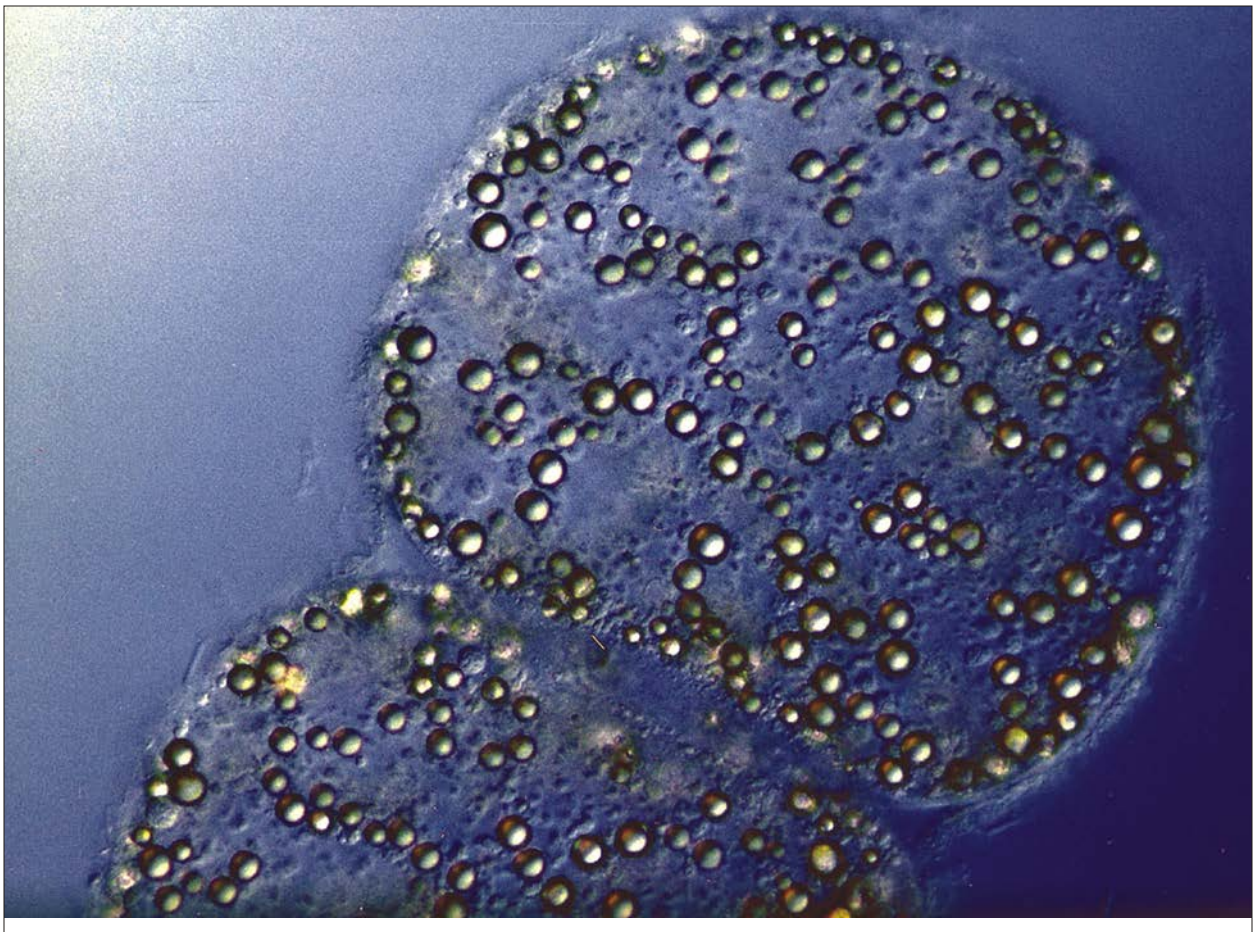

Microbiology

Journal

ISSN 2153-0696

Academic
Journals Inc.

www.academicjournals.com

Microbiology Journal 1 (1): 17-24, 2011
ISSN 2153-0696 / DOI: 10.3923/mj.2011.17.24

