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Fungal colonization of air-conditioning systems and indoor cultivated plants and its relation to human health

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Fungi have been implicated as quantitatively the most important bio-aerosol component of indoor air associated with contaminated air-conditioning systems and soil of indoor cultivated plants. The objectives of this study were not only to assess the level of fungal contamination in the filter dust of air conditioning systems and soil of indoor cultivated potted plants present inside homes, offices and hospitals for one year, but also fungal identification and examination of their potentiality to produce extracellular hydrolytic enzymes. A total of 5740 fungal colony-forming units (CFU) were collected belonging to 57 fungal species. The predominant molds isolated were Aspergillus spp., Penicillium spp., Cladosporium spp. and Fusarium spp. Enzymatic activity test of the isolated fungi revealed that many isolates showed cellulolytic and keratinolytic activity. In addition, some isolates showed lipolytic, proteolytic and hemolytic activity and could grow at 37oC, which indicate their pathogenic potentiality as human opportunistic pathogens. The results of this surveillance study indicated that in the case of CF, the abundance of fungal colonies was much higher in homes than offices and hospitals while in case of CP, it was much higher in hospitals followed by offices and homes. It is important to stress that fungal colonization of air-conditioning systems and soil of potted plants should not be ignored and to educate homes, offices and hospitals about the need of routine cleaning and disinfection of gadgets like air-conditioning systems and soil of cultivated plants for minimizing the chances of proliferation and dispersal of potentially pathogenic fung.

Key words: Contamination, degrading enzymes, diseases, filter dust, genus diversity, micromycetes, soil.

INTRODUCTION

Contemporary lifestyles dictate that people spend between 60 and 90% of their daily lives indoors. For those living in warm climates, air conditioning is thus considered a necessity. Air conditioners function by removing hot and humid air from the building interior and replacing it with cooler air. Microorganisms are

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considered among the most important sources of poor quality of indoor air, and contamination of this air by microbial pollutants is being increasingly recognized as a public health problem and a probable cause of the so-called sick building syndrome. However, recent research has demonstrated that certain microorganisms can colonize panel filter surfaces, particularly fungi can colonize the materials used in heating, ventilation, and air-conditioning systems (Jaakkola et al., 1991; Božić et al., 2019; Al-abdalall et al., 2019).

Indoor environment affects health and indoor air quality (IAQ) is an important issue for occupational and public health. Microbial incidences and the concentrations of fungi are usually higher indoors than outdoors (Božić et al., 2019). IAQ is important in all buildings, especially in hospitals. A wide range of factors affect IAQ; the quality of the outdoor air, building construction and materials (McCunney, 1987), heating, ventilating and air conditioning systems, temperature, humidity, contaminant sources, occupants and possible pollutant pathways are the basic factors that influence indoor air quality (Jaakkola et al., 1991).

Many studies have focused on the sources of fungal contamination in indoor spaces. Pathogenic fungi have been detected in the potting mix of indoor plants; however, it is unclear if plants in indoor work spaces make qualitative or quantitative contributions to the aeromycota within buildings (Torpy et al., 2013). Since soil is one of the most important biotopes for fungi, relatively high concentrations of fungal propagules are to be expected (Haas et al., 2016). Indoor plants could act as a significant source of pathogenic fungal inocula. Relative humidity of indoor conditions is thought to be the leading cause of fungal amplification (Adan and Samson, 2011). Indoor air may vary in humidity due to numerous factors such as seasonal variability and building design, while indoor plants tend to require watering and contain dead organic matter in the potting mix (Torpy et al., 2013).

Fungi are known to elaborate extracellular enzymes based on the substrate they utilize for growth. Cellulases are a group of hydrolytic enzymes, which are capable of degrading cellulose to smaller glucose units. These enzymes are produced mainly by fungi (Hussain et al., 2012; Parveen et al., 2017). In addition, fungi are capable of producing lipase, a principal enzyme involved in the hydrolysis of lipids to free fatty acids and glycerol (El-Diasty and Salem, 2007; Negedu et al., 2012). Keratins are insoluble proteins found in wool, hooves, scales, hair and nails. Due to the strength and stability of keratin, very few organisms can break it down and utilize it. Some fungal strains can produce keratin proteases, which have keratinolytic activity (Ramakrishnaiah et al., 2013; Kumawat et al., 2013). Hemolytic activity of many fungi was previously reported by Taira et al. (2011) and Aboul-Nasr et al. (2013).

Production and secretion of hydrolytic enzymes are very important virulence factors. These enzymes play a role in nutrition, tissue damage, fungal dissemination within the human body. Thus, they affect fungal pathogenicity. Also, these enzymes could enable tissue invasion easier by impairing some mechanisms of the immune system and causing various injuries to the host (Hass et al., 2016; Al-abdalall et al., 2019; Golofit-Szymczak et al., 2019).

The aim of this study was not only to assess the level of fungal contamination in the filter dust of air conditioning systems and soil of indoor cultivated potted plants present inside homes, offices and hospitals for one year but also fungal identification and examination of their potentiality to produce extracellular hydrolytic enzymes, which are important virulence factors involved in fungal pathogenicity and influence people health.

