust (as dry weight base) with 9 ml sterile distilled water in a test tube and one drop of Tween 80 was added and shacked thoroughly for 10 minutes. Serial dilutions up to 10^{-3} were made. Aliquots of 1 ml from 10^{-3} dilution were added to sterile Petri dishes (in triplicates) and about 20 ml of medium was poured over. Plates were incubated at 25°C for two weeks. Isolates from growing colonies were sub cultured on appropriate media for identification.

Identification of Fungi

The majority of detected species were identified to species level based on morphological and cultural characteristics. Keratinophilic and opportunistic pathogenic fungi were identified according to Oorschot (1980), Currah (1985), De Hoog & Guarro (1995), Guarro et al. (2012), Kidd et al. (2016) and Sandoval-Denis et al. (2016a, b). Entomopathogenic fungi were identified according to Tzean et al. (1997) and Bischoff et al. (2009). For other fungi, apart from *Aspergillus* and *Penicillium*, the manuals of Ellis (1971, 1976) and Domsch et al. (1980) were followed.

For identification of species in the genera *Aspergillus* and *Penicillium*, pure colonies were grown on four media according to Klich (2002) and Samson et al. (2000). The media are as follows: Czapek Yeast Extract Agar incubated for seven days at 25°C (CYA25), Czapek Yeast Extract Agar incubated for seven days at 37°C (CYA37), Czapek Yeast Extract Agar with 20% Sucrose incubated for seven days at 25°C (CY20S) and Malt Extract Agar was incubated for seven days at 25°C (MEA).

For each culture, four plates were used, two of CYA and one for each of CY20S and MEA. Each plate was inoculated at the center and incubated in the dark for seven days. One CYA was incubated at 37°C. The rest were incubated at 25°C. All species were identified according to the keys and descriptions provided by Pitt & Hocking (1997), Klich (2002), Samson et al. (2000, 2007) and Visagie et al. (2014a, b).

Measurements were taken after seven days incubation. Microscopic mounts were made in lacto phenol with or without cotton blue. For recognizing the ornamentations of the conidiophores and conidia, microscopic slides were examined with oil immersion.

Frequency of occurrence for each species was calculated according to the following equation:

$$\text{Frequency of occurrence (\%)} = \frac{\text{No. of samples on which a fungus appeared}}{\text{Total number of samples}} \times 100$$

Results and Discussion

A total of 39 species (30 species from the house dust and 24 species from the mosques dust) in addition to yeast and non-sporulating mycelia were isolated and identified using oatmeal agar medium (OMA) amended with 0.5 g/ l cycloheximide and 0.6 g/ l cetyltrimethyl ammonium bromide (CTAB) and 50 mg/ l chloramphenicol (Table 1). These fungi were categorized into three groups, dermatophytes and related keratinophilic fungi, entomopathogenic and other opportunistic pathogenic fungi. The first group included species in the genera *Chrysosporium*, *Gymnoascus* and *Microsporum*. The second group, entomopathogenic fungi were represented by three species: *Beauveria bassiana*, *Metarhizium anisopliae* and *Purpureocillium lilacinum*. The third group, opportunistic pathogenic fungi were represented by those species that can tolerate a higher level of cycloheximide including species in the genera *Acaulium*, *Alternaria*, *Arthrographis*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Cunninghamella*, *Fusarium*, *Geomyces*, *Geotrichum*, *Microascus*, *Myceliophthora*, *Penicillium*, *Phaeoacremonium*, *Rhizopus*, *Scopulariopsis*, *Sordaria*, *Stachybotrys*, *Syncephalastrum*, *Trichoderma* in addition to yeast and non-sporulating mycelia.

Table 1: Frequency isolation (F %) of Entomopathogenic and opportunistic fungi with Cycloheximide, CTAB and Oat meal.

Species	Houses		Mosques	
	No of positive samples	F (%)	No of positive samples	F (%)
Dermatophytes and related keratinophilic				
Chrysosporium keratinophilum	2	4	-	-
C. pseudomerdarium	3	6	1	2
C. pannicola	3	6	-	-
C. tropicum	7	14	3	6
Gymnoascus reesii	1	2	-	-
G. devroeyi	-	-	1	2
Microsporum gypseum	3	6	1	2
Entomopathogenic fungi				
Beauveria bassiana	4	8	8	8
Metarhizium anisopliae	5	10	-	-
Purpureocillium lilacinum	3	6	2	2
Other potentially pathogenic fungi				
Acaulium acremonium	1	2	-	-
Alternaria alternata	18	36	6	12
Arthrographis kalrae	3	6	3	6
Aspergillus flavus	17	34	6	12
A. japonicus	-	-	1	2
A. niger	17	34	5	10
A. niveus	-	-	1	2