Indoor Terrestrial Fungi in Household Dust Samples in Riyadh, Saudi Arabia

S.S. Alwakeel and L.A. Nasser
Scientific Section, Department of Biology, University of Princess Nora Bent Abdul Rahman, Riyadh, Saudi Arabia

Corresponding Author: S.S. Alwakeel, Scientific Section, Department of Biology, University of Princess Nora Bent Abdul Rahman, Riyadh, P.O. Box 876, Saudi Arabia

ABSTRACT

Conditions increasing air humidity, decreased ventilation and increased moisture level increase the proliferation of terrestrial fungi and bacteria which may cause severe illness as a result of indoor mold exposure. No efforts had been made for screening the mycological biodiversity in indoor household dust in Saudi Arabia. Thus, this investigation represents an attempt for studying the occurrence and distribution of terrestrial fungi inhabiting indoor household dust samples in Riyadh, Saudi Arabia. Dust samples were collected in vacuum cleaner plastic bags from 60 different households in Riyadh, Saudi Arabia. These dust samples were sifted, kept at 2-5°C until fungal analysis was completed. Mycological and physico-chemical analysis of terrestrial fungi in the samples were performed. Terrestrial fungal genera and species were identified directly from colony morphology on Rose Bengal media. Seventy-two species belonging to 24 genera of terrestrial fungi were isolated on Glucose and cellulose Czapek's agar media. *Aspergillus* (16 species and one variety) and *Penicillium* (11 species) and *Cladosporium* were the predominating species isolated. *Acremonium*, *Botryodiplodia*, *Circinella*, *Myrothecium* and *Syncephalastrum* were also recovered. Glucose-Czapek's agar medium produced the greatest species diversity (61 species+one variety) compared to cellulose-Czapek's agar medium (59 species+one variety). The most frequently encountered fungal species in dust samples were *Aspergillus niger*, *Aspergillus flavus*, *Penicillium funiculosum* and *Penicillium chrysogenum*.

Key words: Indoor mold, dust, physicochemical, glucophilic, cellulose-decomposing

INTRODUCTION

Dust formation occurs as a result of the ongoing elutriation of airborne organic and inorganic particulate matter that originates from a multiplicity of indoor and outdoor sources. In recent years, the quality of indoor air has been the subject of several studies. Conditions increasing air humidity, decreased ventilation and increased moisture level subsequently increase the proliferation of fungi and bacteria (Ruest, 2004). These fungal elements may cause severe illness as a result of indoor mold exposure including pulmonary, immunologic, neurologic and oncologic disorders. (Kuhn and Ghannoum, 2003). The most common fungal mycotoxins come from the *Penicillium* and *Aspergillus* genera. However, in indoor environments, the array of mycotoxins are quite different, which includes less common mycotoxins from *Stachybotrys* and *Chaetomium* fungi (Jarvis and Miller, 2005). In a study conducted in 2004, *Aspergillus* spp. was the most prevalent fungal isolate present in 31-40% of samples followed by *Penicillium* and *Cladosporium* sp.

(Pieckova and Wilkins, 2004). In a separate report on house dust mycoflora, *Deuteromycota* genera predominated in the isolates (59.4% of total fungi), followed by *Penicillium* and *Cladosporium* (Noritzuna, 2002). Indoor air levels of molds are insignificantly higher compared to that of outdoor levels of molds (Jovanovic, 2001). The results from several studies showed that there is a direct correlation between the levels of mycofloral contamination in household dust to the occurrence of respiratory diseases, especially asthma and sinusitis (Porter *et al*., 2009; Cho, 2008; Woodcock *et al*., 2006; Salo *et al*., 2005; De Blay, 2000). Saudi Arabia is a country with very little rainfall occurrence per year. Most of the time, the country experiences sandstorms thus increasing the propensity of indoor household fungal contamination. Two previous studies on fungal flora in house dust samples in Riyadh conducted in 1990 and 1999 reported higher concentrations of fungal colonies in the room air conditioner and living room compared to other locations of the house, with Aspergillus predominating the isolated colonies (Bahkali and Parvaez, 1999; Saad and El-Gindy, 1990). Al-Sheikh (2008) published a report on the airborne mycoflora in schools environment in Saudi Arabia. In this report, *Aspergillus* predominated the isolated mycoflora. Since then, no follow-up studies had been made for screening the mycological biodiversity in indoor household dust in Saudi Arabia. Hence, this investigation represents an attempt for studying the occurrence and distribution of terrestrial fungi inhabiting indoor household dust samples in Riyadh region, Saudi Arabia.