MATERIALS AND METHODS

Samples collection

Thirty-six dust samples were collected from different air-conditioning filters (CF) from homes, lecturer's offices of Ain Shams University and hospitals at Shoubra, Cairo, Egypt for one year (one sample per month from each site). None of the analyzed filters from these sites had been removed or cleaned for at least one year (Figure 1). Sampling was carried out by removing the filter and collecting its dust. Dust was sampled via manual wiping according to ACV hygienic specification (MOH, 2012; Liu et al., 2021). For each filter, sampling included three sampling points (that is, top surface, bottom surface and side surface) and the area of each sampling point was 100 cm^2 . The manual wiping was conducted with the use of non-woven fabric and dust sampling frame to wipe all dust accumulated at sampling points. A specific procedure was to wear disposable plastic gloves, take 100 mm × 100 mm non-woven and presterilized fabric by tweezers to collect dust. Afterwards, dust samples were sealed in a sterile wild-mouth bottle (Zhou and Gao, 2000; Xu, 2013) and stored at room temperature in dark. Meanwhile, other 36 samples from the soil of different indoor potted cultivated plants (CP) (one sample per month from each site) were collected from the same sites. The samples from potted plants were collected manually from the surface to 2 to 5 cm below the soil surface. Sampling was conducted at monthly intervals from April 2017- March 2018.

Isolation and identification of fungal isolates from CF and CP

For detection of fungi, samples of air-conditioning filters dust and soils from potted plants were suspended and plated onto several culture media. Culturable fungal spores are presented in terms of CFU/g of the dust of air-conditioning filter (CF) and soil of potted cultivated plants (CP). Sub-samples (0.5 g) were taken from each dust sample and suspended in distilled water (0.0425 g/l KH_2PO_4, 0.25 g/L $MgSO_4$, 0.008 g/L NaOH, 0.02% Tween 80 detergent). Dilution series were prepared and three successive dilutions were plated in triplicate according to Pasanen et al. (1997) with some modification using the following media instead of malt agar medium:(i) Sabouraud's dextrose agar (SDA) (20 g dextrose, 10 g peptone, 5 g yeast extract and 20 g agar in 1 Lwater); (ii) potato dextrose agar (PDA) (10 g dextrose, 200 g sliced potato and 15 g

Figure 1. Photograph showing a grossly contaminated filter of the window mounted air-conditioning unit.
Source: Author

agar in 1L water) and (iii) Czapek's agar (10 g dextrose, 3 g sodium nitrate, 1 g KH_2PO_4, 1 g KCl_2, 0.5 g $MgSO_4$, 0.01 g ferrous and ferric sulphate, 20 g agar in 1 L water) with the antibiotic streptomycin to prevent bacterial growth. On the other hand, mold fungi of potted plants soils were estimated using the soil dilution plate method (Johnson and Curl, 1972). The plates were incubated at 28 ± 2°C and observed after 5 to 7 days. Fungal colonies formed on the medium were identified based on both cultural and microscopic characteristics of each isolated colony using various identification keys (Ainsworth et al., 1973; Arx, 1981; Ellis and Ellis, 1985; Pitt, 1979; Samson et al., 2006; Booth, 1971; Carmichael et al., 1980).

Colony counting and microbiological studies

Isolated fungi from different isolation sites were encountered during the four seasons: fall (from October to December), winter (from January to March), spring (from April to June) and summer (from July to September). The following microbiological parameters were estimated: (i) species count, (ii) species and genus richness = the number of species and genera recorded and (iii) species dominance = percentage of each species about the total count of all species. The objective of this methodological stage is to give an idea about fungal species and genus richness and diversity of CF and CP in all isolation sites throughout the different seasons.

Screening of fungal isolates for extracellular enzymes production

From the various isolates, screening for cellulolytic fungi was made using PDA medium supplemented with 5% carboxymethyl cellulose (CMC). Cellulolytic fungi create a clearing zone around the colony on the agar (Gautam et al., 2010). Keratinolytic activity was tested by culturing the isolated fungi on keratin agar medium (gm/250 ml) containing keratin - 2.5, $MgSO_4$ - 0.25, KH_2PO_4 - 0.115, K_2HPO_4 - 0.25 and Agar- 5. Streptomycin 1% was mixed with the medium. Plates were incubated at 28ºC for 5 days. Keratinolytic activity was detected as a clear zone around the colony (Mini et al., 2012).

Protease activity was determined using a casein hydrolysis medium in which skim milk gives an opaque final appearance and hydrolysis of the casein resulted in a clear zone around the fungal colony (Paterson and Bridge, 1994). Lipase activity was measured using the method of Ullmann and Blasius (1974) with some modification using Tween 80 instead of Tween 20. The lipolytic producing ability was observed as a visible precipitate due to the formation of crystals of calcium salt of the oleic acid liberated by the enzyme. Hemolytic activity of fungal isolates was measured using human blood agar medium (Ronald, 2000).

Statistical analysis

Data collected were subjected to one-way ANOVA using the statistical analysis software Minitab V19 of least significant difference test (LSD) at 5% probability level was used to compare the difference among the treatment means. Data are mean of three replicates.

RESULTS AND DISCUSSION

Isolation, identification and microbiological analysis of fungal isolates

The total count of fungal isolates of the investigated air-conditioning systems filter dust (CF) and indoor soil of cultivated plants (CP) from homes, offices and hospitals during the year of isolation was 5740 colony forming units (CFU) (Table 1). Regarding the isolation source, the higher total CFU count from all isolation sites was detected in CP while that of CF was lower. It has been proposed that indoor plants could act as a significant source of fungal inocula. As most fungi require moisture and are saprophytic, and indoor plants tend to require watering and contain dead organic matter in the potting mix, as a result, it harbors large numbers of spores from fungal taxa to the indoor environment (Torpy et al., 2013). Also, indoor relative humidity (RH) levels above 80% are thought to be the leading cause of fungal amplification (Adan and Samson, 2011) and the relative humidity levels around the air-conditioning filters are not always more than 80%, which depends on many factors such as

Table 1. Mean total count in CFU of fungi isolated from the dust of indoor conditioning systems filter (CF) and soil of indoor cultivated plants (CP) from different isolation sites during a year

Isolation site	Isolation source	
	CF	CP
Homes	1278±70.7[a]	750±14.1[c]
Offices	832±7.07[b]	853±4.24[b]
Hospitals	685±2.83[c]	1342±3.54[a]
Significance Level	*	*
LSD (0.05)	41.06	8.7

Values followed by the same letter within the column do not differ statistically (P> 0.05). * = Significant (P≤ 0.05).
Source: Author

environmental conditions (Ljaljevic et al., 2008).

