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**Original article**

## Isolation of dematiaceous fungi from soil of Mashhad, Neyshabur(North-east of Iran) and Isfahan (center of Iran) cities

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### ABSTRACT

Dematiaceous fungi can infect patients who are immunocompetent and as well as may be related to in a wide variety of disorders. This study aimed to isolate and identify dematiaceous fungi from soil of Mashhad, Neyshabur and Isfahan cities. Of 93 strains isolated, the frequency of *Ulocladium*, *Cladosporium*, *Aureobasidium* and *Alternaria* were as 38.7%, 30.2%, 10.7% and 6.4%, respectively by microscopic examination. Also 13.9% were sterile hyphae. In this study, *ulocladiumsp* were the predominant fungi among all of the dematiaceous fungi. Although *ulocladiumsp* that formerly was identified as non-pathogenic saprophyte, have recently been reported in various diseases such as asthma, allergic rhinitis, hypersensitivity pneumonitis, chronic wounds, onychomycosis, cutaneous granulomas and keratitis.

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### 1. Introduction

Dematiaceous fungi are comprised of a huge heterogenous group of organisms characterized by a light to dark brown pigmentation of their hyphal or conidial elements or both. They are widely found in nature but are not considered as common human pathogens. The dematiaceous fungi are widely distributed among the Ascomycetes, Basidiomycetes, and Zygomycetes (Espinel-Ingroff et al., 1982). These fungi can infect individuals who are

immunocompetent and may be related to a wide variety of disorders ( Fothergill, 1996). A broad range of clinical syndromes have been associated with melanized fungi. On the other hand, the pathogenesis of dematiaceous fungi is widespread; including eumycetoma, chromoblastomycosis and pheohyphomycosis. Pheohyphomycosis encompasses a broad spectrum of diseases, such as a lergic disease (allergic fungal sinusitis and allergic bronchopulmonary mycosis [ABPM]), superficial infections (onychomycosis and tinea nigra), deep local infections (subcutaneous lesion, keratitis, bone and joint infections, peritonitis and other miscellaneous infections), Pulmonary, Central nervous system and disseminated infections. The main route of fungal entrance to human body is respiratory tract and micro trauma in the skin. *Alternaria* frequently causes subcutaneous lesions; *Bipolaris* and *Curvularia* are often related to allergic disease. Moreover, *Cladosporium bantiana* and *Scedosporium prolificans* most commonly cause brain abscess and disseminated disease, respectively (Revankar and Sutton, 2010).The aim of this study was the isolation and identification of dematiaceous fungi from soil of Mashhad, Neyshabur and Isfahan cities.

## 2. Materials and methods

### 2.1. Sampling

Fifty-one soil and decaying leave samples (200-400 grams for each, and depth of 15cm) were collected from Mashhad, Neyshabur and Isfahan cities during autumn 2012, for the presence of Dematiaceous fungi.

### 2.2. Isolation

Three grams of each sample was weight by a digital balance and transmitted to a 15ml glass tube and then 10ml normal saline was added to it. The vortex of solution was performed gently to make a homogenous suspension.

### 2.3. Pour plate test

200µl of the suspension was inoculated to Sabouraud Dextrose Agar containing penicillin and gentamicin in a plate and was mixed by shaking. For growth of such slow growing fungi, all the culture media were incubated at 28°C for 3 weeks, being checked every day for colony formation. From pure black colonies and black colonies that overlapped with other saprophytic fungi were sub-cultured and then incubated at 28°C in a dark space.

**Table 1**  
source and frequency of isolated dematiaceous fungi.

| Fungus isolated<br>city | <i>Ulocladium</i> | <i>Cladosporium</i> | <i>Aureobasidium</i> | <i>Alternaria</i> | Sterile hypha |
|-------------------------|-------------------|---------------------|----------------------|-------------------|---------------|
| Mashhad                 | 11                | 7                   | 1                    | 2                 | 8             |
| Neyshabur               | 13                | 10                  | 3                    | 3                 | 1             |
| Isfahan                 | 12                | 11                  | 6                    | 1                 | 4             |
| Total                   | 36                | 28                  | 10                   | 6                 | 13            |
| (%)                     | (38.7%)           | (30.2%)             | (10.7%)              | (6.4%)            | (13.9%)       |

### 2.4. Microscopic test

Finally, all the isolated colonies were tested by slide Riddle method. After Lacto Phenol catton Blue staining, the slides were assessed for morphology identification of dematiaceous fungi with optical microscope.

