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# Determination of the Connection Between the Asthma Patients and Mycobiota in the Environment They Live in

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Abstract: In the 12-month period between February 2014 and January 2015, this study was carried out in the homes of 55 asthma patients living in 14 different regions of Istanbul (Ataşehir, Bulgurlu, Fikirtepe, Hasanpaşa, İçerenköy, Moda, Göztepe, Çekmeköy, Ümraniye, Altayçeşme Neighborhood, Esenkent Neighborhood, Feyzullah Neighborhood, Gülsuyu Neighborhood, Yalı Neighborhood). Air ideal (Biomerieux, France) air vacuuming device was used to determine the fungal flora in the domestic ambient air of the relevant patients. In this context, in order to prevent bacterial growth, Streptomycin antibiotic was added and Rose Bengali potato dextrose agar was placed in the slot of the device and the air filter of the device was installed. The device, which was placed at a height of 75-85 cm from the ground, was operated for 3-5 minutes and 200 liters of domestic ambient air was vacuumed. A total of 1071 microfungi colonies isolated in the study were found to belong to a total of 10 genuses and 23 species. The obtained genera are Alternaria (Arıküfü), Aspergillus (Asper), Aureobasidium (Karamaya), Chaetomium (Günoku), Cladosporium (Havaküfü), Fusarium (Solduran), Mucor (Ekmekküfü), Paecilomyces (Günküfü), Penicillium (Penisilyum) and Rhizopus (Karaküf). Among them, the most isolated genera were Aspergillus, Penicillium, Cladosporium, Alternaria and Fusarium. The most isolated species in this study were Aspergillus niger (Kara asper), Fusarium poae (Buğday solduran), Alternaria alternata (Astımküfü), Cladosporium cladosporioides (Karakökküfü), Penicillium brevicompactum (Sağlam penisilyum), Cladosporium macrocarpum (İrikurutan), Cladosporium sphaerospermum (Güllekurutan) and Penicillium glabrum (Bol penisilyum). In the 12-month period, the lowest microfungi concentration was observed in January and the highest microfungi concentration was observed in May. During the study, the temperature of the sample areas were measured with a thermometer and the humidity rates were measured with a hydrometer. In this study, the types of allergen microfungus that cause the onset of asthma disease or the progression of the degree of disease are stated. These were determined as Alternaria alternariae (Fıstık küfü), Alternaria alternata, Aspergillus fumigatus (Kıran asper), Aspergillus niger, Aureobasidium pullulans (Karamaya), Chaetomium globosum (Top günoku), Cladosporium cladosporioides, Cladosporium herbarum (Yaygınkurutan), Cladosporium sphaerospermum, Penicillium chrysogenum (Penisilyum), Penicillium glabrum.

