Original Article

# Identification of Mucorales isolates from soil using morphological and molecular methods

Ziaee A[1], Zia M[2*], Bayat M[1], Hashemi J[3]

[1] Department of Medical and Veterinary Mycology, Faculty of Veterinary Specialized Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2] Department of Basic Sciences, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran
[3] Department of Mycology and Parasitology, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran

***Corresponding author: Mohammadali Zia,** Department of Basic Sciences, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran. Email: zia.mohammadali@gmail.com

(Received: 17 December 2015; Revised: 5 March 2016; Accepted: 29 March 2016)

## Abstract

**Background and Purpose:** Soil is the main habitat of saprophytic and pathogenic fungi. Mucoromycotina constitutes a large group of soil fungi, with certain opportunistic members causing systemic infections in immunocompromised hosts. The majority of human and animal infections are caused by the members of the genera *Rhizopus*, *Mucor*, *Rhizomucor*, *Lichtheimia* (*Absidia)*, *Cunninghamella,* and *Mortierella*. Accordingly, in the present study, we aimed to isolate and identify the main genera of the order Mucorales, using molecular assays and morphological features.

**Materials and Methods:** In total, 340 soil samples were collected from seven public parks throughout the city and sidewalk gardens in 14 municipal districts in Isfahan, Iran. All the samples were cultured on the appropriate media, incubated at 27°C for 2- 4 days, and examined daily for visible fungal growth. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was applied and macroscopic, microscopic, and physiological characteristics were assessed to identify fungal colonies.

**Results:** 400 pure colonies, belonging to the orders Mucorales and Mortierellales, including the genera *Lichtheimia*, *Rhizopus, Rhizomucor, Mucor, Cunninghamella,* and *Mortierella,* were identified. The genus *Rhizopus* (35.5%) was the most frequent isolate, followed by *Mucor* (32.25%) and *Rhizomucor* (27.5%).

**Conclusion**: The results emphasize the importance of opportunistic fungi in public areas and indicate the risk of exposure for immunocompromised individuals.

**Keywords:** Mucorales, *Mucor, Rhizopus, Lichtheimia, Rhizomucor, Mortierella,* PCR-RFLP

➤ How to cite this paper:
Ziaee A, Zia M, Bayat M, Hashemi J. Identification of Mucorales isolates from soil using morphological and molecular methods. Curr Med Mycol. 2016; 2(1): 13-19.

## Introduction

In the literature, no relationship has been established between the distribution of fungal species and environmental factors [1]. The amount and type of microorganisms in a particular section of soil are associated with several factors, such as sunlight, temperature, moisture, soil pH, nutrients, and reduction potential [2].

Fungi, as a group of microorganisms with a wide distribution in soil, play important roles in the soil ecosystem and soil-borne fungal diseases. Today, it is well established that soil can be a reservoir of most pathogenic and opportunistic fungi [3-6]. In fact, soil-borne pathogenic fungi may enter the human body via direct inoculation into wounds, direct soil ingestion, or indirect ingestion via contaminated food.

Fungal spores can be dispersed in different environments through dust or mud particles from soil disturbances and enter the respiratory tract [2, 6]. Mucorales is an order of mostly saprophytic fungi, growing on organic substances, such as food, dead plants, or animal waste materials in soil [7, 8]. These organisms may survive in the soil for a long time before infecting humans who are in contact with contaminated soil [9].

In 2007, in a taxonomic reclassification, Zygomycetes was abolished as a class, and zygomycosis is now mainly attributed to the order Mucorales of subphylum Mucoromycotina [10]. Therefore, the term "mucormycosis", previously known as zygomycosis, is currently used for opportunistic infections caused by the order Mucorales [11].

Mucormycosis is the third most common invasive fungal infection, following aspergillosis and candidiasis [12]. The importance of

mucormycosis has been highlighted in recent years as a consequence of the dramatic increase in the number of patients with predisposing factors [13-15]. In general, six families, including Cunninghamellaceae, Lichtheimiaceae, Mucoraceae, Saksenaeaceae, Syncephalastraceae, and Thamnidiaceae, have been described to be responsible for human infections [14].

With this background in mind, the main aim of this study was to investigate the diversity of fungi belonging to the subphylum Mucormycotina, particularly the order Mucorales, in the soil of different public places and sidewalk gardens, located in the populated areas of municipal districts of Isfahan, Iran, using molecular and morphological assays.

