Indoor Surveillance of Airborne Fungi Contaminating Intensive Care Units and Operation Rooms in Assiut University Hospitals, Egypt

M. Bassam Aboul-Nasr¹, Abdel-Naser A. Zohri² and Enas Mahmoud Amer²

1. Faculty of Science, Department of Botany, University of Sohag, Sohag 82524, Egypt

2. Faculty of Science, Department of Botany and Microbiology, University of Assiut, Assiut 71515, Egypt

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Abstract: Mycoflora of atmospheric air and dust samples collected from air conditioning systems in 12 of each I.C.U. (intensive care units) and O.R. (operation rooms) were tested using settle and dilution plate methods on four types of agar media and incubated at 25 °C. Forty-five fungal species representing 23 genera were isolated and identified. The most prevalent genera recorded were *Cladosporium*, *Aspergillus*, *Penicillium* and *Fusarium*. The total colony forming units of airborne fungi recovered in I.C.U. and O.R. ranged between 31.13-49.61 colonies/m³ on the four types of media used. The fungal total catch of the dust samples collected from the air conditioning system filters in I.C.U. and O.R. were ranged from 65.5-170 colonies/mg dust. Since, the interest to replace synthetic xenobiotics by natural compounds with low environmental persistence and biodegradable to control such airborne fungal contaminants is needed. In this respect, essential oils showed to possess a broad spectrum of antifungal activity. Fungal static ability of six oils was tested on 30 different fungal isolates. Vapors of common thyme oil exhibited the strongest inhibitory effects on the tested isolates, whereas the headspace vapors of blue gum and ginger had no inhibitory effects on the tested fungal isolates. These data revealed that the air conditioning systems may be an important source of contamination in I.C.U. and O.R. of Assiut university hospitals. Thus, patients may be in risk of being exposed to contaminated atmospheric air by opportunistic fungi and the use of essential oils as an alternative option to control hospital wards from fungal contaminants needs further studies.

Key words: Air conditions, airborne fungi, intensive care units, operation rooms and volatile oils.

1. Introduction

Recently, increased attention has been paid to fungal infections causing mycosis especially aspergillosis and therefore microorganisms in hospital rooms were the subject of many studies as the most important source of fungal infections [1-3]. Most of these studies were performed in intensive care units, surgical units, and other departments where the risk of infections is great [3-6]. *Aspergillus*, *Fusarium* and *Mucor* species were found to be the most common fungal genera that contaminate hospitals with low quantities ranging from 2 CFU/m³ to 26 CFU/m³ [7-10]. Indeed, fungal

contamination of the hospital rooms may occur due to the growth of fungi on the organic matter of building materials. The spores emanating from these colonies could be inhaled by immunosuppressed patients and caused local infections, prior to possible dissemination [11]. Although air-conditioning systems are essential to maintain a comfortable indoor environment but also are often contaminated by microbes which their discharge into the indoor environment with strong air currents may cause fungal contamination of the air. A relation between bronchial asthma and fungal contamination by air conditioning systems was studied [12, 13]. The levels of nosocomial pathogens in the air of hospitals increased due to dirtiness of air ducts without regular replacement [14] in addition to organic

Corresponding author: M. Bassam Aboul-Nasr, Ph.D., associate professor, research field: microbiology. E-mail: mbassamaboulnasr@yahoo.com.

materials such as food, flowers and fruits derived from outdoor by visitors and the interior structures of the hospital [15]. The environmental fungal contamination in hospitals and the incidence of invasive aspergillosis were demonstrated by Ref. [7] and more than 500 cases of post operative aspergillosis in immunocompetent individuals were reported by Ref. [16]. *Aspergillus fumigatus* and *A. flavus* were the leading species of the genus *Aspergillus* causing invasive aspergillosis [17]. Vonberg and Gastmeier [18] reviewed all cases of invasive aspergillosis and reported that the fungus could be able to cause disease in an environment containing less than 1 CFU/m³ of air.

Essential oils showed to possess a broad spectrum of antifungal activity [19]. The advantage of plant essential oils is their bioactivity in their vapor phase which makes them used as possible antifungal fumigants [20-22].

As it is known, very limited studies of the airborne fungi in Egyptian hospitals especially I.C.U. and O.R. were performed. In this context, the aim of this study was focused on examining the spectrum and the level of fungi in the atmospheric air and the dust samples collected from the filters of their air conditioning system in Assiut university hospitals. Also, studying the antifungal activity of some essential plant oils (Blue gum, Clove, Common thyme, Ginger, Lupine and Radish) on the most prevalent fungal isolates collected was assessed.