Keywords: İstanbul, Asthma, Indoor air, Microfungi

# Astım Hastalarının Yaşadıkları Ortamlardaki Mikobiyotanın Astım Hastalığıyla İlişkisinin Belirlenmesi

Öz: Şubat 2014-Ocak 2015 tarihleri arasındaki 12 aylık zaman periyodunda, İstanbul'un 14 farklı bölgesinde (Ataşehir, Bulgurlu, Fikirtepe, Hasanpaşa, İçerenköy, Moda, Göztepe, Çekmeköy, Ümraniye, Altayçeşme Mahallesi, Esenkent Mahallesi, Feyzullah Mahallesi, Gülsuyu Mahallesi, Yalı Mahallesi) yaşayan 55 astım hastasının ev ortamlarında bu çalışma gerçekleştirilmiştir. İlgili hastaların ev içi hava ortamında bulunan fungal floranın belirlenmesi amacıyla Air Ideal (Biomerieux, France) hava vakumlama cihazı kullanılmıştır. Bu bağlamda bakteriyel üremeyi önlemek amacıyla Streptomisin antibiyotiği eklenmiş Rose Bengalli patates dekstroz agarlar cihazın yuvasına yerleştirilmiş ve cihazın hava filtresi takılmıştır. Yerden 75-85 cm yüksekliğe konulan cihaz 3-5 dakika çalıştırılarak 200 litre ev içi ortam havası vakumlanmıştır. Arastırmada izole edilen toplam 1071 mikrofungus kolonisinin toplam 10 genus ve 23 türe ait olduğu saptanmıştır. Elde edilen cinsler Alternaria, Aspergillus, Aureobasidium, Chaetomium, Cladosporium, Fusarium, Mucor, Paecilomyces, Penicillium ve Rhizopus'tur. Bunların içerisinde en fazla izole edilen cinsler; Aspergillus, Penicillium, Cladosporium, Alternaria ve Fusarium olmuştur. Bu çalışmada en fazla izole edilen türler; Aspergillus niger, Fusarium poae, Alternaria Cladosporium cladosporioides, Penicillium brevicompactum, alternata. Cladosporium macrocarpum, Cladosporium sphaerospermum ve Penicillium glabrum olmuştur. 12 aylık zaman periyodunda en az mikrofungus konsantrasyonu Ocak ayında, en fazla mikrofungus konsantrasyonu ise Mayıs ayında görülmüştür. Çalışma süresince örneklem alanlarının termometre ile sıcaklığı, higrometre ile nem oranlarının ölcümleri gerçeklestirilmiştir. Bu çalışmada astım hastalığının başlamasına ya da hastalık derecesinin ilerlemesine neden olan allerjen mikrofungus türleri belirtilmiştir. Bunlar; Alternaria alternariae, Alternaria alternata, Aspergillus fumigatus, Aspergillus niger, Aureobasidium pullulans, Chaetomium globosum, Cladosporium cladosporioides, Cladosporium herbarum, Cladosporium sphaerospermum, Penicillium chrysogenum, Penicillium glabrum olarak tespit edilmiştir.

Anahtar kelimeler: İstanbul, Astım, İç ortam havası, Mikrofungus

## Introduction

The discovery of the existence of microfungi began after the invention of the simple microscope by Anton van Leeuwenhoek and Robert Hooke in the seventeenth century, which primarily relied on the system of lenses. Especially due to the fact that it causes various diseases, the importance given to microfungi has increased, and studies have been started on microfungi in order to diagnose the microfungi infections that cause these diseases and to find a treatment for these infections (Tümbay, 1983). Like all groups of microorganisms, microfungi are present in excess amounts in the air. Microorganisms in the air continue their vital activities on water, soil, plants, animals and humans that provide optimum conditions for their lives. Microfungi are present everywhere on our planet. The increase in the density and number of microfungi depends on basic factors such as high-water activity, high temperature, high carbon dioxide rate (Çolakoğlu, 1996).

Since there are fungal elements that cause various health problems, it is important to detect and diagnose them correctly. Since fungi have very different characteristics, they have morphological and microscopic differences. While the basic criteria in this distinction are counted as macroscopic features such as colony shape and size of fungi, colony colors, observation of exudation on the colony surface, full adhesion of colonies to the agar surface and easy separation from the agar surface in powder consistency, the sports shapes, sizes, surface shapes of fungi being different, the shape and size of phialides and conids being different, and the morphologies of hyphae being different can be counted as microscopic features. All these different macroscopic and microscopic characteristics are due to the difference in the genetic heritage of the fungi. The reasons for the difference can be the environmental conditions, especially the humidity and nutrient status of the environment, and the age status of the culture, apart from the genetic characteristics. So much so that differences are observed between young and old colonies of the same fungus species. Since the characteristics of phialide, conidi and hyphae in different regions of the same colonies may vary, all characteristics should be examined in detail in the differentiation of fungi (Arda. 2000).

It has been observed that the molds in the indoor air of the houses and their fungal spores cause the onset and progression of respiratory system diseases, especially asthma and rhinitis (Miller, 1992). Fungal pathogens in the air that cause respiratory system diseases are also known to cause allergic reactions (Verhoeff and Burge, 1997). *Alternaria, Cladosporium* and *Penicillium* mold were the most isolated pathogen types in the ambient air of the houses where asthma patients live (Şen and Asan, 2001). Species belonging to important genera such as *Aspergillus, Aureobasidium, Paecilomyces, Rhizopus* and *Ulocladium* were also detected in the hospital ambient air (Çolakoğlu and Karaltı, 2011).

This study aims to determine the species of microfungi that make up the fungal flora in the homes of asthma patients living in certain regions of Istanbul and to determine the types of mold that cause or affect the course of the disease and to reveal the relationship between the species isolated in this manner and the disease. Therefore, between February 2014 and January 2015, samples were taken via air sampler from the habitats of 55 asthma patients in 14 different regions of Istanbul for 1 year and isolated species were identified and asthma-related species were specified.