A. ochraceus	7	14	-	-
A. robustus	-	-	2	4
A. ustus	1	2	-	-
A. versicolor	5	10	1	2
Chaetomium longicolleum	-	-	2	4
C. madrasens	-	-	1	2
Cladosporium herbarum	2	4	-	-
Cunninghamella sp.	2	4	1	2
Fusarium sp.	3	6	1	2
Geomyces pannorum	1	2	-	-
Geotrichum candidum	3	6	-	-
Mycliophthora sepedonium	-	-	1	2
Microascus cirrosus	2	4	2	4
M. pyramidus	-	-	3	6
Penicillium chrysogenum	7	14	8	16
Phaeoacremonium sp.	1	2	2	4
Rhizopus stolonifer	5	10	-	-
Scopulariopsis brevicaulis	2	4	-	-
Sordaria lappae	-	-	2	4
Sterile mycelium (White)	1	2	2	4
Stachybotrys atra	1	2	-	-
Syncephalastrum racemosum	2	4	-	-
Trichoderma sp.	2	4	-	-
Yeast	34	68	18	36

Aspergillus was the most common genus with eight species. *A. flavus*, *A. niger* and *A. versicolor* were detected from both dust sources. *A. japonicus*, *A. niveus* and *A. robustus* were detected at lower frequency from the mosques dust. Few species of black aspergilli were encountered from the indoor environment. However, *A. niger* was the most detected species. *A. japonicus* is rarely encountered. Our finding is in consistent with previous reports (Bokhary, 1999; Maghraby et al., 2008; Varga et al., 2014; Visagie et al., 2014a).

Three entomopathogenic fungi were detected of which *B. bassiana* at rates of 8 and 16%, *P. lilacinum* at 6 and 2% were from houses and mosques dusts, respectively. *M. anisopliae* was detected at 10% frequency from house dust only.

Several specific media that contained antifungal and antibiotic agents at appropriate concentration that inhibit rapidly growing saprophytic fungi and allow the growing of target fungi were developed (Goettel & Inglis, 1997; Luz et al., 2007; Fernandes et al., 2010; Abdullah et al., 2015b; Kepler et al., 2015). In the present study, we used a selective medium based on CTAB at 0.6 g/ l and oat meal agar as a basal medium as described by Pasudos et al. (2012) and modified by addition of cycloheximide 0.5 g/ l.

Entomopathogenic fungi of the genera *Beauveria* and *Metarhizium* (Hypocreales: Ascomycetes) are widely used in biological control against a broad spectrum of pest insects (De Faria & Wraight, 2007). Although soil is the main natural habitat for entomopathogenic fungi, there are increased evidences for their occurrence as endophytes (Arnold & Lewis, 2005; Steinwender et al., 2015) and rarely encountered from dust (Maghraby et al., 2008). We successfully detected three entomopathogenic fungi from dusts of houses and mosques.

Although entomopathogenic fungi are well known as insect pathogens. However, few reports documented their pathogenic potential towards humans. *M. anisopliae* can manifests as agents of keratitis (De Garcia et al., 1997; Jani et al., 2001) and disseminated skin lesions (Osorio et al., 2007). Ocular infections caused by *Beauveria bassiana* have been also reported (Low et al., 1997; Kisla et al., 2000). Two species of *Chaetomium* (*C. longicolleum* and *C. madrasens*) were detected from mosque dust at isolation frequency of 4 and 2%, respectively. The two species have been recently reported from Iraqi soil (Abdullah & Azzo, 2015).

Phaeoacremonium sp. was detected in dust sample from houses and mosques at frequency of 2 and 4%, respectively. *Phaeoacremonium* species have been previously reported from Iraq as among the other fungi causing grapevine decline in Iraq (Haleem et al., 2011; Abdullah et al., 2015a).

Phaeoacremonium species are among other dematiaceous hyphomycetes reported as etiologic agent of cutaneous and subcutaneous phaeohyphomycosis (Mostert et al., 2005; Marques et al., 2006).

Other species were isolated at different frequency including *Acaulium*, *Alternaria*, *Fusarium* sp., *Stachybotrys atra* and *Syncephalastrum racemosus*, yeast and sterile mycelium. Most of those above species have been isolated from indoor dusts by several studies (Bahkali & Parvez, 1999; Abdullah & Al-Musa, 2000; Maghraby et al., 2008; Singh et al., 2009; Al-Humiany, 2010; Abdullah & Al-Musa, 2011; Yasser, 2013).

Conclusions

Carpet dusts in residential houses and mosques are rich in diverse types of fungi including dermatophytes, related keratinophilic fungi, entomopathogenic fungi and cycloheximide resistant fungi. Most of the recovered species during this investigation can be considered as potential pathogens to humans. Some of the recovered species, particularly in the genera *Aspergillus*, *Penicillium*, *Fusarium* and *Stachybotrys*, are well known as mycotoxins producers.

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