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Fungi and some mycotoxins producing species in the air of soybean and cotton mills: a case study

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ABSTRACT

The aim of the present study was to identify fungi and their levels in the air of cotton and soybean industry work–places with a focus on the ability of some *Aspergillus, Penicillium* and their teleomorphic species for producing mycotoxins. Air samples were collected by passive sedimentation on Petri plates containing 2% malt extract agar, for 10 min. Various allergenic, opportunistic and toxigenic species were found. *Aspergillus flavus, Aspergillus niger, Penicillium nigricans, Alternaria alternata* and *Cladosporium cladosporoides* were the predominant species. Many of the identified species have aerodynamic diameter (D_{ae}) <5 µm that can deeply penetrate into the lungs. Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) were used to screen some strains for mycotoxin production. *Aspergillus parasiticus I* and *II* were the greatest aflatoxins (AFT) producers in the soybean and cotton mills, respectively. Blue fluorescence (B_1) was the major AFT produced by toxigenic fungi. It is unsure whether inhalation of toxigenic fungal spores leads to subsequent production of mycotoxin into the lungs and further work is required.

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1. Introduction

It is increasingly being suggested that fungi in indoor air are responsible for causing adverse health effects (Ren et al., 2001). Exposure to fungi has been reported to cause irritations, allergies, toxic effects and aspergillosis (Repp and Muller–Wening, 1989; Mota et al., 2008). Mycotoxins are secondary toxic metabolites produced by fungi. There are at least 21 different mycotoxin classes (Cole and Cox, 1981) with over 400 individual toxins produced by at least 350 fungi (Tuomi et al., 2000). Mycotoxins are potentially hazardous to man and animal health (Turner et al., 2009) causing cancer and serious diseases (Hendry and Cole, 1993; McPhaden et al., 2006). *Aspergillus*, *Fusarium*, *Penicillium* and *Stachybotrys* are the major genera producing mycotoxins (Hintikka and Nikulin, 1998). Ochratoxin A (OTA) is produced by some species such as *Aspergillus ochraceous* mainly in tropical regions and by *Penicillium verrucosum* in temperate ones (Thrane, 1989). Aflatoxins (AFT) are produced by *Aspergillus flavus* and *Aspergillus parasiticus* which are common contaminants in agricultural products. Based on their fluorescence under ultraviolet light (blue or green) and relative chromatogenic mobility during TLC analysis, B_1, B_2, G_1 and G_2 are the major AFT (Bennett and Klich, 2003).

Ingestion of mold foodstuff is the usual means of conveying the toxin into the body. AFT and OTA have been implicated in liver cancer and kidney damage, respectively (Hintikka and Nikulin, 1998). In recent times, concerns have been raised about inhalation exposure to mycotoxin–producing fungi in indoor air. Exposure can occur by inhalation of spores, hyphae fragments and substrate materials (Morey, 1993). Sorenson et al. (1984) reported that AFT and other toxins may be airborne in high concentrations in some work environments. Inhaled mycotoxins producing fungi can reach the alveoli which may affect our immune responses; others can interact with foreign particles and may have to be cleared by macrophage cells (ACGIH, 1999).

Workers in agricultural related industries and workplaces are exposed to various allergenic or toxigenic fungal species. The present study aims to identify fungi in the indoor air of cotton and soybean mills to screen the common *Aspergillus*, *Penicillium* and their teleomorphic species for mycotoxins production in order to study the presence of such toxigenic mold in workplaces.

2. Materials and Methods

2.1. Air sampling

Air samples were collected at two agricultural related work–places, soybean and cotton mills located in Giza, Egypt. At the soybean mill the air samples were collected from wet line (producing milk) and store unit. At the cotton mill (producing various types of fine, soft and thick threads) samples were collected from the carding and spinning departments. These mills differ in the type of product, number of workers, mode of the work and raw materials.

Temperature ranged between 15–37 °C and 16–34 °C with mean values of 28.4 °C and 27 °C in the soybean and cotton mills, respectively. Relative humidity ranged between 40–78% and 43–

69% with mean values of 58% and 54% in the corresponding mills, respectively.

A total of 348 samples were taken between January and December, 2006 using passive sedimentation plates containing 2% malt extract agar (Difco, Detroit, MI) supplemented with chloramphenicol at a concentration of 50 ppm, for 10 min. Air samples were collected at a height of 1.5 meter, the breathing zone, at the centre of the indoor workplaces. Fungal plates were incubated at 28°C for 7 days. The fungal concentration was calculated according to Omeliansky (1940) using the following formula:

$$N = 5\ a\ 10^4\ (b\ t)^{-1} \quad (1)$$

where N is the fungal concentration (CFU m^{-3}), a is the number of colonies per Petri dish, b is the area of dish (cm^2), and t is the exposure time (min).