#### **Material and Metod**

Samples were taken from the houses of asthma patients living in the regions specified in Table 1 within the borders of Istanbul province between February 2014 and January 2015 and in the number of asthma patients specified in these regions.

Samples were taken from specific areas of the homes of asthma patients in specified areas each month for one year. Sampling was performed with the Air Ideal (Biomerieux, France) device. The specified device was operated at a height of 75-85 cm from the ground for 3-5 minutes and samples were taken by vacuuming 200 liters of air into the medium. This procedure was applied for a year, in 14 different regions of Istanbul, where 55 asthma patients lived. Rose Bengali Peptone Dextrose Agar was used as the main culture medium in which the samples were taken. 30 mg/l streptomycin was added while preparing cultures to prevent bacterial growth (Salo et al., 2008).

Petri plates, containing Peptone Dextrose Agar with Rose Bengal and streptomycin, used for isolation were kept in the laboratory at room temperature (20-26 °C) for 7 days for incubation. Fungus growths were examined during this period. Each fungus colony where reproduction occurred were passaged to Potato Dextrose Agar (PDA), Malt Extract Agar (MEA) and Czapek's Agar (CZ) culture media. These Petri plates were also incubated at room temperature (20-26 °C) for 7-10 days. Pure cultures of microfungi were obtained as a result of incubation. In this step, all colony macromorphologies such as colony shapes, sizes, structures, diameters and sizes, exudation and pigmentation states were examined and noted from the surface and reverse region of the colonies (Yoltaş et al., 2010).

Preparates were prepared for each fungus colony obtained purely for the purpose of genus and species diagnosis of microfungi. For the microscopic examination of the preparations, a cotton-blue lactophenol solution, which is functional in genus diagnoses and stains the fungus cell walls, and a yellow-colored picric acid-dyed lactophenol solution, which is functional in fungus species diagnoses, were used. A drop of these solutions was dripped on the microscope slide and the micelle and fructification organs of the microfungus were transferred from the previously prepared pure fungus culture to the area where the solution drops were located on the slide with the help of an extract sterilized by passing through the flame and closed with a lamella. Then, the preparate was covered with a colorless-transparent nail polish to prevent contact with air and causing spoilage.

Each of the preparates prepared from the pure cultures of the microfungi was examined separately with an optical microscope. The organs of the microfungi such as hyph, conidiophore, conidi, phialid, etc. were measured 50 times and averaged. The diagnosis of microfungi was carried out using domestic and foreign sources. In order to perform the measurements in the study, an ocular micrometer was placed in the microscope eyepieces.

In order to compare the isolated fungus concentration with the temperature and humidity values criteria, temperature and humidity values were measured with a thermometer and hygrometer in the homes of asthma patients in the sampled regions during the study. These values are given in Table 2.

Within the scope of micro and macromorphological genus identifications Barnett and Hunter (1999) was used as the diagnostic key and as the diagnostic key for species identifications, the relevant literature sources (Ellis, 1965; Klich, 2002; Pitt, 1979; Pitt and Hocking, 2009; Samson et al., 2004) were used. Molecular diagnostic studies of microfungi for gene regions such as its, Beta-Tubulin and Actin also support our study results according to traditional culture and morphological characteristics (Asan et al., 2018).

	NAME OF THE DISTRICT	of regions sampled and numb	NUMBER OF PATIENTS
		NEIGHBORHOOD	
1	Ataşehir	Atatürk Neighborhood	1
2	Çekmeköy	Merkez Neighborhood	4
3	Kadıköy	Fikirtepe Neighborhood	3
4	Kadıköy	Göztepe Neighborhood	5
5	Kadıköy	Hasanpaşa Neighborhood	4
6	Kadıköy	İçerenköy Neighborhood	1
7	Kadıköy	Moda Neighborhood	6
8	Maltepe	Altayçeşme Neighborhood	5
9	Maltepe	Esenkent Neighborhood	1
10	Maltepe	Feyzullah Neighborhood	5
11	Maltepe	Gülsuyu Neighborhood	9
12	Maltepe	Yalı Neighborhood	6
13	Ümraniye	Atakent Neighborhood	4
14	Üsküdar	Bulgurlu Neighborhood	1
	Total		55