2. Materials and Methods

2.1 Media Used

Four types of agar media were used for isolation of fungi (Sabouraud dextrose, Czapek's glucose, Czapek's glucose at pH 8.5, and Czapek's cellulose agar media) [23].

2.2 Determination of Airborne Fungi

Airborne fungi of 12 of each I.C.U. and O.R. in Assiut university hospitals were determined for two consecutive years started from June 2008 to May 2010

using Koch sedimentation method according to Polish Standard PN 89/Z-04008/08. Air mycoflora was settled directly on three plates of the four types of media per month for five minutes exposure time (optimal time for hunting a reasonable and countable number of colony forming units) in I.C.U. and 30 min in O.R. Plates were incubated at 25 ± 2 °C for 7-10 days. The fungal count was calculated as CFU/m³ according to the equation $CFU/m^3 = \text{no. of colonies} \times 10,000 / \text{surface of the Petri dish} \times \text{time of exposure} \times 0.2$ [24].

2.3 Determination of Mycoflora of Air-Conditioning Filter's Dust

Dilution plate method [25-29] was used to determine the mycoflora of dust samples collected from air-conditioning filters of I.C.U. and O.R. on the four types of media used. The plates were incubated on 25 ± 2 °C for 7-10 days and TFC (total fungal colonies) were calculated.

2.4 Identification of Fungi

Fungal genera and species were identified on the basis of both macroscopic and microscopic characteristics identification keys of Refs. [25, 28, 30-38].

2.5 Essential Oils Assay

Six volatile plant oils were purchased from local markets, blue gum (*Eucalyptus globules* Labill.), clove (*Syzygium aromaticum* L. Merr & L.M. Perry), common thyme (*Thymus vulgaris* L.), Ginger (*Zingiber albus* L.), lupine (*Lupinus albus* L.) and radish (*Raphanus sativus* L.) and screened for their bioactivity on the most prevalent fungal isolates collected. Fifty mL of Czapek's glucose agar medium [23] was poured into a sterile Petri dish and left to solidify and inoculated by the tested fungal isolate in its center. A sterile filter paper disc (≈ 2 cm) was loaded with 50 µL of the tested volatile oil and placed on the center of the upper side of the Petri dish. The Plate was sealed with polyethylene film and incubated upside down at 25 ± 2 °C for 7 days and the diameter of the fungal growth was measured in cm [39].

3. Results and Discussion

Results summarized in Tables 1 and 2 revealed that a total of 45 species belonging to 23 genera of filamentous fungi were isolated and identified from atmospheric air and dust samples collected from air conditioning systems in I.C.U. and O.R. of Assiut university hospitals. There were no appreciable differences in air mycobiota of either I.C.U. or those of O.R. as well as those of the filter's dust samples of their air conditions. Taxa isolated were belonging to three taxonomic groups: *Ascomycetes*, *Hyphomycetes* and *Zygomycetes*. The first group was represented by only one species (*Eurotium amstelodami*), whereas the second group comprised the greatest number of fungal genera and species (39 species of 17 genera) and *Zygomycetes* embraced five genera and five species. The airborne total fungal forming units/m³ load in I.C.U. and O.R. were ranged from 31.13-49.61 units/m³, whereas their total fungal catch in the dust samples collected was ranged from 65.5-170 colony/mg. In one year study of fungal air contamination in outdoor and inside two hematological units in France, Sautour et al. [40] found that the mean viable fungal load was 122.1 CFU/m³ in outdoor samples and 4.1 CFU/m³ in the units. More or less similar results were reported by Ekhaïse et al. [41]. They found that the fungal population in the air of five different hospital wards was ranging from 10-53

CFU/m³. Falvey and Streifel [42] reported that the mean recovery of the outdoor air was ranged from 22-122 CFU/m³, whereas the patient care areas in the hospitals in Minnesota university hospitals comprised the half number of the outdoor samples.

Although *Aspergillus* (17 species) and *Penicillium* (five species) showed the greatest spectrum of airborne fungi and dust samples of air conditions on all types of media used, but *Cladosporium* (two species) was recorded as the most common airborne genus (19.35-32.8 CFU/m³ and frequently appeared in 100%) followed by *Aspergillus* (0.97-19.93 CFU/m³ and 67%-100%) and *Fusarium* came third (0.12-1.71 CFU/m³ and 17%-63%). *Penicillium* and *Rhizopus* were collected with moderate to rare frequencies in I.C.U.. However, *Penicillium* replaced *Fusarium* rank in O.R. on all of the media used. Most likely, these results came in agreement with those of Sautour et al. [40] who isolated *Fusarium* with low frequency while Faure et al. [8] isolated *Cladosporium* with 16% of total fungi in the haematologic hospital in France.