The resultant colonies were counted and the concentrations were expressed as colony forming unit per cubic meter of air (CFU m^{-3}). The resultant fungal colonies were studied using macroscopic and microscopic features indicated in the literature to identify their species (Raper and Fennell, 1965; Ellis, 1971; Singh et al., 1991; Barnett and Hunter, 1999). The aerodynamic diameter (D_{ae}) was calculated from the density (1 g cm^{-3}), shape and physical diameter (Hinds, 1982).

2.2. Fungi producing mycotoxin

Representative *Aspergillus, Penicillium* and their teleomorphic strains were selected to study mycotoxins production. The strains were cultured on Potato dextrose agar (PDA), (Difco, Detroit, MI) plates, and incubated at 28°C for 7 days. An inoculum of the cultured PDA was inoculated, in duplicate, in flasks containing 50 mL 2% yeast broth and incubated at 28°C for 15 days. The cultures were filtered through Whatman paper No. 42 and the dry weights were homogenized to release spore contents while the filtrates were used for the qualitative and quantitative deter–mination of mycotoxins.

2.3. Preparation for column chromatography

A ball of glass wool was placed in the bottom of 22 × 300 mm glass column. Five grams of anhydrous sodium sulfate (analytical grade) was added as a bed to give base of the column and then ten grams of silica gel G60 (70–230 mesh) was added using 20 mL chloroform. Twenty five mL of the fungal extract was added to the column and the extract was washed using 100 mL hexane followed by 100 mL diethylether. AFT were eluted from the column using 100 mL methanol: chloroform in ratio of 3:97%. The extract was evaporated to dryness in a ventilated cupboard and the residue was dissolved in 200 µL of benzene:acetonitrile 98:2 (v/v). The extract was used for qualitative and quantitative estimation of mycotoxins using TLC and HPLC, respectively.

2.4. TLC technique

Twenty µL of the extract, AFT and OTA standards (Sigma–Aldrich Laborchemikalien, Germany) were spotted on the baseline (1 cm) of a TLC plate (20 x 20 cm, aluminum sheet precoated with silica gel). The distance between each spot was around 1.2 cm. Mycotoxins were developed with toluene:ethylacetate:formic acid 6:3:1 (v:v:v) solvent system. The plate was removed from the jar and dried at the room temperature. Mycotoxin spots were identified under UV 365 nm lamp fitted in a black cabinet. AFT– B_1 and B_2 show blue fluorescence and G_1 and G_2 show green fluorescence whereas OTA shows green color. The rate of flow (Rf), color and intensity of fluorescence of the extracts were compared with different concentrations of reference of B_1, B_2, G_1, G_2 and OTA (AOAC, 1995). The intensity of the fluorescence was expressed by a variable numbers of "+" signs (Lin and Dianese, 1976).

2.5. HPLC technique

The residue of TLC's positive samples were dried and derivatized by hexane and trifluoroacetic acid (AOAC, 1995). Then 1.95 mL water:acetonitrile (9:1) was added and vigorously mixed for 1.0 minute. The solution was left for 10 min for the 2 layers to separate. The lower aqueous layer was used for HPLC analysis. The HPLC system Agilent (1100–series) equipped with Auto–sampler (model G 1329 A), fluorescence detector (model G 1321A) and C18 column was used to determine B_1, B_2, G_1, G_2 and OTA. The mobile phase was methanol:water:acetonitrile: 30:60:10 v/v/v. The data obtained were integrated and concentrations determined by Chemstation Software (Ayesh et al., 2003).

3. Results

Airborne fungal counts ranged between $6x10^2$–$1.66x10^4$ CFU m^{-3} and $7x10^1$–$2.4x10^3$ CFU m^{-3} with median values of $2.2x10^3$ CFU m^{-3} and $2x10^3$ CFU m^{-3} in the soybean and cotton mills. A total of 50 species ascribed to 32 fungal genera and 42 species ascribed to 28 fungal genera were identified in the air of soybean and cotton mills, respectively (Table 1). *Aspergillus*, *Alternaria*, *Cladosporium*, *Fusarium* and *Penicillium* were the predominant fungal genera in the soybean and cotton mills, respectively. *Aspergillus flavus, Aspergillus niger and Aspergillus fumigatus* were the predominant species in both mills. *Penicillium nigricans*, *Penicillium compactum* were the dominant *Penicillium* species. *Cladosporium* was only represented by *Cladosporium cladosporoides* at both industry mills.