MATERIALS AND METHODS

Collection of dust samples: Dust samples were collected in vacuum cleaner plastic bags from 60 different households in Riyadh, Saudi Arabia in the spring of 2009 during the months of March and April. These dust samples were sifted, under aseptic conditions; through an 80-mesh sieve to remove large dust particles and were kept at 2-5°C until fungal analysis was completed.

Mycological analysis of terrestrial fungi in dust samples: The dilution-plate method (Warcup, 1955; Garrett, 1981) was adopted for the determination of terrestrial mycobiota inhabiting the collected household dust samples. One milliliter of diluted dust sample was transferred under aseptic conditions into each of eight clean and sterilized Petri-dishes (10 cm diameter, each) using a Menziess dipper. Glucose (10 g L^{-1}) and cellulose (20 g L^{-1}) plus Czapek´s Dox agar media were used as isolation media. Glucose or cellulose replaced sucrose (30 g L^{-1}), for the isolation of glucophilic and cellulose-decomposing fungi, respectively. Rose Bengal (1/1500) and Chloromycetin (chloramphenicol) (0.05 mg mL^{-1} medium) were added and served as bacteriostatic agents. Four plates were supplied for each type of media. The plates (after agar solidification) were, then, incubated at 28°C for two weeks and the developing colonies of terrestrial fungi were counted, identified (purely morphologically, depending upon macro- and microscopic characteristics) and the numbers were assessed and calculated per g dry dust particles.

Physico-chemical analysis of dust samples: The collected household dust samples were subjected to physicochemical analysis. The physicochemical characteristics included pH value, total soluble salts and organic matter contents as per Jeffery (2003).

- **pH:** pH values of the dust samples were determined in the laboratory using digital pH meter model: w/w pH 9.0. The electrode was immersed in the dust suspension with a ratio of 1: 5 (w/v) to avoid higher dilution errors

- **Total soluble salts:** A known weight of dust sample was diluted in certain volume of distilled water and shaken for about half an hour, then, the mixture was left overnight to settle down. Thereafter, the dust extract was filtered and a known value was evaporated in an oven at 105°C. The dry residue weighed and the quantity of total soluble salts in oven-dry dust was calculated
- **Organic matter content:** A known weight of dust sample was digested by chromic acid for the oxidation of organic matter to carbon dioxide. The excess of chromic acid was back titrated against standard ferrous sulfate solution using diphenylamine as an indicator

Identification of terrestrial fungi isolated from dust samples: Terrestrial fungal genera and species were identified directly from colonies on Rose Bengal isolation media using features of macroscopic and microscopic examination, standard reference works and appropriate monographs (Moubasher, 1993).

RESULTS AND DISCUSSION

The results of physicochemical analysis revealed that the pH values of the tested dust samples were alkaline (range: 7.47 to 10.16). This is in concordance with the report on El-Nagdy and Nasser, 2000 when they analyzed the soil and mud samples from Riyadh, Saudi Arabia. (El-Nagdy and Nasser, 2000) Furthermore, it was observed that the pH values of the investigated samples did not display any role governing the occurrence and distribution of the recoverable terrestrial fungi (El-Nagdy and Nasser, 2000; Ali and Nasser, 2001).

Most of the tested dust samples contain low amount of total soluble salts ranging from 7.25-0.12% (g/100 g dry dust). Also, the dust samples content of total soluble salts showed no influence on the variety of terrestrial fungi isolated. The chemical analysis of dust samples indicated that these samples were generally poor in organic matter and the amount fluctuate between 0.029 and 0.536% (g/100 g dry dust). Dust samples with relatively higher organic matter content showed greater concentrations of terrestrial fungal species. Similar results were also shown by El-Nagdy and Nasser (2000).