DNA-based molecular assays have been shown to be rapid and highly reliable tools, especially for rRNA genes. These assays have become widely accepted for phylogenetic identification of various fungi [8]. In the present study, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was applied to identify the important genera of the order Mucorales, as successfully performed in previous studies [16, 17].

## Materials and Methods

During August 2014 to October 2015, a total of 340 soil samples were gathered from different sites, i.e., seven public parks and different sidewalk gardens located in 14 municipal districts of Isfahan, Iran. First, all the debris, present on the soil surface, was removed. Then, approximately 400 g of soil at a depth of 5-10 cm was collected from the surface layer and stored in sterile bags, using a sterile stainless steel spoon. All the samples were immediately transferred to the laboratory for the next processing stage.

The samples were crushed in a mortar under sterile conditions and then homogenized. Afterwards, 5 g of each soil sample was suspended in 20 ml of sterile double-distilled water and shaken for 5 min to prepare a soil suspension [18, 19]. Approximately 0.5 ml of the suspension was poured in the bottom of a Petri dish. Then, cooled molten agar medium of Sabouraud dextrose agar (SDA) or potato dextrose agar (PDA), supplemented with chloramphenicol (50 mg/l), was added to the suspension and entirely mixed. All the samples were incubated at 27°C for 2-4 days and were observed daily in terms of fungal colony growth. The primary identification was performed on the basis of macroscopic and microscopic features. Definite identification of *Rhizopus, Mucor, Lichtheimia, and Rhizomucor* at the species level was performed, based on molecular analysis and morphological and physiological studies. Moreover, identification of *Mortierella* and *Cunninghamella* species was based on the morphological characteristics [20].

### *Macroscopic and microscopic features*

Mucorales colonies are typically floccose and dense. These colonies rapidly fill the entire Petri dish with abundant intertwined aerial mycelium, resembling gray cotton candy. The hyphae are predominantly aseptate or very sparsely septate and wide. Features which are most useful for distinguishing Mucorales include the presence of rhizoids, shape of sporangium, length of sporangiophore, shape of columella, presence or absence of apophysis and collarette, and organization and branching of stolons [15, 21].

### *Molecular studies*

Genomic DNA, pertaining to all pure Mucorales colonies, was extracted, using the phenol-chloroform method [22]. A mixture of specific sense primers (MucL1: 5’ TGATCTACGTGACATATTTCT 3’; AbsL1: 5’ TGA TCTACACGGCATCAAAT 3’; RpL1: 5’ TGATCTACGTGACAAATTCT 3’; and RmL1: 5’ TGATCTACGCGAGCGAACAA 3’) was used, corresponding to *Mucor*, *Lichtheimia*, *Rhizopus*, and *Rhizomucor* species, respectively. Also, an antisense degenerate primer (MR1: 5’ AGTAGTTTGTCTTCGGTCAA 3’) corresponding to Mucorales was applied for the amplification of the purified extracted DNA. In a previous study by Machouart and colleagues, these primers had been successfully applied for the detection of the abovementioned genera belonging to the order Mucorales [17].

The following temperature program was applied for the amplification of the extracted DNA: primary denaturation at 94°C for 3 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 7 min [23]. The PCR products of Mucorales were digested individually with the selected restriction enzymes, as presented in Table 1.

For PCR-RFLP analysis, a mixture was prepared, using the following components in a final volume of 25 µl: restriction enzyme (2 µl), enzyme buffer (2.5 µl), PCR product (10 µl), and

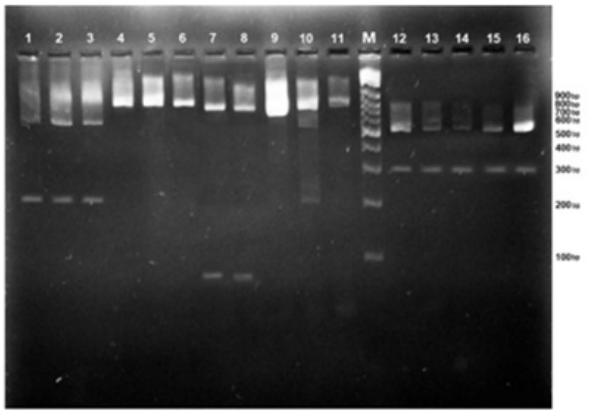


**Figure 1.** Agarose gel electrophoresis of 18S rRNA PCR products of different species of Mucorales after restriction digestion with *XhoII* and *CspsI*. Lanes 1, 2, & 3: digestion with *XhoII* (*Rhizomucor pusillus*); Lanes 4, 5, & 6: undigested PCR products; Lanes 7 & 8: digestion with *AseI* (*Rhizopus microsporus or R. azygosporus*); Lanes 9, 10, & 11: undigested PCR products; Lanes 12, 13, 14, 15, & 16: digestion with *AclI* (*Lichtheimia corymbifera* or *L. blakesleeana*); Lane M: 100 bp molecular-size marker