Concerning various isolation sites, in the case of CF, results indicated that the abundance of fungal colonies was much higher in homes (46%) than offices and hospitals (30 and 24%, respectively). In this study, none of the investigated sites was following a maintenance program for the air-conditioning (AC) units. The abundance of fungal species isolated from different air-conditioning systems from different isolation sites can be attributed to (i) different ways of maintaining the systems themselves. Unfortunately, bad maintenance of AC systems or their low efficiency can often lead to unintentional contamination of indoor spaces (Gołofit-Szymczak and Górny, 2010). Window mounted AC unit draws in atmospheric air from an air vent and the chances of filters acquiring a higher volume of dust and fungal spores from the atmosphere are therefore high and variable. The filters utilized in these units if left unattended can act as a suitable nidus for the growth and proliferation of fungi (Kelkar and Kulkarni, 2011). Also, the study of Kalwasińska et al. (2012) emphasizes the fact that rooms with efficient ventilation or air-conditioning systems and guaranteed air-tightness are less contaminated than rooms where air-conditioning was not installed. However, (ii) the differences in relative humidity (RH) levels around the air-conditioning filters depend on many factors such as environmental conditions (Ljaljevic et al., 2008). Moreover, (iii) ventilation air-conditioning systems moderate heat and moisture in buildings produce environmental conditions such that indoor RH is generally different (between 60 and 80%) (Torpy et al., 2013). Finally, (iv) it was reported that air-conditioning systems are highly linked with fungal pollution of indoor air, and the infiltration of outdoor air into the building envelope air through its filters can be the major mechanism responsible for fungal contamination (Gołofit-Szymczak and Górny, 2010).

On the other hand, results of the fungal concentration of CP in various isolation sites indicated that the abundance of fungal colonies was much higher in hospitals (46%) followed by offices and homes (29 and 25%, respectively). Generally, the highest CFU total count from CF and CP was detected in homes and hospitals which was near equals, while that of offices was lower (Table 1). The high fungal concentration of homes can be discussed as homes contain the toilet and kitchen. The toilet contains the toilet-bowl, washbasin and humidifier and apart from the people who produce large amounts of microorganisms in the air. However, in the kitchen, there are tiny particles that may form a suspension in bio aerosols are released into the air during food preparation. On the other hand, hospitals are characterized by a large circulation of people and many visitors who discuss the appearance of a new significant microbiological contamination source. Finally, a strong relationship between occupant density, human activity and microorganisms concentration in the indoor air was previously reported (Fleischer et al., 2006; Stryjakowska-Sekulska et al., 2007). It was also reported that the fungal spectrum in potted soils may also be affected by factors such as the cultivated plant, substrate, ambient temperatures, or watering habits (Haas et al., 2016).

Generally, the variability of total fungal concentration in CF and CP and in various isolation sites may be because indoor air may vary in humidity due to numerous physical, chemical and biological factors, microbial pollutants reservoirs (people, plants, animals, to some extent soil and water as well as human-made materials), seasonal variability and building design (Skowroń et al., 2004; Torpy et al., 2013).

Concerning the diversity and concentration of fungal isolates, results indicated that 57 species belonging to 20 genera were obtained (Table 2). Quality characteristics of fungal flora isolated from CF and CP showed dominating contributions of the genera: *Aspergillus* (37.3%), *Penicillium* (9.3%), *Cladosporium* (7.2%), *Fusarium* (5.9%) and *Scopulariopsis* (5.5%) in which *A. flavus, P. spinulosum, C. herbarum, F. solani* and *S. brevicaulis* were the most dominant species of these genera. In terms of number of species isolated (species richness),

Table 2. Fungal genera, total count (CFU), species count and most dominant species isolated from CF and CP from different isolation sites

Fungal genus	Most dominant species	No. of species	Total count (CFU)	Abundance (%)
Aspergillus	*A. flavus*	17	2145±7.07[a]	37.3
Penicillium	*P. spinulosum*	6	537±9.8[b]	9.3
Cladosporium	*C. herbarum*	3	417±4.24[c]	7.2
Fusarium	*F. solani*	6	340±14.1[d]	5.9
Scopulariopsis	*S. brevicaulis*	2	318±2.7[e]	5.5
Alternaria	*Al. alternata*	2	286±8.49[f]	4.9
Curvularia	*Cu. Lunata*	2	276±8.5[f]	4.8
Rhizopus	*R. stolonifera*	1	240±7.2[g]	4.1
Chrysosporium	*Ch. Tropicum*	2	203±6.96[h]	3.5
Acremonium	*Ac. Curtips*	2	185±7.07[i]	3.2
Geotrichum	*G. candidum*	1	167±9.90[j]	2.9
Trichoderma	*T. viride*	2	160±2.9[j]	2.7
Mucor	*M. circinilloides*	2	99±5.66[k]	1.7
Phialophora	*Ph. Bubakii*	2	70±1.41[l]	1.2
Bipolaris	*B. specifera*	1	67±2.83[l]	1.15
Cunninghamella	*c.* sp.	1	65±7.1[l]	1.1
Syncephalastrum	*Sy. Racemosum*	1	50±2.6[m]	0.87
Ulocladium	*U. atrum*	2	42±2.83[mn]	0.73
Trichothecium	*Tr. Roseum*	1	38±2.8[mn]	0.66
Nigrospora	*N. spherica*	1	35±4.5[n]	0.6
Total		**57**	**5740***	**100**

Values followed by the same letter within the column do not differ significantly (P>0.05); * = Significant (P≤0.05).
Note: Sr. No. = Serial number**.**
Source: Author

the genus *Aspergillus* was the highest and represented by 17 species followed by the genera *Penicillium* and *Fusarium* (6 species for each) and *Cladosporium* (3 species) (Table 2). Similar results were obtained by Torpy et al. (2013) and Mousavi et al. (2016). Moreover, many of *Aspergillus* species and members of the order Mucorales proliferate in the air-conditioning units (Ljaljevic et al., 2008; Kelkar and Kulkarni, 2011).