In the present work, the total fungal catch units/mg dust samples *Aspergillus* was the most dominant genus where its total fungal count and frequency level of appearance ranged between (43.5-147 colony/mg dust and 100% respectively) followed by *Fusarium* (two species), whereas *Penicillium* occupied the third place in both I.C.U. and O.R.. All of these fungal genera were similarly recorded by Refs. [5, 8, 43-45].

Table 1 Total fungal forming units (CFU/m³) and frequency levels (F%) of fungal genera and species of airborne fungi recovered on A-Czapek's glucose, B-Czapek's glucose at pH 8.5, C-Sabouraud dextrose, D-Cellulose agar media in 12 of each I.C.U. & O.R. at Assiut university hospitals.

Media used Parameters Fungal species	A				B				C				D			
	I.C.U.		O.R.		I.C.U.		O.R.		I.C.U.		O.R.		I.C.U.		O.R.	
	CFU	F%	CFU	F%	CFU	F%	CFU	F%	CFU	F%	CFU	F%	CFU	F%	CFU	F%
<i>Absidia corymbifera</i>	0.03	4														
<i>Acremonium strictum</i>	0.18	17	0.09	13	0.29	25	0.06	8			0.06	8				
<i>Alternaria alternata</i>	0.58	38	0.21	25	0.47	38	0.15	17	0.44	21	0.21	21	3.42	58	0.27	25
<i>Aspergillus spp.</i>	0.97	67	5.37	100	1.83	67	3.36	88	19.93	100	2.22	88	8.73	88	6.33	88
<i>A. aculeatus</i>			0.09	13			0.57	42	0.03	4	0.03	4	0.03	4	1.56	42
<i>A. awamori</i>	0.22	17	0.81	100	0.47	25	0.27	21	8.77	54	0.15	13	0.11	8	0.03	4
<i>A. candidus</i>			0.18	8			0.45	38	0.03	4	0.48	38	0.03	4	2.52	79
<i>A. flavipes</i>			0.09	13							0.09	13				

(Table 1 continued)