## 3. Results

In this study, approximately 86.1% of fungi were identified and other colonies were reported as sterile hyphae. Four fungi genera instead fungi including *Ulocladium*, *Cladosporium*, *Aureobasidium* and *Alternaria* were isolated from soil samples. After microscopic examination of the 93 isolated strains, the frequency of *Ulocladium*,

*Cladosporium*, *Aureobasidium* and *Alternaria* were as 38.7%, 30.2%, 10.7% and 6.4% respectively. Also 13.9% were as sterile hyphae. The following table 1 shows the abundance of fungi studied here, regarding the source.



Fig. 1. *Ulocladium sp* in the microscopic examination.



Fig. 2. *Cladosporium sp*.



Fig. 3. *Aureobasidium sp*.



Fig. 4. *Alternaria sp*.

#### 4. Discussion

Because of the fungal spores are present in soil, at the beginning of autumn and increasing wind, the exposure of individuals with spores will increase. On the other hand, the main ways for entrance of spores include respiratory tract and inoculation into the skin. It should be noted, although *ulocladium sp* that formerly was identified as non-pathogenic saprophyte, have recently been reported in various diseases. Multiple evidence suggest that *U.chartarum* evokes symptoms of asthma, allergic rhinitis and hypersensitivity pneumonitis in sensitized persons (Metzge et al., 2010). *Ulocladium sp* are opportunistic fungi and cause infections such as chronic wounds (Sun, 2010). Furthermore, these non-dermatophytic filamentous fungi are causative agents of onychomycosis (Hilmioğlu-Polat et al., 2005). *U.chartarum* has been reported as etiologic agent of cutaneous granulomas in a 58-year-old woman (Altmeyer and Schon, 1981). also, this organism caused cutaneous mycoses in an Immunodeficient heart transplant recipient (Teresa Duran et al., 2003). On the other hand, the related species of *U.atrum* were associated with keratitis in a 43-year-old man (Badenoc et al., 2006). Shokouhi and coworkers have isolated 47 species of fungi from different areas of sari countryside in which *Alternaria* was the most frequent among other genera. According to research of chomel and colleagues, variation in frequency of fungi can be dependent on type and depth of soil, climate, vegetation, moisture and salinity of soil (Chmel and Vláčilíková, 1977). Some elements such as carbon, nitrogen, potassium, phosphorous and also vegetation (due to high levels of carbon and nitrogen) can increase fungal populations in soil, while the presence of calcium in the soil is an inhibitor of fungal populations (Chauhan, 1982). Considering the wide spectrum of fungal infections, increasing incidence of diseases with poor prognosis and also enhancement of such target groups (immunocompromised patients) has augmented the importance of these fungi in society and individual health (Brandt and Warnock, 2003; Derber et al., 2010). The results of antifungal therapy remain poor, with approximately mortality rate of 79%. The presence two types of melanin including 1,8-dihydroxynaphthalene (DHN)–melanin and 3,4-dihydroxyphenylalanine (DOPA)–melanin in their cell walls may be a virulence factor for these fungi (Revankar et al., 2002). This pathogenic factor protects fungi from reactive oxygen, nitrogen radicals and macrophage-mediated killing (Missall et al.,

2004). The laboratory diagnosis of these fungi is mainly based on microscopic and pathological examinations. The number of published articles relating to these fungi has risen steadily in recent years. Due to the presence of dematiaceous fungi in the soil and daily exposure of people to these fungi, they may cause life-threatening diseases; especially in the immunocompromised patients. Also these species are capable of causing various disorders even in immunocompetent individuals. Researches shows that overall incidence of 4.6 cases of Primary Immunodeficiency Diseases (PIDs) per 100,000 person-years. However, there has been an increasing temporal trend in incidence rates during the past 31 years, with a rate of 10.3 per 100,000 person-years rate in 2000-2006 compared with 2.4 per 100,000 person-years in 1976-1980 (Joshi et al., 2009). Hence the importance of dematiaceous fungi and their pathogenicity has increased more than before.

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