In this study, a total of 71 species belonging to 24 genera of terrestrial fungi were isolated from the 60 indoor dust samples on glucose-and cellulose-Czapek´s Dox agar media at 28°C. The genera *Aspergillus* (16 species and one variety) and *Penicillium* (11 species) contributed the majority of the isolated mycoflora of terrestrial fungi. *Acremonium*, *Botryodiplodia*, *Circinella*, *Myrothecium* and *Syncephalastrum* were represented by only one species. This is in exact concordance with the report made by Pieckova and Wilkins (2004) wherein *Aspergillus* and *Penicillium* predominate among the mycoflora isolated from house dust. This is in contrast to the report by Jarvis and Miller (2005) that *Penicillium* and *Aspergillus* may not be the predominant species in indoor dusts but rather *Stachybotrys* and *Chetomium* (Table 1).

Also in this study, 61 species in addition to one variety representing twenty genera of terrestrial fungi were identified and recovered on glucose-Czapek´s Dox agar medium (Table 1). Cellulose produced a narrower spectrum of species than on glucose-agar where 59 species and one species variety attributing 22 terrestrial fungal genera were identified and recovered on cellulose-Czapek´s agar medium. This result is to be expected since glucose is more easily utilizable as source of carbohydrate and by fungi. The most frequently isolated species of terrestrial fungi during this on both media of isolation were *Aspergillus niger*, *Aspergillus flavus*, *Penicillium funiculosum* and *Penicillium chrysogenum*, all belonging to the two major predominating mycoflora, *Aspergillus* and *Penicillium*.

Table 1: Total counts (TC, calculated per mg dust sample) No. of cases of isolation (NCI, out of sixty dust samples) and Occurrence Remarks (OR) of genera and species of terrestrial fungi recovered on glucose- and cellulose- Czapek´s agar at 28°C from sixty indoor dust samples, which were collected from Riyadh region

	Media of isolation			
	Glucose		Cellulose	
Fungal genera and species	TC	NCI and OR	TC	NCI and OR
Acremonium strictum	-	-	9.2	9L
Alternaria		24M	15.6	20M
A. alternatar	0.0	20M	12	16M
A. tenuissima		5R	2.8	2R
A. chlamydospora	6.8	-	0.8	2R
Aspergillus		56H	223.7	51H
A. aculeatus	0.2	3R	-	-
A. candidus		5R	1.6	3R
A. carneus		8L	4.4	3R
A. flavipes	78.6	15M	6.4	11L
A. flavus		38H	67.6	41H
A. flavus var. columnaris	0.4	9L	13.6	6R
A. fumigatus		8L	16.8	17M
A. japonicus	0.4	3R	-	-
A. melleus	5.4	2R	4.8	6R
A. niger	15.2	47H	93.3	43H
A. ochraceous	52.8	6R	3.6	4R
A. oryzae	28.0	-	3.2	2R
A. sydowii	7.6	6R	5.2	8L
A. tamarii	7.2	2R	-	-
A. terreus	4.4	2R	-	-
A. versicolor	204.4	9L	-	-
A. wentii	10.0	11L	3.2	3R
Botryodiplodia theobromae	-	-	9.6	8L
	5.6	17M	22.8	23M
	4.0			
	2.4			
	6.0			
	18.8			
	-			
	9.9			
Botryotrichum				
B. atrogriseum	4.8	11L	15.6	18M
B. piluliferum	5.1	6L	7.2	5R
Chaetomium	60	10L	49.2	21M
C. globosum .	60	10L	42.8	16M
C. spirale	-	-	6.4	5R
Circinella muscae	-	-	3.2	3R
Cladosporium	26.4	31H	40.4	29M
C. cladosporioides	20.4	27M	36	22M
C. herbarum	6	4R	4.4	7L
Cunninghamella	6.8	8L	5.6	11L