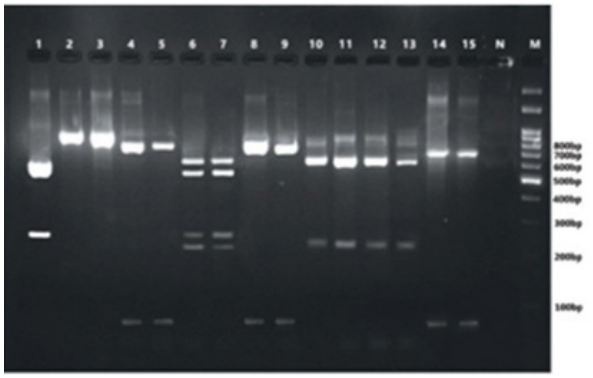


**Figure 2.** Agarose gel electrophoresis of 18S rRNA PCR products of different species of Mucorales after restriction digestion with *BmgBI* (Lanes 1, 2, & 3), *AflII* (Lanes 4, 5, 8, & 9), *CspsI* (Lanes 6 & 7), *XmnI* (Lanes 10, 11, 12, & 13), and *PpumI* (Lanes 14 & 15); Lane 1: *Rhizopus* spp. (except *R. stolonifer*); Lanes 2 & 3: undigested PCR products; Lanes 4, 5, 8, & 9: *Mucor* spp.; Lanes 6 & 7: *Rhizopus arrhizus*; Lanes 10, 11, 12, & 13: *M. circinelloides*, or *M. racemosus*, or *M. ramosissimus,* or *M. plumbeus*; Lanes 14 & 15: *Rhizomucor* spp.; Lane N: negative control; Lane M: 100 bp molecular-size marker

distilled water (10.5 µl). The reaction mixture was incubated at 37°C for 2 h. The PCR-RFLP products were run on agarose gel electrophoresis (1.5% and 2%, respectively) in tris/borate/EDTA (TBE) buffer and photographed under ultraviolet transillumination (Figures 1 & 2).

## Results

A total of 393 and seven pure colonies, belonging to the orders Mucorales and Mortierellales, were obtained, respectively. *Rhizopus* was the most frequently detected species with a frequency rate of 35.5%, followed by *Mucor* (32.25%), *Rhizomucor* (27.5%), *Lichtheimia* (2.5%), *Mortierella* (1.75%), and *Cunninghamella* (0.5%) (Table 2).

**Table 1.** Restriction enzymes, species specificity, and fragment size used in the present study

| Enzyme (restriction site) | Specificity | Fragment size (bp) |
|---|---|---|
| *PpuMI* | *Rhizomucor* spp. (except *R. variabilis*) | 68+720 |
| *XhoII* | *Rhizomucor pusillus* | 215+573 |
| *BmgBI* | *Rhizopus* spp. (except *R. stolonifer*) | 593+235 |
| *AseI* | *Rhizopus microsporus* and *R. azygosporus* | 750+75 |
| *CspCI* | *Rhizopus oryzae* | 214+249+579+614 |
| *AflII* | *Mucor spp.* | 750+87 |
| *XmnI* | *Mucor circinelloides, M. racemosus, M. ramosissimus,* and *M. plumbeus* | 613+224 |
| *AclI* | *Lichtheimia corymbifera* and *L. blakesleeana* | 518+306 |

As presented in Table 2, *R. stolonifer* with a frequency of 3% was the predominant species, followed by *R. arrhizus* and *R. pusillus* with frequency rates of 2.5% and 1.5%, respectively; the lowest frequency rate was attributed to *M. wolfii* (0.25%). *M. racemosus* with a frequency rate of 1% was the predominant isolate among the identified species of the genus *Mucor*. Also, two *C. bertholletiae* isolates were identified in pure Mucorales cultures.