The main reason for the dominance of *Aspergillus, Penicillium* and *Cladosporium* is that they produce numerous small (2-3.5 µm) and light spores that generally remain in the air, whereas *Alternaria* and some other fungal genera produce fewer, larger and heavier spores that tend to have faster settling (Gołofit-Szymczak and Górny, 2010).

Seasonal variation of fungal concentrations

Results of this study revealed that fungal concentrations in the CF and CP vary not only throughout various isolation sites but also in the course of the season. The average number of fungi present in indoor CF and CP in different isolation sites during the year of study are represented in Figure 2, which showed that winter represented the highest fungal concentration followed by the fall and spring, whereas summer represented the lowest CFU count. Similar results were reported by Hariri et al. (1978) and Bunnag et al. (1982). The possible explanation of these results is that, during fall and winter the relative humidity often rises to levels of 80-90%. Moisture gets dehumidified (converted to water) when it comes in an air-conditioned environment. These conditions could create a suitable nidus for the proliferation of fungi (Kelkar and Kulkarni, 2011).

Looking at the seasonal variations of fungal concentrations in homes, offices and hospitals investigated (Figure 2), it is easy to notice a sharp difference in the different seasons. In winter, the concentration of fungal isolates in homes was the highest followed by offices then hospitals. This sequence of fungal concentration (homes > offices > hospitals) was recorded in fall, spring and summer. These results were confirmed by Mentese et al. (2009) who found that the highest fungal counts obtained in high-humidity indoor spaces such as home kitchens and bathrooms and also

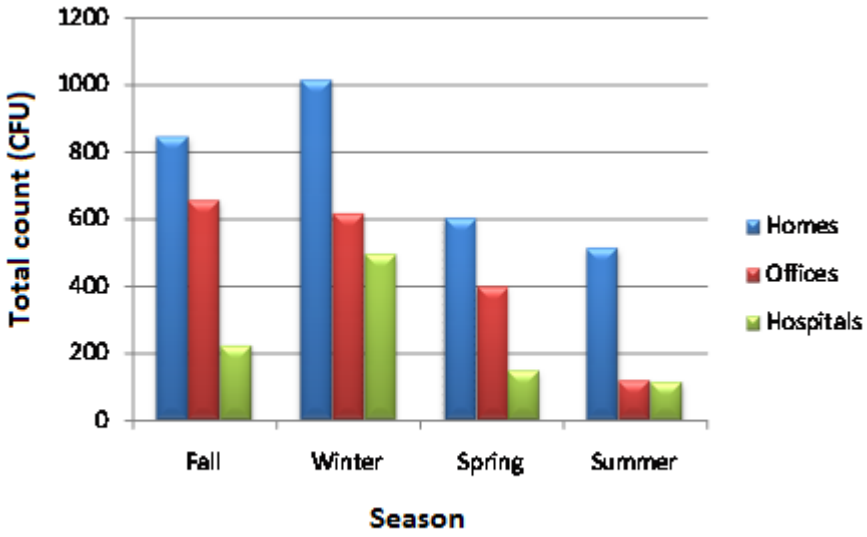

Figure 2. Mean seasonal total count (CFU) of fungi from CF and CP of homes, offices and hospitals.
Source: Author

agreed with those of Božić et al. (2019) who stated that there was a positive correlation between the concentrations of fungi and relative humidity.

Fungal diversity of CF and CP in all isolation sites throughout the different seasons are represented in Table 3. In fall, thirty-three fungal species belonging to 15 genera were isolated in which *Aspergillus* was the most dominant genus (10 species) followed by the genus *Penicillium* (5 species). On the other hand, forty-five fungal species belonging to 17 genera were isolated in winter. *Aspergillus niger*, *Cl. herbarum*, *A. flavus*, *A. fumigatus*, *A. terreus*, *F. solani*, *P. citrinum*, *P. spinulosum*, *R. stolonifer*, *S. brevicaulis* and *S. candida* were the most dominant species. *Aspergillus* was the most dominant genus followed by the genera *Fusarium* and *Penicillium* (Table 3).

Forty-four fungal species belonging to 19 genera were isolated in spring in which *A. niger, Al. alternata*, *Curvularia lunata, A. flavus, A. wentii, C. tropicum, M. circinelloides* and *P. spinulosum* were the most dominant species. On the other hand, *Aspergillus* was the most dominant genus followed by *Penicillium.* Concerning summer, 29 fungal species belonging to 16 genera were isolated and *A. niger*, *C. tropicum*, *C. lunata*, *P. chrysogenum, A. flavus* and *S. brevicaulis* were the most dominant species. However, *Aspergillus* was the most dominant genus followed by *Penicillium.* On the other hand, *A. niger* was the most dominant species isolated from CF and CP from all sites of isolation in all seasons, while *Aspergillus* and *Penicillium* were the most dominant genera found in all seasons with high frequency (Table 3). In contrast to this, Gonçalves et al. (2010) found *Penicillium* and *Aspergillus* species to be dominant across all seasons both indoors and outdoors, the results which confirm the present results.