Media used	A				B				C				D			
Parameters	I.C.U.		O.R.		I.C.U.		O.R.		I.C.U.		O.R.		I.C.U.		O.R.	
Fungal species	CFU	F%	CFU	F%	CFU	F%	CFU	F%	CFU	F%	CFU	F%	CFU	F%	CFU	F%
<i>A. flavus</i>	0.33	21	0.93	79	0.58	33	0.36	33	6.29	50	0.39	33	4.51	63	0.72	21
<i>A. fumigates</i>	0.03	4	0.06	4			0.06	8	0.22	13	0.03	4	1.35	21	0.27	17
<i>A. melleus</i>			0.18	8			0.09	13			0.27	21	0.15	8	0.03	4
<i>A. niger</i>	0.15	13	0.24	21	0.55	17	0.36	25	2.87	29	0.21	25	2.18	42	0.3	21
<i>A. niveus</i>	0.03	4	0.03	4			0.03	4	0.06	8	0.03	4	0.03	4	0.78	38
<i>A. ochraceous</i>	0.03	4	1.41	17	0.06	8	0.09	8	0.73	33	0.15	17	0.25	25	0.03	4
<i>A. sulphureus</i>			0.15	21			0.03	4	0.06	8	0.03	4	0.03	4	0.03	4
<i>A. sydowii</i>	0.15	17	1.08	54	0.11	13	0.78	33	0.15	17	0.18	21	0.06	8	0.03	4
<i>A. tamarii</i>							0.03	4								
<i>A. terreus</i>	0.03	4	0.06	8	0.06	8	0.09	13	0.69	25	0.12	8			0.03	4
<i>A. wentii</i>			0.06	4			0.06	8	0.03	4	0.06	8				
<i>A. versicolor</i>							0.09	13								
<i>Botryotricum piluliferum</i>									0.03	4			0.03	4		
<i>Circinella muscae</i>			0.33	13					0.03	4	0.06	8				
<i>Cladosporium spp.</i>	28.74	100	27.3	100	25.06	100	24.57	100	19.35	100	26.34	100	32.8	100	27.84	100
<i>C. cladosporioides</i>	18.37	100	20.01	100	16.11	100	17.67	100	12.00	100	18.36	100	19.71	100	17.28	100
<i>C. herbarum</i>	10.37	100	7.29	100	8.95	100	6.9	100	7.35	100	7.98	100	13.09	100	10.56	100
<i>Cunninghamella echinulata</i>													0.15	13	0.03	4
<i>Curvularia lunata</i>															0.06	8
<i>Drechslera spicifera</i>	0.03	4			0.06	8	0.12	17					0.15	13	0.09	13
<i>Epicoccum nigrum</i>	0.15	13			0.06	8	0.06	13	0.29	8						
<i>Eurotium amstelodami</i>			0.09	13							0.42	38	0.03	4		
<i>Fusarium spp.</i>	0.58	58	0.3	29	0.29	29	0.12	17	0.65	38	0.24	33	1.71	42	0.96	63
<i>F. oxysporum</i>	0.29	33	0.09	13	0.11	13			0.36	17	0.18	21	0.95	38	0.51	50
<i>F. solani</i>	0.29	33	0.21	13	0.18	21	0.12	17	0.29	29	0.06	8	0.76	38	0.45	46
<i>Mucor circinelloides</i>			0.12	4							0.21	25			0.09	8
<i>Myrothecium roridum</i>	0.15	13	0.06	17	0.18	17			0.06	8			0.03	4		
<i>Penicillium spp.</i>	0.54	38	2.58	83	0.55	33	2.13	71	2.55	54	3.06	75	2.11	63	1.71	58
<i>P. chrysogenum</i>	0.15	17	0.21	25	0.15	17	0.6	38	0.69	42	0.24	25	0.62	33	0.39	29
<i>P. corylophilum</i>	0.11	13	0.72	50	0.11	13	0.69	54	0.29	21	0.24	13	0.65	33	0.3	25
<i>P. duclauxii</i>	0.11	13	1.08	71	0.11	13	0.36	38	0.73	29	0.39	33	0.51	25	0.33	25
<i>P. italicum</i>	0.11	13	0.51	46	0.15	8	0.3	21	0.44	25	1.65	54	0.22	13	0.42	33
<i>P. oxalicum</i>	0.06	8	0.06	4	0.03	4	0.18	25	0.40	13	0.54	33	0.11	4	0.27	17
<i>Rhizopus stolonifer</i>	0.06	8	0.03	4	0.06	8	0.15	21	0.33	42	0.09	13	0.18	21	0.15	17
<i>Scopulariopsis spp.</i>	0.36	29	0.33	21	0.47	29	0.45	38			0.9	25			0.06	8
<i>S.brevicaulis</i>	0.18	21	0.21	17	0.25	25	0.27	33			0.42	29			0.03	4
<i>S. brumptii</i>	0.18	21	0.12	4	0.22	25	0.18	17			0.48	21			0.03	4
<i>Stachybotrys elegans</i>	0.18	21			0.33	38			0.15	17			0.15	13		
<i>Stemphylium vesicarium</i>	1.82	63			1.42	50	0.12	17	0.98	38			0.06	4	0.06	8
<i>Talaromyces luteus</i>							0.06	8								
<i>Ulocladium atrum</i>	2.29	17			0.06	8			0.11	13			0.06	4		
<i>Verticillium albo-atrum</i>			0.03	4							0.09	13				
Total CFU	36.48		36.84		31.13		31.35		44.9		33.9		49.61		37.65	
Number of genera (23)	15		13		14		11		13		12		14		12	
Number of species (45)	29		33		26		32		30		32		30		30	

Table 2 Total fungal catch (TFC/mg dust) and frequency levels (F%) of fungal genera and species of air conditioning filter's dust samples recovered on A-Czapek's glucose, B-Czapek's glucose at pH 8.5, C-Sabouraud dextrose and D-Cellulose agar media, in 8 of each ICU and OR at Assiut university hospitals.