Table 1: Continued

	Media of isolation			
	Glucose		Cellulose	
Fungal genera and species	TC	NCI and OR	TC	NCI and OR
C. echinulata	6.8	8L	4.4	9L
C. elegans	-	-	1.2	2R
Curvularia	4.7	5R	7.8	8L
C. lunata	4.4	4R	6.4	7L
C. ovoidea	0.3	1R	1.4	1R
Emericella	10.8	11L	5.6	6R
E. nidulans	6.8	8L	4.4	5R
E. quadrilineata	2	3R	1.2	1R
E. rugulosa	2	2R	-	-
Eurotium	11.6	11L	4	7L
E. amestelodami	8.4	8L	2.4	4R
E. montevidensis	1.2	2R	0.8	2R
E. repens	1.2	1R	0.8	1R
E. rubrum	0.8	1R	-	-
Fusarium	16.8	23M	28	30M
F. equiseti	3.6	6R	5.6	9L
F. moniliforme	8	9L	15.6	13L
F. oxysporum	5.2	8L	5.6	6R
F. solani .	-	-	1.2	2R
Humicola	7.6	10L	-	-
H. fuscoatra	2.4	2R	-	-
H. grisea	5.2	8L	-	-
Mucor	9.6	19M	8	13L
M. circinelloides	7.6	15M	4.4	9L
M. hiemalis	0.8	2R	2.8	3R
M. racemosus	1.2	2R	0.8	1R
Paecilomyces	11.2	9L	17.6	17M
P. lilacinus	2.8	3R	1.2	1R
P. variotii	8.4	6R	16.4	16M
Penicillium	112.4	42H	60.8	38H
P. brevicompactum	15.2	18M	13.2	16M
P. chrysogenum	24.8	31H	14.4	17M
P. citrinum	14.4	11L	8.8	8L
P. funiculosum	24.4	35H	10.8	16M
P. glabrum	10	8L	-	-
P. islandicum	4.4	5R	2.8	3R
P. janthinellum	2.8	3R	1.2	2R
P. jensenii	2	2R	-	-
P. purpurogenum	2.8	4R	8.8	8L
P. verruculosum	4.4	5R	-	-
P. waksmanii	7.2	8L	0.8	1R
Phoma herbarum	-	-	30.4	10L
Rhizopus	9.6	18M	7.6	11L

Table 1: Continued

	Media of isolation			
	Glucose		Cellulose	
Fungal genera and species	TC	NCI and OR	TC	NCI and OR
R. rhizopodiformis	8.4	16M	5.6	8L
R. stolonifer	1.2	2R	2	3R
Scopulariopsis	2	3R	4.4	8L
S. brevicaulis	2	3R	3.2	6R
S. konigii	-	-	1.2	2R
Syncephalastrum racemosus	5.6	8L	-	-
Trichoderma	13.2	18M	19.6	22M
T. hamatum	2.8	4R	6.8	8L
T. viride.	10.4	14L	12.8	14L
Ulocladium	2.8	4R	7.2	10L
U. alternariae (CKE) Simmons	0.8	2R	1.2	1R
U. atrum preuss	2	2R	6	9L

OR: Occurrence remarks; H: High occurrence (> 30 samples out of sixty); M: Moderate occurrence (between 15-30 samples); L: Low occurrence (between 7-14 samples); R: Rare occurrence (<7 samples)

Glucophilic fungi (recovered on glucose Czapek´s Dox agar at 28ºC): Among the glucophilic fungi, three genera were recorded and classified as high frequency of occurrence; *Aspergillus* (15 species+ one variety), *Penicillium* (11 species) and *Cladosporium* (2 species). The genera *Alternaria* (2 species), *Fusarium* (3 species), *Mucor* (3 species), *Rhizopus* (2 species) and *Trichoderma* (2 species) and *Botryotrichum* (2 species) were recovered in moderate frequency. Seven genera of terrestrial fungi were regarded as of low frequency of occurrence namely; *Eurotium* (4 species) and *Emericella* (3 species), *Humicola, Paecilomyces* (2 species each), *Chaetomium, Cunninghamella, Syncephalastrum* (one specie each). The remaining three genera; *Curvularia, Ulocladium* (2 species each) and *Scopulariopsis* (one specie) of terrestrial fungi were encountered (3-5 dust samples out of 60 tested samples) and recovered in rare occurrence.