Generally, winter was the highest in species richness (45 species) followed by spring (44 species), while fall and summer represented lower species richness (33 and 29 species, respectively). However, spring represented the highest genus richness (19 genera) followed by winter, summer and fall (17, 16 and 15 genera, respectively). According to earlier studies, the microbiological quality of indoor air is formed by two main factors: the microbiological composition of outdoor air and indoor air microbial sources (Stryjakowska-Sekulska et al., 2007). Outdoor air is very much influenced by environment, season, the weather and even daytime. Some pathogenic *Aspergillus* and *Fusarium* spp. isolated from CF and CP throughout the isolation period are presented in Figure 3.

Screening of fungal isolates for extracellular enzymes production

Fungal isolates obtained from CF and CP from various isolation sites were tested for their ability to produce extracellular enzymes on solid media. Data presented in Table 4 indicated that 65.4% of tested isolates could produce cellulase. The genus *Aspergillus* represented the highest percentage of cellulase production (27.9%) followed by *Penicillium* (7.5%), *Cladosporium* (5.0%), *Scopulariopsis* (4.2%) and *Fusarium* (3.6%). Other isolates were less in their cellulose activity (Table 4).

Table 3. Fungi isolated from the dust of conditioning systems filters and soil of indoor cultivated plants from different isolation sites during different seasons

Ge. S.N.	Sp. S.N.	Fungal isolate	Season																							
			Fall						Winter						Spring						Summer					
			Home		Offices		Hospitals		Home		Offices		Hospitals		Home		Offices		Hospitals		Home		Offices		Hospitals	
			CF	CP	CF	CP	CF	CP	CF	CP	CF	CP	CF	CP	CF	CP	CF	CP	CF	CP	CF	CP	CF	CP	CF	CP
1	1	***Acremonium***																								
		Ac. alternatum	+	+	-	-	-	+	+	-	+	-	+	-	+	-	+	-	+	-	-	-	-	-	-	-
	2	*Ac. Curtips*	+	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-	-
2	3	***Alternaria***																								
		Al. alternata	+	-	-	+	-	+	+	-	-	+	-	+	+	+	+	+	+	+	-	+	-	-	-	-
	4	*Al. solani*	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	-	+	-	+	+	+	+	-	-
3	5	***Aspergillus***																								
		A. avenaeus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-
	6	*A. awamori*	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
	7	*A. carneus*	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-
	8	*A. clavatus*	+	-	-	+	-	-	+		+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	9	*A. duricaulis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
	10	*A. effusus*	-	-	-	-	-	-	+	-	+	-	+	-	-	-	-	+	-	-	+	-		+	-	-
	11	*A. flavus*	+		+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+
	12	*A. fumigatus*	+	+	-	+	-	-	+	+	+	+	-	+	+	-	+	-	+	-	+	-	-	-	+	-
	13	*A. nidulans*	+	-	+	-	-	+	+	+	-	+	+	-	-	-	-	-	-	-	+	-	+	-	+	-
	14	*A. niger*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	15	*A. oryzae*	-	-	-	+	-	-	+	+	+	-	+	-	+	+	+	-	+	-	+	-	+	-	+	-
	16	*A. sulphureus*	-	-	-	-	-	-	+	+	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-
	17	*A. tamarii*	-	-	+	+	-	-	+	-	+	+	+	-	+	+	-	-	+	-	+	+	-	-	-	-
	18	*A. terreus*	+	-	-	-	-	-	+	+	+	-	+	+	+	-	-	-	+	-	-	-	-	-	-	-
	19	*A. ustus*	-	-	-	+	-	-	+	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
	20	*A. versicolor*	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	21	*A. wentii*	+	-	+	+	+	-	-	-	-	-	-	-	+	+	+	-	+	+	+	+	+	-	+	-
4	22	***Bipolaris***																								
		B. spicifera	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	+	+	+	-
5	23	***Chrysosporium***																								
		C. queenslandicum	-	-	-	-	-	-	+	+	-	+	+	-	-	-	-	-	+	-	+	-	+	-	+	+
	24	*C. tropicum*	-	-	-	-	-	-	+	-	-	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+
6	25	***Cladosporium***																								
		Cl. herbarum	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+		+	-	+	-	-	-	-	+
	26	*Cl. oxysporum*	+	+	+	+	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-
	27	*Cl. sphaerospermum*	-	-	+	-	+	-	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
7	28	***Cunninghamella***																								
		Cunninghamella sp.	-	-	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-
8	29	***Curvularia***																								
		Cu. brachyspora	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+
	30	*Cu. lunata*	+	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 3. Contd.