Media used	A				B				C				D			
Parameters	I.C.U.		O.R.		I.C.U.		O.R.		I.C.U.		O.R.		I.C.U.		O.R.	
Fungal species	TFC	F%	TFC	F%	TFC	F%	TFC	F%	TFC	F%	TFC	F%	TFC	F%	TFC	F%
<i>Alternaria alternata</i>					0.5	13										
<i>Aspergillus spp.</i>	114.5	100	104.5	100	147	100	88.5	100	85.5	100	53.5	100	77.5	100	43.5	100
<i>A. awamori</i>	96	88	77.5	88	123	88	47	50	45	75	28	75	28.5	50	18	38
<i>A. flavus</i>	14	63	18.5	63	19	50	24.5	75	16	88	23.5	75	7	63	16	75
<i>A. fumigates</i>	0.5	13	0.5		1	13	15	25	2.5	25	0.5	13	12	50	0.5	13
<i>A. niger</i>	0.5	13	1	13	0.5	13	1	13	20	63	1	13	29.5	63	4	13
<i>A. ochraceous</i>	1	25	1.5	13	3.5	13	1	38	2	25	0.5	13	0.5	13	5	38
<i>A. tamari</i>	1	25														
<i>A. terreus</i>	0.5	13	1	25												
<i>A. ustus</i>	0.5	13														
<i>A. versicolor</i>	0.5	13														
<i>Fusarium spp.</i>	6.5	50	11	75	3.5	50	27.5	100	3.5	38	21	88	15	63	12	63
<i>F. oxysporum</i>	2.5	25	3	75	0.5	12	10.5	88	1	13	7.5	75	3	38	3	38
<i>F. solani</i>	4	50	8	63	3	50	17	100	2.5	38	13.5	88	12	50	9	63
<i>Mucor circinelloides</i>	1.5	38	1	25	2.5	25	0.5	13	3	50	2	38	0.5	25	1.5	38
<i>Penicillium spp.</i>	7	63	41	75	15.5	38	25.5	75	28	63	28.5	88	23.5	50	4	50
<i>P. chrysogenum</i>	1.5	38	5.5	75	4	25	10	50	3.5	50	8.5	50	3	38	3	38
<i>P. corylophilum</i>	1.5	38	8	50	4.5	25	3.5	63	5	50	10	50	8	38	0.5	25
<i>P. duclauxii</i>	1.5	25	10.5	50	3	38	2.5	63	7.5	25	3.5	25	7.5	38	0.5	13
<i>P. italicum</i>	2.5	38	17	38	4	13	9.5	50	12	38	6.5	38	5	25	1	13
<i>Rhizopus stolonifer</i>	3.5	13	1.5	38	1	50	1.5	38	3	50	1.5	25	2	13	4	50
<i>Stachybotrys elegans</i>	1	13	0.5	13					1	25	0.5	13	2.5	38	0.5	13
Total TFC	134		159.5		170		143.5		124		107		121		65.5	
Number of genera (7)	6		6		6		5		6		6		6		6	
Number of species (19)	18		15		14		13		14		14		14		14	

Panagopoulou et al. [5] studied the environmental fungal load of air surfaces and tap water in three hospitals in different regions in Greece and found that *A. niger* was the most prevalent fungal species in the air of all hospitals followed by *A. flavus* and *A. fumigates*. In contrast, Augustowska and Dutkiewicz [46] isolated *A. fumigates* as a dominant species (77% of total fungal isolates) in air of a hospital in Poland.

Most of these fungi isolated from air or dust samples collected in this study may cause different kinds of mycosis. *Aspergillus fumigatus* is a known hazardous agent which may cause allergic alveolitis, asthma, pulmonary aspergillosis and mycotoxicoses [46]. Pegues et al. [47] reported systemic infection *A. fumigatus* in patient was happened at eleven days after

liver transplantation in France. Also, they found that lung aspergillosis caused by the same fungal species was detected in two patients at an intensive care unit. *Penicillium* species were reported occasionally to cause human penicilloles, pulmonary infection and fungemia [48, 49]. Moreover *Fusarium* was reported as causative agents of superficial and systemic infections in humans [50].

In the second part of the study, the antifungal activity of six types of volatile plant oils (blue gum, clove, common thymus, ginger, lupine and radish) was tested on 30 selected isolates belonging to six fungal species (five/each). The results in Table 3 reflected that common thymus oil completely inhibited the growth of all fungal isolates studied but clove oil inhibited only

Table 3 The effect of volatile plant oils on the mean diameter/cm of the colony growth of different fungal isolates tested.