Table 1. Names of regions sampled and number of patients
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Table 2. Temperature and humidity values measured in the homes of asthma patients (February 2014-January 2015)
Months

	Months											
Name of the	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan
District-Region												
Ataşehir-Atatürk	14°C	17°C	17°C	19°C	22°C	26°C	27°C	22°C	15°C	16°C	14°C	15°C
Neighborhood	53%	51%	45%	79%	70%	78%	69%	74%	91%	89%	57%	48%
Ataşehir-	17°C	20°C	19°C	20°C	20°C	26°C	25°C	24°C	22°C	19°C	18°C	16°C
İçerenköy	42%	54%	45%	78%	70%	73%	64%	69%	80%	74%	61%	40%
Neighborhood												
Çekmeköy-	16°C	14°C	15°C	22°C	21°C	28°C	26°C	24°C	16°C	17°C	17°C	12°C
Merkez	51%	53%	46%	78%	68%	73%	69%	74%	88%	78%	61%	44%
Neighborhood												
Kadıköy-	15°C	18°C	18°C	20°C	23°C	25°C	26°C	24°C	16°C	17°C	15°C	13°C
Fikirtepe	54%	55%	46%	79%	72%	76%	69%	75%	90%	89%	58%	40%
Neighborhood												
Kadıköy-	14°C	17°C	17°C	19°C	22°C	26°C	27°C	23°C	15°C	16°C	14°C	15°C
Göztepe	53%	51%	44%	78%	70%	77%	68%	74%	92%	90%	56%	47%
Neighborhood												
Kadıköy-	16°C	18°C	19°C	20°C	23°C	24°C	27°C	24°C	18°C	18°C	14°C	11 °C
Hasanpaşa	50%	51%	46%	80%	68%	78%	70%	72%	93%	90%	55%	40%
Neighborhood												
Kadıköy-Moda	16°C	17°C	17°C	19°C	22°C	26°C	27°C	24°C	15°C	16°C	14°C	14°C
District	53%	55%	44%	80%	67%	73%	65%	75%	92%	90%	57%	42%
Maltepe-	17°C	20°C	19°C	20°C	20°C	25°C	24°C	24°C	22°C	19°C	18°C	18°C
Altayçeşme	42%	53%	44%	79%	71%	73%	64%	69%	80%	73%	60%	40%
Neighborhood												
Maltepe-	16°C	14°C	15°C	22°C	21°C	28°C	26°C	24°C	16°C	17°C	17°C	14°C
Esenkent	51%	53%	44%	77%	69%	72%	68%	74%	87%	79%	60%	44%
Neighborhood												
Maltepe-	15°C	15°C	16°C	20°C	21°C	27°C	26°C	25°C	17°C	17°C	19°C	17°C
Feyzullah	49%	53%	47%	80%	72%	70%	68%	72%	86%	80%	63%	40%
Neighborhood												
Maltepe-	9°C	11 °C	13°C	20°C	20°C	26°C	24°C	21°C	14°C	15°C	9°C	4°C
Gülsuyu	52%	58%	40%	82%	73%	78%	66%	73%	85%	74%	59%	43%
Neighborhood												
Maltepe-Yalı	17°C	17°C	19°C	19°C	22°C	24°C	25°C	24°C	17°C	16°C	15°C	15°C
Neighborhood	49%	54%	43%	82%	73%	77%	66%	73%	91%	90%	56%	40%
Ümraniye-	16°C	18°C	19°C	20°C	23°C	25°C	27°C	24°C	17°C	18°C	16°C	13°C
Atakent	49%	51%	45%	80%	69%	77%	69%	75%	90%	88%	56%	41%
Neighborhood												
Üsküdar-	15°C	18°C	18°C	21°C	24°C	26°C	28°C	24°C	17°C	18°C	15°C	12°C
Bulgurlu	55%	57%	48%	80%	72%	77%	70%	75%	88%	85%	58%	39%
Neighborhood												

#### Results

From the aerial samples taken from the houses of 55 asthma patients living in 14 different regions of Istanbul, 23 species of 10 genera were isolated and a total of 1071 colonies were examined. The most isolated microfungus genus was *Aspergillus* with 23.34%, followed by Penicillium with 22.53%, Cladosporium with 21.48%, Alternaria with 11.57%, Fusarium with 8.87%,

Aureobasidium with 4.38%, Mucor with 4.11%, Rhizopus with 2.42%, Paecilomyces with 1.21%, and Chaetomium with 0.09% (Table 3).