According to the findings, 51.81% of the

**Table 2.** Distribution of Mucorales and Mortierellales in the soil of different public parks and municipal districts

| Genus | Species | No. (%) | Total (%) |
|---|---|---|---|
| *Mucor* | *M. Circinelloides* | 3 (0.75%) | 129 (32.25%) |
| | *M. Racemosus* | 4 (1%) | |
| | *M. Plumbeus* | 2 (0.5%) | |
| | *Mucor* spp. | 120 (30%) | |
| *Rhizomucor* | *R. Pusillus* | 6 (1.5%) | 110 (27.5%) |
| | *Rhizomucor* spp. | 104 (26%) | |
| *Rhizopus* | *R. Arrhizus* | 10 (2.5%) | 142 (35.5%) |
| | *R. Stolonifer* | 12 (3%) | |
| | *Rhizopus* spp. | 120 (30%) | |
| *Lichtheimia* | *L. Corymbifera* | 2 (0.5%) | 10 (2.5%) |
| | *Lichtheimia (Absidia)* spp. | 8 (2%) | |
| *Cunninghamella* | *C. bertholletiae* | 2 (0.5%) | 2 (0.5%) |
| *Mortierella* | *M. Wolfii* | 1 (0.25%) | 7 (1.75%) |
| | *Mortierella* spp. | 6 (1.5%) | |
| Total (%) | | 400 (100%) | 400 (100%) |

**Table 3.** Distribution of Mucorales and Mortierellales in the soil of different public parks

| Genus | Park No. Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | No. (%) | Total (% ) |
|---|---|---|---|---|---|---|---|---|---|---|
| | *M. circinelloides* | - | - | - | 1 | - | 1 | - | 2 (1.20) | |
| ***Mucor*** | *M. racemosus* | 1 | - | - | - | - | - | - | 1 (0.60) | 23(13.86) |
| | *Mucor* spp. | 2 | 9 | 1 | 2 | 1 | 3 | 2 | 20 (12.06) | |
| ***Rhizomucor*** | *R. pusillus* | - | 1 | - | - | 1 | - | - | 2 (1.20) | 47(28.31) |
| | *Rhizomucor* spp. | 12 | 20 | 3 | 3 | 4 | - | 3 | 45 (27.11) | |
| | *R. oryzae* | 1 | | 1 | 1 | - | 1 | 1 | 5 (3.01) | |
| ***Rhizopus*** | *R. stolonifer* | 1 | 2 | 1 | | - | 3 | 2 | 9 (5.42) | 86(51.81) |
| | *Rhizopus* spp. | 20 | 1 | 2 | 17 | - | 30 | 2 | 72 (43.38) | |
| ***Lichtheimia*** | *L. corymbifera* | 1 | - | - | - | - | - | - | 1 (0.60) | 5 (3.01) |
| | *Absidia* spp. | 2 | - | - | - | 1 | 1 | - | 4 (2.41) | |
| ***Cunninghamella*** | *C. bertholletiae* | 1 | - | - | - | - | - | - | 1 (0.60) | 1(0.60) |
| ***Mortierella*** | *Mortierella* spp. | 1 | 1 | 1 | - | - | 1 | - | 4 (2.41) | 4(2.41) |
| **Total (%)** | | 42 (25.30) | 34 (20.48) | 9 (5.42) | 24 (14.46) | 7 (4.22) | 40 (24.10) | 10 (6.02) | 166 (100) | 166(100) |

**Table 4.** Distribution of Mucorales and Mortierellales in the soil of different municipal districts

| Genus | District No. Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | No. (%) | Total (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Mucor* | *M. circinelloides* | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - | 1 (0.43) | 106 (45.30) |
| | *M. racemosus* | 1 | - | - | - | - | - | 1 | - | - | - | - | - | 1 | - | 3 (1.28) | |
| | *M. plumbeus* | - | - | 2 | - | - | - | - | - | - | - | - | - | - | - | 2 (0.85) | |
| | *Mucor* spp. | 5 | 13 | 22 | 5 | - | 4 | 21 | 2 | 6 | 3 | 7 | 3 | 2 | 7 | 100 (42.74) | |
| *Rhizomucor* | *R. pusillus* | - | 2 | - | - | - | - | - | - | - | - | - | 1 | - | 1 | 4 (1.71) | 63 (26.92) |
| | *Rhizomucor* spp. | 7 | 8 | 10 | 2 | 1 | 1 | 3 | 10 | 5 | 2 | 1 | 3 | 5 | 1 | 59 (25.21) | |
| *Rhizopus* | *R. oryzae* | - | - | - | - | 1 | - | - | 1 | - | 1 | - | - | 1 | 1 | 5 (2.14) | 56 (23.93) |
| | *R. stolonifer* | - | - | - | 1 | - | 1 | - | - | - | - | - | 1 | - | - | 3 (1.28) | |
| | *Rhizopus* spp. | 4 | 5 | 9 | 6 | - | 4 | 12 | - | 1 | - | 3 | 2 | - | 2 | 48 (20.51) | |
| *Lichtheimia* | *L. corymbifera* | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | 1 (0.43) | 5 (2.14) |
| | *Absidia* spp. | 3 | - | - | - | - | - | - | - | - | - | - | 1 | - | - | 4 (1.71) | |
| *Cunninghamella* | *C. bertholletiae* | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | 1 (0.43) | 1(0.43) |
| *Mortierella* | *M. wolfii* | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | 1 (0.43) | 3 (1.28) |
| | *Mortierella* spp. | - | - | - | - | - | - | - | - | 1 | 1 | - | - | - | - | 2 (0.85) | |
| Total (%) | | 20 (8.55) | 28 (11.96) | 43 (18.38) | 14 (5.98) | 3 (1.2) | 11 (4.7) | 37 (15.81) | 13 (5.56) | 13 (5.56) | 7 (2.99) | 11 (4.70) | 11 (4.70) | 9 (3.85) | 14 (5.98) | 234 (100) | 234 (100) |