Fungal species	Plant oils	A	B	C	D	E	F	G
<i>Aspergillus flavus</i>		7.2	4.2	4	4.6	3.6	4.8	-
<i>Aspergillus fumigates</i>		6.2	4	4.6	4.6	2.4	4.8	-
<i>Aspergillus niger</i>		7.4	5.4	5.4	4.8	4.2	3.4	-
<i>Cladosporium cladosporioides</i>		2	1	1	-	-	1.8	-
<i>Fusarium solani</i>		4.4	3.2	2.4	3.2	3.25	-	-
<i>Stachybotrys elegans</i>		2.6	1.8	1.8	1.75	-	2.6	-

A = Control, B = Blue gum, C = Ginger, D = Radish, E = Clove, F = Lupin, G = Common thyme.

the growth of *C. cladosporioides* and *S. elegans*. Lupine and radish oils inhibitory action affected only *F. solani* and *C. cladosporioides* respectively. On the other hand, volatile oils of both blue gum and ginger had a partially or no inhibitory effects on all of the tested fungal isolates. Montes-Belmont and Carvajal [51] and Lee et al. [52] found similar results with those in this study. Moreover, the essential oil of thymus inhibited the growth of various fungi involved in food spoilage, mycotoxin producers, pathogenic and wood decay fungi [53, 54]. In contrast, Mourad et al. [55] found that thymus oil exhibited moderate activity against wood rot fungi. These results supported the concept that plant oils could be used as fungicidal components.

4. Conclusions

Aspergillus, *Cladosporium*, *Penicillium*, *Fusarium* and several other fungal genera were recorded in the air of different I.C.U. and O.R. in addition to the dust samples of their air conditions at Assiut university hospitals. These fungi are harmless for healthy people, but they may be dangerous for patients of risk groups, including those who are treated in O.R. and I.C.U. even if their total count will be less than 1 CFU/m³ of air. Therefore, air monitoring is important in hospitals particularly in O.R. and I.C.U.. And the routine maintenance of air-conditioning systems should not be ignored. On the other hand, thymus oil had the greatest ability to inhibit the growth of all fungal isolates studied but still the use of essential oils as an alternative option to control airborne fungi that contaminate hospital wards need further studies.

References

- [1] S.G. Kelsen, M. McGuckin, The role of airborne bacteria in the contamination of fine particle nebulizers and the development of nosocomial pneumonia, Annual NY Academic Science 353 (1980) 218-229.
- [2] J. Rainer, U. Peintner, R. Poder, Biodiversity and concentration of airborne fungi in a hospital environment, Mycopathologia 149 (2001) 87-97.
- [3] C.S. Li, P.A. Hou, Bioaerosol characteristics in hospital clean rooms, Total Environment 305 (2003) 169-176.
- [4] J.C. Gould, The use of cephaloridine in an antibiotic policy trial, Postgraduate 46 (1970) 83.
- [5] J. Panagopoulou, G. Filioti, P. Petrikkosy, M. Giakouppiy, E. Farmaki, A. Kantay, et al., Environmental surveillance of filamentous fungi in three tertiary care hospitals in Greece, Hospital Infection 52 (2002) 3-8.
- [6] G. Pini, R. Donato, E. Faggi, R. Fanci, Two years of a fungal aerobiocontamination survey in a Florentine haematology ward, Epidemiology 19 (7) (2004) 693-698.
- [7] C. Alberti, A. Bouakline, P. Ribaud, C. Lacroix, P. Rousselot, T. Leblanc, Relationship between environmental fungal contamination and the incidence of invasive aspergillosis in haematology patients, Hospital Infection 48 (2001) 198-206.
- [8] O. Faure, H. Fricker-Hidalgo, B. Lebeau, M.R. Mallaret, P. Ambroise-Thomas, R. Grillot, Eight year surveillance of environmental fungal contamination in hospital operating rooms and haematologic units, Hospital Infection 50 (2002) 155-160.
- [9] F. Perdelli, M.L. Cristina, M. Sartini, A.M. Spagnolo, M. Dallera, G. Ottria, Fungal contamination in hospital environments, Infection Control Hospital Epidemiology 27 (2006) 44-47.
- [10] M. Vackova, V. Buchta, R. Prymula, J. Cerman, A. Kubatova, P. Hamal, et al., The occurrence of microscopic fungi in air samples from a transplant intensive care unit, Indoor and Built Environment 15 (2006) 115-124.
- [11] N. Singh, D.L. Paterson, *Aspergillus* infections in transplant recipients, Clinical Microbiology Reviews 18 (2005) 44-69.