The Turkish nomenclature of the genus and species of microfungi isolated within the scope of thestudy was made according to the Turkish Fungus List (Sesli et al., 2020) (Table 3-4).

Table 3. Colon	v count and percentag	e ratios of isolated r	nicrofungus genuses

Name of the Genus	Number of Colonies	Percentage of the Colonies
Alternaria (Arıküfü)	124	11,57
Aspergillus (Asper)	250	23,34
Aureobasidium (Karamaya)	47	4,38
Chaetomium (Günoku)	1	0,09
Cladosporium (Havaküfü)	230	21,48
Fusarium (Solduran)	95	8,87
<i>Mucor</i> (Ekmekküfü)	44	4,11
Paecilomyces (Günküfü)	13	1,21
Penicillium (Penisilyum)	241	22,53
Rhizopus (Karaküf)	26	2,42
Total	1071	100

The percentages of species isolated throughout the study were Aspergillus niger with 15.96%, Fusarium poae with 8.87%, Alternaria alternata with 7.93%, Penicillium brevicompactum with 7.47%, Cladosporium cladosporioides with 7.47%, Penicillium glabrum with 6.54%, Cladosporium sphaerospermum with 6.54%, Cladosporium macrocarpum with 6.54%, Aspergillus fumigatus with 4.58%, Aureobasidum pullulans with 4.38%, Penicillium citrinum (Limon penisilyum) with 2.80%, Aspergillus acidus (Ekşi asper) with 2.80%, Mucor

circinelloides (Halkaküf) with 2.71%, Rhizopus microsporus (Küçükküf) with 2.42%, Penicillium chrysogenum with 2.36%, Alternaria alternariae with 2.24%, Penicillium commune (Zonlu penisilyum) with 2.24%, Mucor racemosus (Salkımküf) with 1.40%, tenuissima (Narinküf) 1.40%. Alternaria with Paecilomyces variotii (El günküfü) with 1.21% Penicillium digitatum (Yeşil penisilyum) with 1.12%, Cladosporium herbarum with 0.93%, Chaetomium globosum with 0.09% (Table 4).

Table 4. Colon	v number and	percentage i	rates of isolated	microfungus species

Name of the Species	Number of	Percentage of the
	Colonies	Colony
Alternaria alternariae (Fıstık küfü)	24	2,24
Alternaria alternata (Astımküfü)-Pathogen	85	7,93
Alternaria tenuissima (Narinküf)	15	1,40
Aspergillus acidus (Ekşi asper)	30	2,80
Aspergillus fumigatus (Kıran asper)-Pathogen	49	4,58
Aspergillus niger (Kara asper)-Pathogen	171	15,96
Aureobasidium pullulans (Karamaya)-Pathogen	47	4,38
Chaetomium globosum (Top günoku)-Pathogen	1	0,09
Cladosporium cladosporioides (Karakökküfü)-Pathogen	80	7,47
Cladosporium herbarum (Yaygınkurutan)-Pathogen	10	0,93
Cladosporium macrocarpum (İrikurutan)	70	6,54
Cladosporium sphaerospermum (Güllekurutan)-Pathogen	70	6,54
Fusarium poae (Buğday solduran)	95	8,87
Mucor circinelloides (Halkaküf)	29	2,71
Mucor racemosus (Salkımküf)	15	1,40
Paecilomyces variotii (El günküfü)	13	1,21
Penicillium brevicompactum (Sağlam penisilyum)	80	7,47
Penicillium chrysogenum (Penisilyum)-Pathogen	25	2,36
Penicillium citrinum (Limon penisilyum)	30	2,80
Penicillium commune (Zonlu penisilyum)	24	2,24
Penicillium digitatum (Yeşil penisilyum)	12	1,12
Penicillium glabrum (Bol penisilyum)-Pathogen	70	6,54
Rhizopus microsporus (Küçükküf)	26	2,42
Total	1071	100

Within the scope of the study, the most microfungus isolation by months was realized in May with a maximum rate of 19.61%. This was followed by June with a rate of 14.00%, November with a rate of 10.74%, October with a rate of 9.15%, July with a rate of 7.47%,

April with a rate of 7.00%, February with a rate of 6.54%, March with a rate of 6.07%, August with a rate of 5.60%, September with a rate of 4.95%, and December with a rate of 4.67%. The least microfungal isolation occurred in January with a rate of 4.20% (Table 5).