The most prevalent species of terrestrial fungi which were repeatedly recovered on glucose-Dox agar medium were; *Aspergillus niger, Aspergillus flavus, Penicillium funiculosum, Penicillium chrysogenum, Cladosporium cladosporioides, Alternaria alternata, Penicillium brevicompactum, Rhizopus rhizopodiformis, Aspergillus flavipes, Mucor circinelloides.* The most commonly occurring species found in the present investigation are consistent findings on the species composition of households dust by other workers worldwide (Jarvis and Miller, 2005; Pieckova and Wilkins, 2004; Noritzuna, 2002) However, It is worth mentioning that 3 species of terrestrial fungi were identified and isolated only once (from only one dust sample) and these namely; *Curvularia ovoidea, Eurotium repens* and *E. rubrum.*

Cellulose-decomposing fungi (recovered on cellulose Czapek´s Dox agar at 28°C): Table 1 also shows that 59 species in addition to one variety representing 22 terrestrial fungal genera were identified and isolated on cellulose-Czapek´s Dox agar medium at 28°C. *Aspergillus* (11 species+one variety), *Penicillium* (8 species) and *Fusarium* (4 species) were the most prevalent genera of terrestrial fungi. They occurred very high (51, 38 and 33 samples out of 60 tested dust samples). Six genera were recorded, identified and isolated in moderate occurrence (20-29 dust

samples out of sixty samples) namely; *Alternaria* (3 species), *Botryotrichum*, *Chaetomium*, *Cladosporium*, *Paecilomyces* and *Trichoderma* (2 species each). *Eurotium*, *Mucor* (3 species), *Cunninghamella*, *Curvularia*, *Rhizopus*, *Scopulariopsis*, *Ulocladium* (2 species), *Acremonium*, *Botryodiplodia* and *Phoma* (1 specie each), were occurred in low frequency (recovered from 7-13 out of 60 tested dust samples). *Circinella* (1 specie in 3 samples) and *Emericella* (2 species in 6 dust samples) were the less frequently isolated.

The most prevalent terrestrial fungal species on cellulose-Dox agar medium were; *A. niger*, *A. flavus*, *Cladosporium cladosporioides*, *Botryotrichum atrogriseum*, *A. fumigatus*, *P. chrysogenum*, *Chaetomium globosum*, *Alternaria alternata*, *Fusarium moniliforme*, *Pacilomyces variotii*, *Penicillium brevicompactum*, *Penicillium funiculosum* (43, 41, 22, 18, 17, 17, 16, 16, 16, 16, 16 and 16 out of 60 dust samples, respectively). The majority of the preceding species of terrestrial fungi have been previously isolated on agar plates and they were reported to be cellulose decomposers by several workers (Pieckova and Wilkins, 2004, Woodcock et al.,2006). Our findings is in accordance with the findings of Pieckova and Wilkins, 2004 that showed Penicillium species and Aspergillus species as the predominating species up to 40% of the samples. Other genera including Cladosporium, Alternaria, Chaetomium, Paecilomyces were isolated in less than 8%. It is interesting to notice that some species of terrestrial fungi were isolated only once (from only one dust sample) and these were; *Curvularia ovoidea*, *Emericella quadrilineata*, *Eurotium repens*, *Mucor racemosus*, *Paecilomyces lilacinus*, *Penicillium waksmanii* and *Ulocladium alternariae*.

In summary, present study on mycological analysis of the tested indoor household dust samples showed the occurrence of a large number of potential human pathogenic fungi including members of *Alternaria* and *Emericella*, aside from the more common *Aspergillus* and *Penicillium*. Furthermore, this study confirms the reliability of house dust sampling as a complementary tool for diagnosis and assessment of mycoflora contamination of households. Dust from unhealthy homes thus, can contain more molds than their healthy counterparts. Therefore, it is advised to take extra precautionary measures to determine the presence of molds in houses. Odors and visible signs of moisture may be tell-tale signs that a mold problem may be present.