isolates from the soil samples of public parks belonged to the genus *Rhizopus,* followed by *Rhizomucor* with a frequency rate of 28.31%, while in the soil samples from municipal districts, the genus *Mucor* (45.30%) was the most frequent isolate, followed by *Rhizomucor* (26.92%) (Tables 3 & 4).

## Discussion

Mucorales are known as thermotolerant moulds, widely found on organic substrates and soil. Temperature of 27°C and high humidity are the optimal environmental conditions, required for the growth and sporulation of Mucorales on these substrates. According to the literature, most Mucorales, as thermophilic species, have been isolated from composting plant materials [9, 24]. In addition, the outbreak of rhinocerebral or pulmonary mucormycosis has been associated with the inhalation of sporangiospores in dust due to excavation, construction, or contaminated air-conditioning filters [9].

Today, mucormycosis is identified as an emergent disease, owing to the increasing incidence and mortality over the past two decades [25]. The diagnosis and management of mucormycosis remain challenging tasks and no clinical or radiological signs specific for mucormycosis have been identified. Moreover, standardized serological or antigen detection tests

are not currently available [26].

Researchers have attempted to compare the identification of Mucorales fungi, using phenotypic and molecular methods [11]. In the present study, all the phenotypic features of Mucorales were adapted in the molecular analysis for the definite identification of fungi. Different regions of rRNA operon have been frequent targets for the detection of Zygomycetes. Also, different fungal primers from 18S or ITS regions of rRNA gene have been used for PCR amplification [27]. In our study, primers selected from the 18S region were used to detect major genera of the order Mucorales.

Species involved in mucormycosis belong to the order Mucorales of subphylum Mucoromycotina. In addition, Mucoraceae is the most important family, comprising of *Rhizopus, Mucor,* and *Lichtheimia* as the most common species and *Rhizomucor, Mortierella, Saksenaea, Syncephalastrum, Cunninghamella,* and *Apophysomyces* as less common agents of mucormycosis [25].

In the present study, *Rhizopus, Mucor*, and *Rhizomucor* were the dominant genera with frequency rates of 35.5%, 32.25%, and 27.5%, respectively (Table 2). These organisms are ubiquitous saprophytes in nature, rarely infecting organisms with an intact immune system [28]. Members of the genus *Rhizopus* are the most common isolates, recovered from clinical mucormycosis samples. Additionally, members of the genus *Mucor* are second to *Rhizopus* in terms of frequency [12].

*R. arrhizus* (*oryzae*) is the most common cause of mucormycosis. Other less frequent etiological agents include *L. corymbifera, A. elegans, C. bertholletiae, R. pusillus,* and *S. vasiformis* [9]. In the present study, *R. arrhizus (oryzae)*, *R. pusillus, L. corymbifera,* and *C. bertholletiae* were isolated from the soil samples of public parks and municipal districts, with the genus *Rhizopus* showing the highest frequency (Tables 3 & 4).

*R. arrhizus* was previously diagnosed as the causative agent of rhinocerebral mucormycosis in a man with diabetes mellitus in Isfahan, Iran. Accordingly, it was concluded that debilitated individuals are predisposed to mucormycosis [29]. Also, *R. pusillus* is the main etiological agent for infection in immunocompromised hosts. In the literature, 22 cases of *R. pusillus* infection were reported before 2013 [30]. However, *Rhizopus* species, which are responsible for almost 80% of infections, remain as the major cause of most mucormycosis cases. Also, a considerable number of mucormycosis cases have been associated with *M. circinelloides* [31].