- [12] E.F. Banaszak, W.H. Thiede, J.N. Fink, Hypersensitivity pneumonitis due to contamination of an air conditioner, *Medicine* 283 (1970) 271-276.
- [13] M.J. Hodgson, P.R. Morey, J.S. Simon, T.D. Waters, J.N. Fink, An outbreak of recurrent acute and chronic hypersensitivity pneumonitis in office workers, *Epidemiology* 125 (1987) 631-638.
- [14] K. Anderson, G. Morris, H. Kennedy, J. Croall, J. Michie, M.D. Richardson, et al., Aspergillosis in immunocompromised pediatric patients: association with building hygiene, design, and indoor air, *Thorax* 51 (1996) 256-261.
- [15] S. Schabrun, L. Chipchase, Healthcare equipment as a source of nosocomial infection: a systematic review, *Hospital Infection* 63 (2006) 239-245.
- [16] A.C. Pasqualotto, D.W. Denning, Post-operative aspergillosis, *Clinical Microbiology Infection* 12 (2006) 1060-1076.
- [17] A.C. Pasqualotto, Differences in pathogenicity and clinical syndromes due to *Aspergillus fumigatus* and *Aspergillus flavus*, *Medical Mycology* 47 (2008) 261-270.
- [18] R.P. Vonberg, P. Gastmeier, Nosocomial aspergillosis in outbreak settings, *Hospital Infection* 63 (2006) 246-254.
- [19] D.P. Thompson, Fungitoxic activity of essential oil components on food storage fungi, *Mycologia* 81 (1989) 151-153.
- [20] N. Paster, M. Menasherov, U. Ravid, B. Juven, Antifungal activity of oregano and thyme essential oils applied as fumigants against attacking stored grain, *Food Protection* 58 (1) (1995) 81-85.
- [21] K.A. Hammer, C.F. Carson, T.V. Riley, Antimicrobial activity of essential oils and other plant extracts, *Applied Microbiology* 86 (1999) 985-990.
- [22] P. Tripathi, N.K. Dubey, A.K. Shukla, Use of some essential oils as post-harvest botanical fungicides in the management of grey mould of grapes caused by *Botrytis cinerea*, *Microbiology and Biotechnology* 24 (2008) 39-46.
- [23] M.A. Ronald, Hand book of Microbiological Media, 10th ed., CRC Press Inc., USA, 2008, pp. 137, 333, 785, 279.
- [24] Polish Standard, PN 89/Z-04008/08, Polish Standardization Committee, Warsaw, Poland, 1989.
- [25] B.K. Raper, D.I. Fennel, The Genus *Aspergillus*, The Williams and Wilkins Company, Baltimore, 1965.
- [26] G.C. Ainsworth, Ainsworth and Bisby's Dictionary of the Fungi, Commonwealth Mycological Institute, Kew, Surrey, England, 1971.
- [27] J.A. Von Arx, The Genera of Fungi Sporulating in Pure Culture, 2nd ed., Vaduz: Cramer, Switzerland, 1974.
- [28] J.I. Pitt, The Genus *Penicillium* and Its Teleomorphic States *Eupenicillium* and *Talaromyces*, Academic Press, United States, 1979.
- [29] M.B. Ellis, P.J. Ellis, Microfungi on Land Plants: An Identification Handbook, The Richmond Publishing Co. Ltd., London, 1997.
- [30] H.L. Barnett, Illustrated Genera of Imperfect Fungi, Burgess Publishing Company, Minneapolis, United States, 1960.
- [31] G.C. Ainsworth, F.K. Sparow, S.A. Sussman, Taxonomic Review with Keys: Ascomycetes and Fungi Imperfecti, The Fungi, Volume IVA, Academic Press, New York and London, 1973.
- [32] C. Booth, The genus *Fusarium*, CMI, Kew, Surrey, England, 1971.
- [33] K.H. Domsch, W. Gams, T.H. Anderson, Compendium of Soil Fungi, Academic press, New York, 1980, p. 859.
- [34] C. Ramirez, Manual and Atlas of *Penicillia*, Elsevier Biomedical Press, Amsterdam, the Netherlands, 1982.
- [35] J.I. Pitt, A Laboratory Guide to Common *Penicillium* Species, Commonwealth Scientific and Industrial Research Organization, Division of Food Research, North Ryde, N.S.W., Australia, 1985.
- [36] M.A. Klich, J.I. Pitt, A Laboratory Guide to the Common *Aspergillus* Species and Their Teleomorphs, Commonwealth Scientific and Industrial Research Organization, Division of Food Processing, North Ryde, Australia, 1992.
- [37] A.H. Moubasher, Soil Fungi in Qatar and Other Arab Countries, The Scientific and Applied Research Centre, University of Qatar, Doha, Qatar, 1993.
- [38] A.F. Mousafa, Zygomycetes of Egypt, in: Fungi of Egypt, Report, AUMC Descriptions, No. 1, Assiut, Seaward, 2006.
- [39] S. Sirirat, R. Wimolpun, S. Sanit, Antifungal activity of essential oils derived from some medicinal plants against grey mould (*botrytis cinerea*), *Asian Journal of Food and Agro-Industry* (2009) 229-233.
- [40] Sautour, Nathalie Sixt, Frédéric Dalle, Coralie L'Ollivier, Vitalie Fourquenot, Céline Calinon, et al., Profiles and seasonal distribution of airborne fungi in indoor and outdoor environments at a French hospital, *Science of the Total Environment* 407 (2009) 3766-3771.
- [41] F.O. Ekhaize, O.U. Ighosewe, O.D. Ajakpori, Hospital indoor airborne microflora in private and government owned hospitals in Benin City, Nigeria, *World Medical Science* 3 (1) (2008) 34-38.
- [42] Falvey, Streifel, Ten year air samples analysis of *Aspergillus* prevalence in a university hospital, *Hospital Infection* 67 (1) (2007) 35-41.
- [43] P.C. Wu, H.J. Su, C.Y. Lin, Characteristics of indoor and outdoor airborne fungi at suburban and urban homes in two seasons, *Science of the Total Environment* 253 (2000) 111-118.
- [44] A. Gniadek, A.B. Macura, Intensive care unit environment