9	31	***Fusarium***																								
		F. chlamydosporum	-	-	-	-	-	-	+	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
	32	*F. moniliforme*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
	33	*F. oxysporum*	+		+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	34	*F. solani*	+	+	+	-	+	-	+	-	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-
	35	*F. sulphureum*	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-
10	36	***Geotrichum***																								
		G. candidum	+	-	+	+	+	-	+	-	+	+	-	-	+	-	+	-	+	+	-	-	-	-	-	-
11	37	***Mucor***																								
		M. circinelloides	+	-	+	+	+	-	+	-	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-
	38	*M. racemosus*	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-
12	39	***Nigrospora***																								
		N. sphaerica	-	-	-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-
13	40	***Penicillium***																								
		P. chrysogenum	+	+	+	-	-	-	+	-	-	+	-	-	+	-	+	-	+		+	+	+	+	+	+
	41	*P. citrinum*	-	-	-	-	-	+	+	+	+		+	+	+	-	-	-	-	-	-	-	-	-	-	-
	42	*P. madriti*	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	+	+	+	-	+	
	43	*P. rugulosum*	+		+	-	-	-	-	+	-	+	-	-	+	-	+	-	-	-	+	-	+	-	+	+
	44	*P. restrictum*	-	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	-	-	-
	45	*P. spinulosum*	+	-	+	-	-	+	+	+	+	+	+	-	+	+	+	-	+	+	-	-	-	-	-	-
14	46	***Phialophora***																								
		Ph. bubakii	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	-	-	-	-	-	-	-	-
	47	*Ph. lagerbergii*	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	48	***Rhizopus***																								
		R. stolonifer	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	-	-	-	+	-	+	+	+	-
16	49	***Scopulariopsis***																								
		S. brevicaulis	+	+	+	+	-	-	+	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	-	+
	50	*S. candida*	-	-	-	-	-	-	+	+	+	+	-	+	+	-	-	-	-	+	+	-	+	+	+	-
17	51	***Syncephalastrum***																								
		Syncephalastrum sp.	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	52	*S. racemosum*	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	+	-	-	-	-	-	-
18	53	***Trichoderma***																								
		T. koningii	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	54	*T. viride*	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	-	+	-	+	+	+	-
19	55	***Trichothecium***																								
		Trichothecium sp.	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-
20	56	***Ulocladium***																								
		U. atrum	-	-	-	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	57	*U. chartarum*	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	+	-

Note: Ge. S.N. = Genus serial number. Sp. S. N. = Species serial number. Positive (+) = Isolated. Negative (-) = Not isolated.
Source: Author

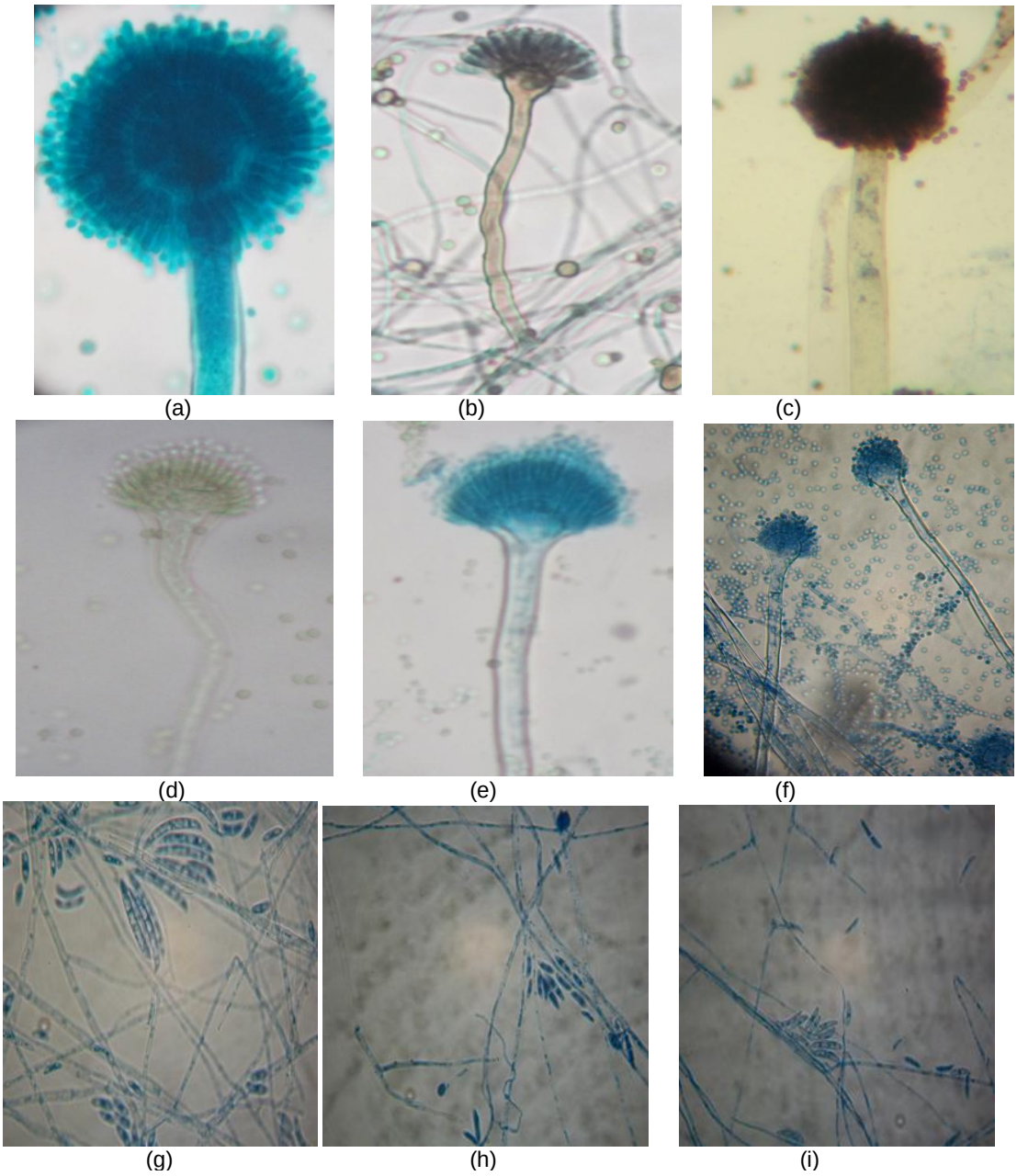

Figure 3. Some pathogenic species of the genera *Aspergillus* and *Fusarium* isolated from CF and CP: (a) *Aspergillus flavus*; (b) *A. nidulans*; (c) *A. niger*; (d) *A. terreus;* (e) *A. carneus* (f) *A. fumigatus;*(g) *Fusarium solani*; (h) *F. moniliforme* and (i) *F. oxysporum.*
Source: Author

Fungi are well-known agents for the decomposition of organic matter in general and of cellulosic substrate in particular (Gautam et al., 2010; Raveendran et al., 2018; Barone et al., 2019). Many studies reported that the most common and potent cellulase producers were *Aspergillus, Penicillium* and *Fusarium* species and that there were some differences in the cellulase activity of different members of fungal genera (Rana and Kaur, 2012; Coronado-Ruiz et al., 2018). This finding indicates that the cellulase system of these fungal forms contains enzymes complexes for the effective hydrolysis of cellulose (Gautam et al., 2010; Hussain et al., 2012).