Table 5. Distribution of isolated microfungi colonies by months and percentage ratios
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Months	Number of Colonies	Percentage of the Colonies		
February	70	6,54		
March	65	6,07		
April	75	7,00		
May	210	19,61		
June	150	14,00		
July	80	7,47		
August	60	5,60		
September	53	4,95		
October	98	9,15		
November	115	10,74		
December	50	4,67		
January	45	4,20		
Total	1071	100		

Within the scope our study, if we specify the relationship between the measurements of temperature and humidity values that vary according to the seasons and isolated allergen-pathogen microfungus species according to the spring, summer, autumn and winter seasons, respectively; Alternaria alternata species at 27,06%, 29,41%, 34,12% and 9,41% ratios, Aspergillus fumigatus species at 26,53%, 30,61%, 28,57% and 14,29% ratios, Aspergillus niger species at 19,88%, 41,52%, 13,45% and 25,15% ratios, Aureobasidium pullulans species at 2,13%, 29,79%, 53,19% and 14,89% ratios, Chaetomium globosum species at 100%, 0,00%, 0,00% and 0,00% ratios, Cladosporium cladosporioides species at 42,50%, 12,50%, 40,00% and 5,00% ratios, Cladosporium herbarum species at 66,67%, 0,00%, 22.22% and 11,11% ratios. Cladosporium sphaerospermum species at 45,72%, 35,71%, 2,86% and 15,71% ratios, Penicillium chrysogenum species at 48,00%, 36,00%, 16,00% and 0,00% ratios and Penicillium glabrum species were isolated at 41,43%, 42,86%, 14,29% and 1,42% ratios

#### Discussions

Microfungi, whose existence was detected after the discovery of the microscope, continue their vital activities all over the world. Microfungi are known to spread the most through the air. Due to the increasing number of diseases and infections caused by microfungi, there is a lot of scientific research and studies on microfungi that spread with air.

In studies on the subject in the literature, it has been stated that the microfungus concentration in the environment is directly related to the temperature and humidity values, so that the mould density is high in seasons with high temperature and humidity values, on the contrary, the mould density is low in seasons with low air temperature and humidity values (Çolakoğlu and Karaltı, 2011). The relevant situation was revealed within the scope of the study (Table 2,5).

Depending on the temperature and humidity values, microfunguses belonging to the *Alternaria* genus were isolated mostly in the spring and autumn period in March, April, May, September, October and November, while microfunguses belonging to the *Cladosporium* genus were isolated in February, March, April, May and November, and microfunguses belonging to the *Aspergillus* and *Penicillium* genuses were isolated much more in the spring and autumn seasons throughout the year (Karaltı and Çolakoğlu, 2012). The results obtained in our study are in line with this condition.

Alternaria, Aspergillus, Cladosporium, Fusarium, Mucor, Penicillium, Rhizopus have been reported to be the main microfungi that cause respiratory system infections in humans, especially asthma (Zock et al., 2002). The increase or decrease in mold flora in the air environment in the house directly affects the course of lower respiratory tract diseases, especially asthma (Ünlü et al., 2003). Even in immunocompetent patients, too much exposure to an allergen-pathogenic microfungus species such as Aspergillus fumigatus can reveal the clinical picture of pulmonary aspergillosis (Huseynov et al., 2020). Within the scope of our study, the genus and species of microfungus isolated from the air throughout the year were specified, and pathogenic mold life forms that cause respiratory diseases such as allergies and asthma; Alternaria alternata, Aspergillus fumigatus, Aspergillus niger, Aureobasidium pullulans, Chaetomium globosum, Cladosporium cladosporioides, Cladosporium herbarum, Cladosporium sphaerospermum, Penicillium chrysogenum and Penicillium glabrum were reported in the light of current scientific data (Table 4).

## **Author Contributions**

All authors contributed equally to this work.

#### **Conflict of Interests**

There is no conflict of interest with any person, institution or organization.

Ethical Statement: It is declared that scientific and ethical principles have been followed while carrying out

and writing this study and that all the sources used have been properly cited (Aras Fahrettin KORKMAZ, Günay Tülay ÇOLAKOĞLU, İskender KARALTI).

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