- contamination with fungi, *Advances in Medical Sciences* 52 (2007) 283-287.
- [45] Y. Kim, K.I.M. Shin, K.I.M. Daekeun, Distribution characteristics of airborne bacteria and fungi in the general hospitals of Korea, *Industrial Health* 48 (2010) 236-243.
- [46] M. Augustowska, J. Dutkiewicz, Variability of airborne microflora in a hospital ward within a period of one year, *Annual Agricultural Environmental Medicine* 13 (2006) 99-106.
- [47] D.A. Pegues, M.E. Ohl, S.I. Miller, *Salmonella*, Including *Salmonella Typhi*, in: M.J. Blaser, R.B. Smith, J.I. Ravdin, H.B. Greenberg, R.L. Guerrant (Eds.), *Infections of the Gastrointestinal Tract*, 2nd ed., Lippincott Williams and Wilkins, Philadelphia, PA, 2002, pp. 669-698.
- [48] J.C.C. Goh, J. Juliana, O. Malina, Z.U. Ngah, O. Norhaf-izalen, Prevalence of *Penicillium* specific Ig E level and allergy symptoms among office workers in a selected company in Bangi, Malaysia, *Tropical Biomedicine* 24 (1) (2007) 37-46.
- [49] S. Haleem Khan, C. Mohan Karuppayil, I.K. Manoharachary, S. Kunwar, Waghay, Isolation, identification and testing for allergenicity of fungi from air-conditioned indoor environments, *Aerobiologia* 25 (2009) 119-123.
- [50] D.H. Howard, *Pathogenic Fungi in Humans and Animals*, 2nd ed., Vol. 16, Marcel Dekker Inc., New York, 2003.
- [51] R. Montes-Belmont, M. Carvajal, Control of *Aspergillus flavus* in maize with plant essential oils and their components, *Food Protection* 61 (1998) 616-619.
- [52] S.B. Lee, K.H. Cha, S.N. Kim, S. Altantsetseg, S. Shatar, O. Sarangerel, C.W. Nho, The antimicrobial activity of essential oil from *dracocephalum foetidum* against pathogenic microorganisms, *Microbiology* 45 (2007) 53-57.
- [53] J. Nguefack, V. Leth, A. Zollo, S.B. Mathur, Evaluation of five essential oils from aromatic plants of Cameroon for controlling food spoilage and mycotoxin producing fungi, *International Food Microbiology* 94 (2004) 329-334.
- [54] M. Zyani, D. Mortabit, M. Mostakim, M. Iraqui, A. Haggoud, M. Ettayebi, et al., Cellulolytic potential of fungi in wood degradation from an old house at the Medina of Fez, *Annual Microbiology* 59 (1) (2009) 699-704.
- [55] Z. Mourad, Dounia Motabit, Soumya El Abed, Adnane Remmal, Saad Ibsouda, Antifungal activity of five plant essential oils against wood decay fungi isolated from an old house at the Medina of Fez, *Microbiology* 2 (3) (2011) 104-108.