Concerning keratinase activity, about 42.3% of the tested isolates could produce keratinase. The genus

Table 4. Extracellular cellulolytic and keratinolytic activity of the tested fungal genera grown at 28°C and collected from CF and CP from all sites

Fungal genera	Cellu. A.	Kera. A.
Acremonium	12±2.83^{ij}	7±1.41^{gh}
Alternaria	25±1.41^{gh}	13±2.83^{g}
Aspergillus	340±5.66^{a}	265±5.66^{a}
Bipolaris	9±1.39^{jk}	4±1.41^{hi}
Chrysosporium	30±2.83^{g}	35±1.41^{d}
Cladosporium	63±2.83^{c}	30±2.83^{e}
Cunninghamella	4±1.4^{kl}	1^{hi}
Curvularia	38±4.24^{f}	17±4.24^{f}
Fusarium	47±2.7^{e}	42±2.83^{c}
Geotichum	9±1.41^{jk}	4±1.41^{hi}
Mucor	23±1.41^{h}	5±1.41^{hi}
Nigrospora	10±2.83^{jk}	0^{i}
Penicillium	90±4.25^{b}	60±7.07^{b}
Phialophora	11±1.41^{ij}	0^{i}
Rhizopus	17±1.41^{i}	1±1.41^{hi}
Scopulariopsis	50±2.83^{d}	40±2.83^{c}
Syncephalastrum	8±1.4^{jk}	0^{i}
Trichoderma	30±2.7^{g}	9±1.41^{g}
Trichothecium	1^{l}	1^{hi}
Ulocladium	5±1.39^{kl}	0^{i}
Significance Level	*	*
LSD (0.05)	2.72	2.70

Values followed by the same letter within the column do not differ significantly (P>0.05); * = Significant (P≤0.05).
Note: S.N. = serial number, Cellu. A. = Cellulolytic activity and Kera. A. = Keratinolytic activity.
Source: Author

Aspergillus represented the highest percentage of keratinase production (21.2%) followed by *Penicillium* (4.5%), *Scopulariopsis* (3.4%), *Fusarium* (3.2%) and *Chrysosporium* (2.8%) (Table 4). The data are coincident with those reported by Singh et al. (2009), who isolated keratinophilic fungi from soil of planted pots in indoor environments. However, *Chrysosporium, Alternaria, Cladosporium*, *Scopulariopsis*, *Curvularia lunata* and *Fusarium solani* has been reported for their keratinolytic activity (Franca and Caretta, 1984; Mukesh and Meenakshi, 2010). It is recorded that the organic matter content of soils is one of the major factors affecting the presence of keratinophilic fungi in them (Chmel et al., 1972). Evidence of keratinolysis lies in the ability of fungi to release soluble sulphur-containing amino acids and polypeptides into the medium (Mini et al., 2012).

In this study, all fungal isolates have been tested for growth at 37°C and a percentage of 1.14% of the total tested fungi were recorded as lipase producers and belonging to the genus *Aspergillus* (Figure 4a,b), the results were supported by Negedu et al. (2012) and Raveendran et al. (2018). On the other hand, a percentage of 13.6% had proteolytic activity and also belonging to the genus *Aspergillus.* Several species of *Aspergillus* are known to secrete protease as reported by Aboul-Nasr et al. (2013) and Raveendran et al. (2018). Results also indicated that 16.9% of the screened fungal isolates exhibited lysis activity (hemolysis) on human blood (Figure 4c). Several studies have reported fungal hemolytic activity and characterized fungal hemolysins (Greenhill et al., 2010; Nayak et al., 2013; Aboul-Nasr et al., 2013).

The isolated Aspergillus spp., Penicillium spp. and Cladosporium spp. were identified as well-known agents of mycosis, acting as opportunistic pathogens in immunocompromised hosts. They are known to contain glucan, a compound with inflammatory properties and they contain allergens and chemicals that have toxic properties (Ljaljevic et al., 2008; Mousavi et al., 2016; Haas et al., 2016).

With different species of Aspergillus isolated, A. fumigatus is a primary concern as it can cause a range of saprophytic, severe invasive diseases with high mortality (O'Gorman, 2011). Aspergillus fumigatus can also cause