In the present study, *M. circinelloides* and *M. racemosus* were isolated from the soil samples gathered from public parks and municipal districts, while *M. plumbeus* was only isolated from two municipal districts (Tables 2, 3, & 4). Although *M. plumbeus* is extensively used for research purposes in biotransformation of natural products, no cases of mycosis have been so far linked to this species [31].

In the present study, different *Lichtheimia* species including *L. corymbifera* were isolated from the soil samples of parks and municipal districts (Tables 2, 3, & 4). In general, the genus *Lichtheimia* (*Absidia*) consists of fungal species, which are ubiquitous soil inhabitants and important causative agents of mucormycosis in humans and animals [32]. In Europe, members of the genus *Lichtheimia* are the second or third most important cause of mucormycosis [33].

Malek *et al*. [34] isolated 17 different fungal genera from the soil of parks in Gorgan, Iran. Overall, 36% of the isolates were related to Zygomycetes, and the genus *Mucor* was the most frequent isolate among other Zygomycetes genera. In the present study, the genus *Rhizopus* (51.81%) was the most frequent isolate detected in soil, followed by *Rhizomucor* (28.31%), *Mucor* (13.86%), *Lichtheimia* (*Absidia)* (3.01%), *Mortierella* (2.41%), and *Cunninghamella* (0.60%), respectively (Table 3). The discrepancy between the findings can be attributed to the differences in temperature, humidity, organic content of soil, and plant diversity in each area.

The Mortierellaceae species can be distinguished from Mucoraceae with respect to their very delicate features. In these species, sporangia are small and contain few or no columella; moreover, the mycelium is dichotomously branched. Considering these delicate features, species belonging to this family can be classified in a family other than Mucoraceae [21].

Isolation of the genus *Mortierella* from soil samples has been studied in the literature [35]. In the present study, different *Mortierella* species including *M. wolfii* were isolated from the soil of parks and municipal districts with frequency rates of 2.41% and 1.28%, respectively (Tables 3 & 4). According to previous research, *M. wolfii* is probably the only

pathogenic species and an important etiological agent of bovine mycotic abortion, pneumonia, and systemic mycosis [21].

Yazdanparast *et al*. [36] reported the isolation of some saprophytic and keratinophilic fungi from the soil samples of parks and municipal districts of Tehran, Iran. They isolated the genera *Cunninghamella*, *Mucor,* and *Rhizopus* from the soil samples of several parks. Based on the findings, the isolation rate of *Cunninghamella* was higher than other genera.

So far, several researchers have isolated different fungi from various types of soil. In this regard, Agamirian *et al*. investigated the prevalence of fungi in soil of Qazvin, Iran and reported 14 genera including *Rhizopus* and *Mucor* [6]. Although the present study focused on Mucorales, genera belonging to other classes of fungi (e.g., *Fusarium, Cladosporium, Aspergillus, Penicillium, Trichophyton mentagrophytes, Chrysosporium,* and *Scopulariopsis*) were also isolated.

In a previous study, Hedayati *et al*. (2004) isolated *Mucor* and *Rhizopus* species from the soil samples of potted plants in hospitals of Sari, Iran. Other isolated genera included *Acromunium, Penicillium, Cladosporium, Paecilomyces, Chrysosporium, Alternaria, Aspergillus, Verticillium, Geotrichum,* and yeasts [37]. The obtained findings were almost in agreement with the results of the present study.

## Conclusion

In conclusion, Mucoromycotina representatives are recognized as ubiquitous organisms in the soil all over the world. These organisms are majorly found in clinical samples and are used in food fermentation [38]. Therefore, knowledge about the pathogenic ecology of Mucorales is essential to the understanding of the epidemiology of mucormycosis in high-risk patients.

## Acknowledgements

We would like to thank the biotechnology and mycology laboratory of Islamic Azad University of Isfahan for the technical assistance and valuable help.

## Authors' contributions

A.Z. carried out the practical and laboratory examinations of the study. M.Z. participated in the study design, conducted practical and laboratory examinations, and drafted the manuscript. M.B. and J.H. participated in the study design and helped draft the manuscript.

## Conflicts of interest

There was no conflict of interest in the present study.

## Financial disclosure

There was no financial interest related to the materials of the manuscript.