REFERENCES

Al-Sheikh, H., 2008. Air-borne mycoflora in the schools environment in hofuf, Al Hassa province of Saudi Arabia. Saudi J. Biol. Sci., 15: 237-241.

Ali, E.H. and L.A. Nasser, 2001. Incidence of mycobiota (Zoosporic and terrestrial fungi) in accumulated rainfall water and submerged mud from three common valleys in the South-Eastern region, Saudi Arabia. Bull. Fac. Sci. Assiut Univ., 30: 195-208.

Bahkali, A.H. and S. Parvaez, 1999. Fungal flora in house dust in Riyadh, Saudi Arabia. Mycoses, 42: 339-343.

Cho, S.J., G. Ramachandran, J. Grengs, A.D. Ryan, L.E. Eberly and J.L. Adgate, 2008. Longitudinal evaluation of allergen and culturable fungal concentrations in inner-city households. J. Occup. Environ. Hyg., 5: 107-118.

De Blay, F., S. Casel, F. Colas, F. Spirlet and G. Pauli, 2000. Elimination of airborne allergens from the household environment. Rev. Mal. Respir., 17: 29-39.

El-Nagdy, M.A. and L.A. Nasser, 2000. Occurrence of zoosporic and terrestrial fungi in accumulated rainfall water in the Riyadh region (Saudi Arabia). Fungal Diversity, 5: 175-183.

Garrett, S.D., 1981. Soil Fungi and Soil Fertility. 2nd Edn., Pergamon Oxford, New York.

Jarvis, B.B. and J.D. Miller, 2005. Mycotoxins as harmful indoor air contaminants. Applied Microbiol. Biotechnol., 66: 367-772.

Jeffery, N.L., M.S.C. D'Andrea and J. Leighton, 2003. Potential exposures to airborne and settled surface dust in residential areas of lower Manhattan following the collapse of the World Trade Center-New York City, Nov. 4-Dec. 11,2003. Morbidity and Mortality Weekly Report, pp: 52. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5207a3.htm.

Jovanovic, S.I., I. Piechotowski, T. Gabrio, U. Weidner and I. Zollner *et al.*, 2001. Assessment of mould pollution in residences in Southwest Germany. Gesunheitswesen, 63: 404-411.

Kuhn, D.M. and M.A. Ghannoum, 2003. Indoor mold, toxigenic fungiand Stachybotrys chartarum: Infectious disease perspective. Clin. Microbiol. Rev., 16: 144-172.

Moubasher, A.H., 1993. Soil Fungi in Qater and other Arab Countries. The Scientific and Applied Research, University of Qatar, Doha, Qatar.

Noritzuna, T., 2002. A study of fungal flora of house dust and its importance to public health. J. Jap. Assoc. Mycotic, 52: 45-55.

Pieckova, E. and K. Wilkins, 2004. Airway toxicity of house dust and its fungal composition. Ann. Agric. Environ. Med., 11: 67-73.

Porter, P., S.C. Susarla, S. Polikepahad, Y. Qian and J. Hampton *et al.*, 2009. Link between allergic asthma and airway mucosal infection suggested by proteinase-secreting household fungi. Mucosal. Immunol., 2: 504-517.

Ruest, K., 2004. House dust: A useful tool to assess microbial contamination in homes. Research Highlight Technical Series 2004. www.cmhc-schl.gc.ca/odpub/pdf/63407.pdf.

Saad, R.R. and A.A. El-Gindy, 1990. Fungi of the house dust in Riyadh, Saudi Arabia. Zentrabl Microbiol., 145: 65-68.

Salo, P.M., M. Yin, S.J. Arbes, R.D. Cohn and M. Sever *et al.*, 2005. Dustborne alternaria alternate antigens in US homes: Results from the national survey of lead and allergens in housing. J. Allergy Clin. Immunol., 116: 623-629.

Warcup, J.H., 1955. On the origin of colonies of fungi developing on soil dilution plates. Trans. Br. Mycol. Soc., 38: 298-301.

Woodcock, A.A., N. Steel, C.B. Moore, S.J. Howard, A. Custovic and D.W. Denning, 2006. Fungal contamination of bedding. Allergy. 61: 140-142.