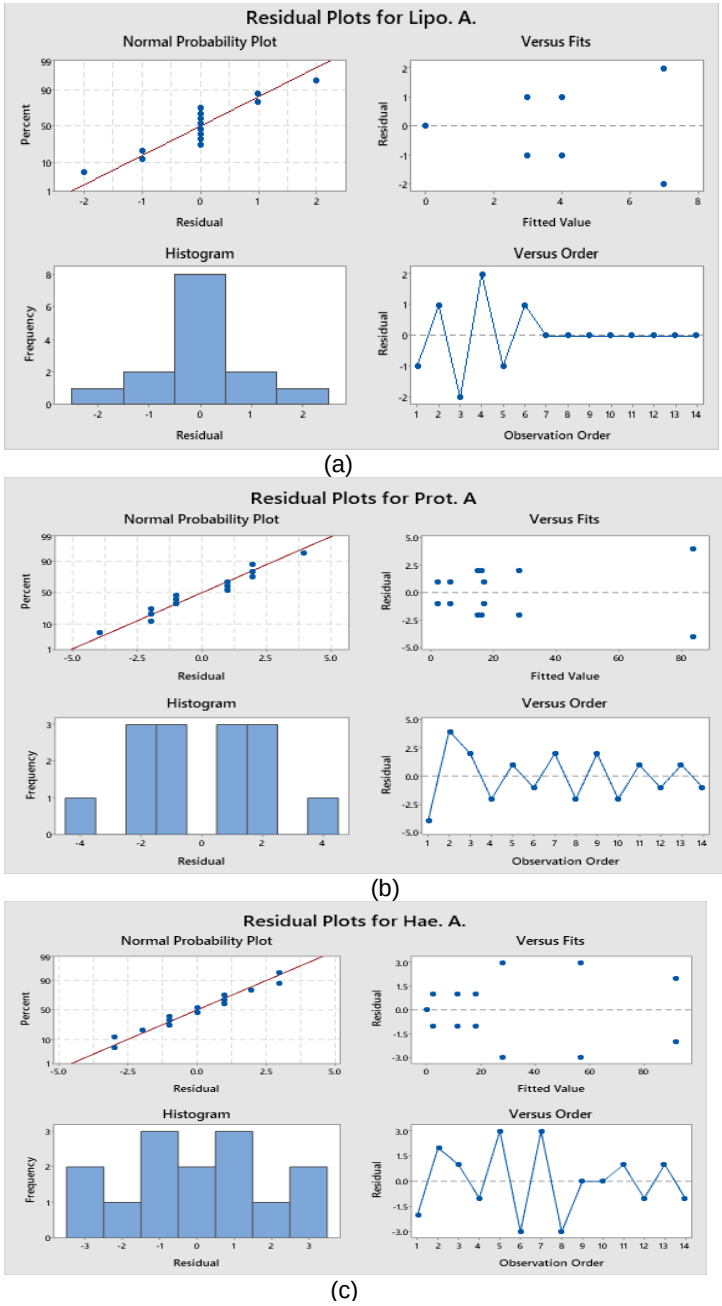

Figure 4. Extracellular lipolytic, proteolytic and hemolytic activity of *Aspergillus* spp. grown at 37°C and collected from CF and CP from all sites (a, b and c, respectively).
Source: Author

opportunistic infection in immunocompromised and healthy individuals and severe allergic diseases (Knutsen and Slavin, 2011). On the other hand, Aspergillus flavus, Fusarium moniliforme, *F. oxysporum*, *Chrysosporium* sp. and others were among the isolated species. Most of these saprophytic isolates are potential pathogens causing skin mycosis (Bernardo et al., 2005; Avasn et al., 2015). Also, exposure to some species of the genus Penicillium, which were isolated in this study, has been associated with a variety of adverse health outcomes including respiratory, hematological, immunological, and neurological system disorders and diseases (Gołofit-

Szymczak and Górny, 2010), while exposure to species of the genus Acremonium causing fungemia (Mattei et al., 2003).

Dematiaceous fungi as Alternaria, Cladosporium and Curvularia were isolated in the present study. Some species have been reported as causing human infections such as subcutaneous mycosis by *Alternaria* spp. (Taira et al., 2011). However, it was reported that cladosporin was produced by Cladosporium and that some species of this genus can cause skin lesions, keratitis and pulmonary infections. On the other hand, Curvularia species may cause infections in humans and has been described as a pathogen that causes respiratory tract, cerebral, cutaneous and corneal infections (Aboul-Nasr et al., 2013).

Virulence factors are properties that increase the survival, growth, and propagation of fungi in human and animal tissue. Some factors are well known, such as the ability of the organism to grow at 37°C and to excrete enzymes (Taira et al., 2011; Aboul-Nasr et al., 2013). It was reported that microbial cells secrete hydrolytic enzymes that are considered the most important virulence factors influencing the pathogenicity of opportunistic fungal infections and destroy the constituents of host cell membranes leading to membrane dysfunction and the invasion of host tissues (Aboul-Nasr et al., 2013) and have immunomodulating activity in humans (Ljaljevic et al., 2008).

Conclusion

Most fungi isolated in this study were considered saprobionts, but depending on the situation they might have the potential to become opportunistic pathogens. Fungal flora can be hazardous for health, particularly in rooms with heating, ventilation and air-conditioning systems and indoor potted plants as potential sources of human diseases. To avoid and reduce potential fungal pollution (infections) in homes, offices and hospitals, the air-conditioning systems must be subjected to regular maintenance. Potted plants, on the other hand, have to subjected to regular cleaning of their soil and elimination eradication of the dead and infected plant parts with the treatment of the soil with a suitable fungicide with a controlled cultivation system. The results show that potentially pathogenic fungi are present in soils. Immunocompromised individuals should avoid handling soils or potted plants in their immediate vicinity.

This surveillance study recommends homes, offices and hospitals about the need for routine cleaning and disinfection of gadgets like air-conditioning systems and soil of cultivated plants for minimizing the chances of proliferation and dispersal of potentially pathogenic fungi. Also, immunocompromised individuals should avoid handling soils of potted plants in their immediate vicinity. Finally, exposure to bioaerosols through air-conditioning systems filter dust can cause various adverse health effects, including infectious and respiratory diseases and hypersensitivity. Consequently, controlling the exposure to bioaerosols constitutes an important aspect of disease control and prevention.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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