## References

1. Edel-Hermann V, Gautheron N, Mounier A, Steinberg C. Fusarium diversity in soil using a specific molecular approach and a cultural approach. J Microbiol Methods. 2015; 111:64-71.
2. Baumgardner DJ. Soil-related bacterial and fungal infections. *J Am Board Fam Med*. 2012; 25(5):734-44.
3. Moallaei H, Zaini F, Pihet M, Mahmoudi M, Hashemi J. Isolation of keratinophilic fungi from soil samples of forests and farm yards. Iran J Public Health. 2006; 35(4):62-9.
4. Suhail M, Irum F, Jatt T, Korejo F, Abro H. *Aspergillus* mycoflora isolated from soil of Korti Barrage Sandy, Pakistan. Pak J Bot. 2007; 39(3):981-4.
5. Zarrin M, Haghgoo R. Survey of keratinophilic fungi from soils in Ahvaz, Iran. Jundishapur J Microbiol. 2011; 4(3):191-4.
6. Aghamirian MR, Ghiasian SA. The prevalence of fungi in soil of Qazvin, Iran. Jundishapur J Microbial. 2012; 6(1):76-9.
7. Kim MJ, Park PW, Ahn JY, Kim KH, Seo JY, Jeong JH, et al. Fatal pulmonary mucormycosis caused by *Rhizopus microsporus* in a patient with diabetes. Ann Lab Med. 2014; 34(1):76-9.
8. Nagao K, Ota T, Tanikawa A, Takae Y, Mori T, Udagawa S, et al. Genetic identification and detection of human pathogenic Rhizopus species, a major mucormycosis agent, by multiplex PCR based on internal transcribed spacer region of rRNA gene. J Dermatol Sci. 2005; 39(1):23-31.
9. Richardson M. The ecology of the Zygomycetes and its impact on environmental exposure. Clin Microbiol Infect. 2009; 15(Suppl 5):2-9.
10. Neto FM, Camargo PC, Costa AN, Teixeira RH, Carraro RM, Afonso JE Jr, et al. Fungal infection by Mucorales order in lung transplantation: 4 case reports. Transplant Proc. 2014; 46(6):1849-51.
11. Yang M, Lee HJ, Kim YK, Ki CS, Huh HJ, Lee NY. Identification of mucorales from clinical specimens: a 4-year experience in a single institution. Ann Lab Med. 2016; 36(1):60-3.
12. Bala K, Chander J, Handa U, Punia RS, Attri AK. A prospective study of mucormycosis in north India: experience from a tertiary care hospital. Med Mycol. 2015; 53(3):248-57.
13. Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, et al. A higher-level phylogenic classification of the fungi. Mycol Res. 2007; 111(Pt 5):509-47.
14. Iwen PC, Thapa I, Bastola D. Review of methods for the identification of Zygomycetes with an emphasis on advances in molecular diagnostics. Lab Med. 2011; 42(5):260-6.
15. Lackner M, Caramalho R, Lass-Florl C. Laboratory diagnosis of mucormycosis: current states and future perspectives. Future Microbiol. 2014; 9(5):683-95.
16. Larche J, Machouart M, Burton K, Collumb J, Biava MF, Gerard A, et al. Diagnosis of cutaneous