Aspergillus carneus, *Aspergillus ustus*, *Cephalosporium irregularis*, *Cunninghamella elegens, Phoma suadae, Syncephalastrum, Scopulariopsis brevicualis,* and *Trichoderma koningin* were only detected in the soybean mill while, *Botrytis cinerae*, and *Stachybotryis atra* were only detected in the cotton mill.

Storage (fungi adept at growing in bad storage conditions), allergenic (fungal spores which induce respiratory allergy symptoms), opportunistic (fungal species capable of causing infection under favorable condition) and immunotoxic (fungi produce mycotoxins which suppress immune system) fungal species were found in the air of both plants. Allergenic and toxigenic fungi were represented by *Aspergillus flavus*, *Aspergillus parasiticus*, *Alternaria chlamydosporae*, *Penicillium nigricans* and *Penicillium brevicompactium*. Storage fungi included *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus candidus*, *Absidia*, *Botrytis*, *Eurotium*, *Mucor racemosus* and *Rhizopus stolonifer* (Table 1).

A total of 16 and 12 *Aspergillus*, *Penicillium* and their teleomorphic species were found to be mycotoxin producers in the cotton and soybean mills, respectively (Table 2). The low AFT producers included *Emericella nidulans* and *Penicillium roqueforti* in the soybean mill as well as *Aspergillus flavus* V and VII and *Penicillium brevicompactum* in the cotton mill. Medium AFT producers included *Aspergillus flavus* VI; *Aspergillus versicolor*; *Aspergillus nidulans*; *Penicillium brevicompactum*; *Penicillium roqueforti* and *Penicillium rubrum* as well as *Aspergillus flavus* VI and VII*; Aspergillus versicolor; Penicillium funicuolosum; Aspergillus nidulans; Penicillium brevicompactum* and *Penicillium roqueforti* in the soybean and cotton mill, respectively. High AFT producers included *Aspergillus parasiticus* I and II, *Aspergillus flavus* I, II, III and V, *Emericella nidulans*, *Penicillium rubrum*, *Penicillium funicolosum*, and *Paecilomyces variotii* as well as *Aspergillus parasiticus* III, *Emericella nidulans*, *Penicillium brevicompactum*, *Penicillium rubrum* and *Penicillium funicolosum* in the corresponding workplaces, respectively (Table 2).

Table1. *Range and median counts of airborne fungi and the numbers, percentages and D_{ae} of the identified species in the soybean and cotton mills*