mucormycosis due to *Rhizopus microsporus* by an innovative PCR-restriction fragment-length polymorphism method. Clin Infect Dis. 2005; 41(9):1362-5.
17. Machouart M, Larche J, Burton K, Collomb J, Maurer P, Cintrat A, et al. Genetic identification of main opportunistic Mucorales by PCR-restriction fragment length polymorphism. J Clin Microbiol. 2006; 44(3):805-10.
18. Mohamed MS, Abdel-Motaleb HY, Mobarak FA. Management of rhino-orbital mcormycosis. Saudi Med J. 2015; 36(7):865-8.
19. Gaddeyya G, Niharika PS, Bharathi P, Kumar PR. Isolation and identification of soil mycoflora in different crop fields at Salur Mandal. Adv Appl Sci Res. 2012; 3(4):2020-6.
20. Ziaee A, Zia M, Bayat M, Hashemi J. Occurrence of important mucormycosis agents in the soil of populous areas of Isfahan and their pathogenicity in immunocompromised patients. J Pure Appl Microbiol. 2016; 10(1):81-8.
21. Ribes JA, Vanover-Sams CL, Baker DJ. Zygomycetes in human disease. Clin Microbiol Rev. 2000; 13(2):236-301.
22. Mirhendi SH, Kordbacheh P, Kazemi B, Samiei S, Pezeshki M, Khorramizadeh MR. A PCR-RFLP method to identification of the important opportunistic fungi: *Candida* species, *Cryptococcus neoformans*, *Aspergillus famigatus* and *Fusarium solani*. Iran J Pub Health. 2001; 30(3-4):103-6.
23. Ziaee A, Zia M, Bayat M, Hashemi J. Molecular identification of Mucor and Lichtheimia species in pure cultures of Zygomycetes. Jundishapur J Microbiol. 2016; 9(4):e35237.
24. Walsh TJ, Gamaletsou MN, McGinnis MR, Hayden RT, Kontoyiannis DP. Early clinical and laboratory diagnosis of invasive pulmonary, extrapulmonary, and disseminated mucormycosis (Zygomycosis). Clin Infect Dis. 2012; 54(Suppl 1):S55-60.
25. Bonifaz A, Triado-Sanchez A, Calderon L, Ponce RM. Cutaneous mucormycosis: mycological, clinical and therapeutic aspects. Curr Fungal Infect Rep. 2015; 9(4):229-37.
26. Dannaoui E, Schwarz P, Slany M, Loeffler J, Jorde AT, Cuenca-Estrella M, et al. Molecular detection and identification of Zygomycetes species from paraffin-embedded tissues in murine model of disseminated Zygomycosis: a collaborative European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Fungal Infection Study Group (EFISG) evaluation. J Clin Microbiol. 2010; 48(6):2043-6.
27. Kasai M, Harrington SM, Francesconi A, Petraitis V, Petraitiene R, Beveridge MG, et al. Detection of a molecular biomarker for Zygomycetes by quantitative PCR assays of plasma, bronchoalveolar lavage, and lung tissue in rabbit model of experimental pulmonary Zygomycosis. J Clin Microbiol. 2008; 46(11):3690-702.
28. Paramythiotou E, Frantzeskaki F, Flevari A, Armaganidis A, Dimopoulos G. Invasive fungal infections in the ICU: how to approach, how to treat. Molecules. 2014; 19(1):1085-119.
29. Mohammadi R, Nazari M, Sayedayn SM, Ehteram H. A successful treatment of rhinocerebral mucormycosis due to *Rhizopus oryzae*. J Res Med Sci. 2014; 19(1):72-4.
30. Bala K, Chander J, Handa U, Punia RS, Raj R, Aggarwal S. Isolation and identification of *Rhizomucor pusillus* from rhinofacial mucormycosis in a diabetic patient. JJM Case Rep. 2014; 1(3):1-4.
31. Granja LF, Pinto L, Almeida CA, Alviano DS, Da Silva MH, Ejzemberg R, et al. Spores of Mucor ramosissimus, Mucor plumbeus and Mucor circinelloides and their ability to activate human complement system in vitro. Med Mycol. 2010; 48(2):278-84.
32. Hoffmann K, Discher S, Voigt K. Revision of the genus Absidia (Mucorales, Zygomycetes) based on physiological, phylogenetic, and morphological characters; thermotolerant Absidia spp. form a coherent group, Mycocladiaceae fam. nov. Mycol Res. 2007; 111(Pt 10):1169-83.
33. Schwartz V, Jacobsen ID. Mucormycoses caused by *Lichtheimia* species. Mycoses. 2014; 57(Suppl 3):73-8
34. Malek E, Moosazadeh M, Hanafi P, Nejat ZA, Amini A, Mohammadi R, et al. Isolation of keratinophilic fungi and aerobic actinomycetes from park soils in Gorgan, North of Iran. Jundishapur J Microbiol. 2013; 6(10):e11250.
35. Ellegaard-Jensen L, Aamand J, Kragelund BB, Johnsen AH, Rosendahl S. Strains of the soil fungus Mortierella show different degradation potentials for the phenylurea herbicide diuron. Biodegradation. 2013; 24(6):765-74.
36. Yazdanparast SA, Dargahi H, Shahrokhi S, Farahani RH. Isolation and investigation of keratinophilic fungi from municipality districts of Tehran. Thrita. 2013; 2(3):2-5.
37. Hedayati MT, Mohseni-Bandpi A, Moradi S. A survey on the pathogenic fungi in soil samples of potted plants from Sari hospitals, Iran. J Hosp Infect. 2004; 58(1):59-62.
38. Muszewska A, Pawlowska J, Krzysciak P. Biology, systemics, and clinical manifestations of Zygomycota infections. Eur J Clin Microbiol Infect Dis. 2014; 33(8):1273-87.