	Soybean mill		Cotton mill		
	Number	**(%)**	**Number**	**(%)**	**D_{ae} (μm)**
Aspergillus	**6 626**	**54.4**	4 825	**46.17**	
Asp. flavus [a,b]	2 782	22.83	**1 860**	17.80	4.5
Asp. niger [a,b]	2 603	21.45	2 289	21.91	3.8
Asp. fumigatus [b,c,e]	570	4.67	244	2.34	2.7
Asp. terreus [a]	270	2.22	47	0.45	2.1
Asp .parasiticus [b,c]	231	1.9	229	2.19	4.3
Asp. versicolor	75	0.62	89	0.85	2.5
Asp. ochraceus [c]	34	0.28	23	0.22	3.7
Asp. sydowii	31	0.25	35	0.33	3.2
Asp. nidulans	10	0.08	–	–	3.5
Asp. candidus [a,b]	7	0.05	9	0.09	3.0
Asp. ustus [d]	9	0.07	–	–	3.5
Alternaria	**774**	**6.1**	**1 138**	**10.89**	
A. alternata [b,c]	501	4.11	610	5.84	$9^{f} \times 17^{g}$
A.chlamydosporae [b,c]	273	2.24	528	5.05	$10^{f} \times 18^{g}$
Absidia sp.[a]	31	0.25	8	0.08	5.5
Acremonium terricola [e]	155	1.27	187	1.79	2.5
Actinomucor cicinelloides	8	0.06	3	0.03	5.2
Botryotrichum album	4	0.03	6	0.06	4.3
Botrytis cinerae [a]	–	–	3	0.03	6.0
Chaetomium globosum [d]	12	0.09	7	0.07	13
Cephalosphora irregularis [d]	2	0.016	–	–	6.0
Cunninghamella elegans	6	0.049	–	–	4.0
Curvularia lunata	24	0.19	11	0.11	$10^{f} \times 16^{g}$
Cladosporium cladosporoides [b]	2 340	19.19	1 740	16.65	3.5
Drechslera spicifera	97	0.79	126	1.21	$12^{f} \times 28^{g}$
Epicoccum nigrum	52	0.42	92	0.88	17
Emericella nidulans	110	0.90	2	0.02	4.0
Eurotium amstelodami [a]	7	0.05	3	0.03	5.2
Fusarium	**739**	**6. 06**	**772**	**7.39**	
F. chlamydospora [c]	352	2.88	225	2.15	$2.5^{f} \times 10^{g}$
F. poae [c]	226	1.85	328	3.14	$2^{f} \times 6^{g}$
F.monilifera [c]	161	1.32	219	2.10	$2.6^{f} \times 8^{g}$
Monillia sp.[d]	4	0.03	2	0.02	3.5
Mucor racemosus[a]	35	0.28	21	0.20	5.4
Nigrospora oryzae	42	0.34	95	0.91	11.2
Paecilomyces variotii	21	0.17	81	0.78	2.5
Penicillium	**624**	**5.11**	**1 059**	**10.13**	
P.nigricans [b,c]	187	1.53	129	1.23	2.5
P.brevicompactum [b,c]	144	1.18	165	1.58	2.3
P.funicuolosum [b,c]	119	0.97	269	2.57	2.5
P.rubrum [b,c]	101	0.82	332	3.18	2.5
P.roqeforti	73	0.59	164	1.57	2.5
Phoma	6	0.04	–	–	–
Rhizopus stolonifer [a]	80	0.65	28	0.27	6.5
Syncephalastrum sp.	3	0.024	–	–	5.5
Sepedomium album	8	0.065	4	0.04	8
Trichothecium album	13	0.10	43	0.41	$7^{f} \times 13^{g}$
Stemphylium botryosum	8	0.07	31	0.29	$9^{f} \times 15^{g}$
Scopulariopsis candida [b]	9	0.07	2	0.02	5.3
Scopulariopsis brevicaulis [b]	2	0.16	–	–	3.5
Stachybotrys atra [c]	–	–	3	0.03	$10^{f} \times 16^{g}$
Trichoderma koningii	4	0.03	–	–	3.5
Trichoderma koningii	4	0.03	–	–	3.5
Ulocladium atrum	18	0.15	27	0.26	$9^{f} \times 16^{g}$
Yeast	324	2.65	130	1.24	4.0
Total isolates	**12 188**	**100**	**10 449**	**100**	
Total count CFU/m^3 x 10^3	**Range**	**Median**	**Range**	**Median**	
	0.60–16.60	2.20	0.07–24.0	2.00	

[a] *storage fungi,* [b] *allergenic,* [c] *immunotoxic,* [d] *infectious,* [e] *opportunistic,* [f] *short axis,* [g] *long axis, D_{ae}: aerodynamic diameter*

Table 2. *Screening of some mycotoxins producing fungal species isolated from soybean and cotton mills using TLC*

Species	AFT– types intensity				OTA intensity
	G_1	B_1	G_2	B_2	
Soybean mill					
Aspergillus flavus I	> ++	++	–	> ++	
Aspergillus flavus II	++	> ++	–	++	
Aspergillus flavus III	+	> ++	–	++	
Aspergillus flavus VI	–	++	–	+	
Aspergillus flavus V	–	> ++	–	> ++	
Aspergillus parasiticus I	> ++	> ++	> ++	> ++	
Aspergillus parasiticus II	> ++	> ++	> ++	> ++	
Aspergillus versicolor	++	–	–	–	
Aspergillus ochraceous	–	–	–	–	> ++
Aspergillus nidulans	–	++		++	–
Emericella nidulans	+	–		> ++	> ++
Paecilomyces variotii	–	++		++	> ++
Penicillium brevicompactum	++	–			–
Penicillium funicuolosum	–	++			> ++
Penicillium roqefortii	–	++		+	–
Penicillium rubrum	–	++		++	> ++
Cotton mill					
Aspergillus flavus V	–	+		+	
Aspergillus flavus VI	–	++		++	
Aspergillus flavus VII	–	++		+	
Aspergillus parasiticus II	> ++	> ++	> ++	> ++	
Aspergillus versicolor	–	–			++
Aspergillus ochraceus	–	–			> ++
Emerciella nidulans	–	>++			+
Penicillium nigricans	–	++			> ++
Penicillium brevicompactum	+	> ++		> ++	> ++
Penicillium funicuolosum	–	++		> ++	++
Penicillium roqeforti	–	–		++	> ++
Penicillium rubrum	–	–		> ++	++

(–) None, (+) Low, (++) Medium, (> ++) High, I, II and III different varieties of the same fungus species

High OTA producers included *Aspergillus ochraceus, Emericella nidulans, Paecilomyces variotii, Penicillium funicolosum* and *Penicillium rubrum* in the soybean mill and *Aspergillus ochraceus, Penicillium nigricans, Penicillium roqueforti* and *Penicillium brevicompactum* in the cotton mill. Low OTA producers included *Emericella nidulans* whereas medium producers included *Aspergillus versicolor, Penicillium funicolosum* and *Penicillium rubrum* in the cotton mill (Table 2).

The quantitative measurements of AFT using HPLC are shown in Table (3). In soybean, *Aspergillus parasiticus* I produced the greatest AFT in concentration of 183.91 μg mL^{-1} whereas *Aspergillus flavus* I produced 7.93 μg mL^{-1}. *Aspergillus flavus* II and III produced AFT at concentration levels of 4.42 μg mL^{-1} and 1.01 μg mL^{-1}, respectively. *Aspergillus flavus* IV produced AFT at concentration level of 0.56 μg mL^{-1} while *Paecilomyces variotii* produced AFT in concentration of 0.27 μg mL^{-1}. *Penicillium funicolosum* only produced B_1 at concentration level of 0.40 μg mL^{-1} (Table 3).

In the cotton mill, the greatest AFT produced by *Aspergillus parasiticus* II was in the amount of 200.06 μg mL^{-1}. *Penicillium rubrum* produced AFT at a concentration level of 0.11 μg mL^{-1}. *Aspergillus flavus* VI and VII produced B_1 in concentrations of 0.98 μg mL^{-1} and 0.18 μg mL^{-1}, respectively. In both mills, *Aspergillus nidulans* produced AFT at concentration of 1.81 μg mL^{-1} (Table 3).

4. Discussion

Sedimentation method gives a rough approximation of the counts (Pelczar et al., 1993) and its reliability is affected by the size and the shape of the particles and the motion of the surrounding air (Nevalainen et al., 1993). In the present study airborne fungi exceeded the guideline limit value of 500 CFU m^{-3} recommended by the World Health Organization (WHO, 1990). Su et al. (1990) reported that there was consistent correlation between hay fever symptoms and log colony forming unit concentrations of several dark spore fungi at geometric mean levels below 500 CFU m^{-3}. Moreover a field guide published by the American Industrial Hygiene Association recommended that the presence of some toxigenic fungi requires urgent risk management decisions (Dillon et al., 1996). Gorny and Dutkiewicz (2002) suggested $4x10^4$ CFU m^{-3} for fungi as occupational exposure limit for industrial settings. In addition the risk is increased by the presence of airborne fungal species reported as causative agents of allergenic, immunotoxic and toxigenic diseases (Lacey and Dutkiewicz, 1994).

In the present study *Aspergillus, Cladosporium*, *Penicillium* and *Alternaria* were the predominant airborne fungi. *Aspergillus* and *Penicillium* are normally found indoors whereas *Cladosporium* and *Alternaria* are normally found outdoors (Miller, 1992). *Aspergillus*, *Penicillium*, smuts and *Cladosporium* species were found to constitute up to 90% of the total fungal spores load in a bakery (Singh et al., 1999). *Alternaria alternate*, *Aspergillus candidus*, *Aspergillus clavatus*, *Aspergillus niger*, *Penicillium brevicompactum*, *Penicillium chrysogenum*, *Rhizopus stolonifer* and *Phoma* were the common genera found in wheat grain milling (Dutkiewicz et al., 2001).

The presence of *Aspergillus* in the air is a major risk for invasive aspergillosis (Denning, 1989). Some *Aspergillus*/ *Penicillium* species are toxigenic while others are pathogenic (Rati et al., 1980). Dimitrov et al. (1990) found high concentrations of potentially allergenic and toxigenic fungi (*Alternaria, Aspergillus, Cladosporium, Fusarium* and *Penicillium*) in the air of plants processing cotton and hemp, posing a risk of respiratory disease for the exposed workers. *Aspergillus flavus* and *Aspergillus fumigatus* cause human aspergillosis (Campbell, 1994).

Table 3. *Quantitative measurements of aflatoxins produced by fungal species using HPLC*

Species	Aflatoxin concentration (μg mL^{-1})				Total concentration
	G_1	B_1	G_2	B_2	
Soybean mill					
Aspergillus flavus I	3.10	1.33	ND	3.50	7.93
Aspergillus flavus II	0.56	3.55	ND	0.31	4.42
Aspergillus flavus III	0.22	0.67	ND	0.12	1.01
Aspergillus flavus IV	ND	0.51	ND	0.05	0.56
Aspergillus parasiticus I	65.50	72.60	25.01	20.01	183.91
Aspergillus nidulans	ND	1.63	ND	0.18	1.81
Paecilomyces variotii	ND	0.12	ND	0.15	0.27
Penicillium funicuolosums	ND	0.40	ND	ND	0.40
Cotton mill					
Aspergillus flavus V	ND	ND	ND	ND	ND
Aspergillus flavus VI	ND	0.98	ND	ND	0.98
Aspergillus flavus VII	ND	0.18	ND	ND	0.18
Aspergillus parasiticus II	72.35	86.52	22.36	18.83	200.06
Aspergillus nidulans	ND	1.63	ND	0.18	1.81
Peicillium rubrum	ND	0.08	ND	0.03	0.11

ND: Not detected

Aerodynamic diameter affects the landing site of an organism (Abdel Hameed et al., 2003). Particles with diameters ≥5 μm such as *Alternaria* and *Epicoccum* are usually deposited in the upper respiratory tract causing allergic rhinitis and asthma (Seltzer, 1995). Particles ≤5 μm can deeply penetrate into the alveoli causing allergies. *Aspergillus* and *Penicillium* spores are important allergenic and toxigenic fungal species (Mullins and Seaton, 1978).

There is increasing evidence that inhaled spores and mycelial fragments may be the potential source of mycotoxins. Mortality occurring between 1963 and 1980 in a small cohort of Dutch oil–press workers exposed between 1961 and 1969 to AFT primarily via the respiratory route was assessed (Hayes et al., 1984). Acute toxicosis and cancer were attributed to respiratory exposures to mycotoxins (Croft et al., 1986). On the other hand, Dvorackova (1976) concluded that the lungs did not seem to be a target for carcinogenic effect of inhaled AFT in humans.

In the present study TLC and HPLC were used to screen mold strains for mycotoxins production. Most mycotoxins determination must rely on correct extraction and cleaning methods (Chu, 1992). TLC is still the most popular method used for mycotoxins analysis which offers the ability to screen large numbers of samples economically and ease of identification of target compounds (Sokolovi and Simpraga, 2006). However one of the major requirements for TLC is that there is an inherent need for sample preparation which requires protocols dependent upon the properties and the type of the toxins being studied (Turner et al., 2009). HPLC is the industry standard for mycotoxins detection (Valenta, 1998) because it gives excellent results in terms of accuracy, precision, reproducibility and high resolution (Cinquina et al., 2004). Modern analysis of mycotoxins relies heavily on HPLC employing various adsorbents depending on the physical and chemical structures of the mycotoxins.

Fusarium, *Aspergillus* and *Penicillium* are the main mycotoxin producers (Chelkowski, 1991). *Penicillium verrucosum* and *Aspergillus ochraceous* produce OTA (Smith and Solomons 1994) which is often higher in grains (Jorgensen et al., 1999).

In various occupational environments the occurrence of toxigenic airborne fungi is well known, however it is still unclear whether their inhalation exposures would result in adverse effects (Gareis and Meussdoerffer, 2000). The question is, do mold capable of producing mycotoxins in the laboratory, produce them under workplace conditions and their inhalation can produce health effects? This is because the ability of mold to produce mycotoxins under the laboratory conditions may not be equivalent to the potential workplace exposures. Detection of toxin production on certain medium does not necessarily indicate that the fungal strain had produced toxin in the natural environment (Cvetnic and Pepeljnjak, 1997).

5. Conclusion

Aspergillus, *Penicillium* and *Cladosporium* were the dominant airborne fungi in the cotton and soybean mills. Mycotoxins producing fungi are found in the air of the studied mills. At these mills, workers are routinely exposed to various dangerous fungal species. Many of them have D_{ae} <5 μm which can reach the alveoli. In this study it was found that *Aspergillus parasiticus* produced the highest amount of AFT at soybean and cotton mills. However it is unclear whether inhalation of toxigenic fungal spores can lead to potential health effects as in vitro results are not synonymous of the studied atmospheres. Further research is required to relate the length of exposure with worker's health and determination of the levels of mycotoxins in the inhalable suspended dust.

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