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IN VITRO TOXICITY OF INDOOR *CHAETOMIUM* KUNZE EX FR.

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Abstract: Microscopic fungi in the indoor environment present a serious health risk for people living in affected buildings. The potentially toxic ascomycete genus *Chaetomium* is supposed to be the third most frequent indoor fungal contaminant. Its brief mycological, toxicological and ecological characterization is given. The work was aimed at *in vitro* study of toxicity of endo- and exometabolites of 14 strains of *Chaetomium* spp., including 4 strains of *Ch. globosum*, isolated from mouldy buildings in Slovakia and Denmark, and 3 *Ch. globosum* strains from the Czechoslovak Collection of Microorganisms (CCM). The endometabolites of 10 isolates of *Chaetomium* spp. were active: 7 isolates (41% of total strain number) stopped tracheal ciliary movement of 1-d-old chickens after 24 h, 9 isolates (53%) after 48 h and 10 strains (59%) after 72 h. In the case of exometabolites, the extracts of 6 *Chaetomium* strains showed some ciliostatic activity: 2 isolates (12% of strains tested) after 24 h, 5 isolates (29%) after 48 h and 6 isolates (35%) after 72 h. In general, 5 isolates of Danish origin (83%) produced ciliostatically active exometabolites and 2 isolates (33%) produced such endometabolites, while only 4 strains isolated in Slovakia (50%) and 3 strains (37%) respectively did the same under experimental conditions. Most toxic metabolites were produced by *Chaetomium* spp. isolated from dwellings, whereas hospital isolates were not able to produce active compounds. Chaetomia as indoor contaminants can contribute to ill health of occupants of mouldy damp buildings.

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INTRODUCTION

Microscopic filamentous fungi colonizing indoor environments can cause serious damage of health, including allergies, acute and chronic respiratory illnesses, as well as non-specific syndromes in occupants. Young children are the main group at risk. Fungi from the ascomycete genus *Chaetomium* Kunze ex. Fr. belong to the most frequently isolated indoor moulds.

Currently, the genus comprises more than 100 species, but the taxonomic data given by different authors vary greatly, e.g. Udagawa *et al.* [29] characterized more than 200, Domsch *et al.* [5] distinguished 160–180, while von Arx *et al.* [1] accepted only about 80 species. All chaetomia produce typical dark-coloured ascomata with a short neck and a wide apical pore, covered with ascomatal setae or hairs, often spirally coiled, with or without branches (Fig. 1, 2). An ascomatal wall is often composed of elongated cells. The superficial ascomata are usually connected to the substrate by rhizoids. The stalked, clavate, fusiform, obovate or narrow cylindrical asci have a relatively thin wall (Fig. 3). The ascospores are aseptate, brown or grey-olivaceous, never black, without ornamentation, extruded in a dark mass (Fig. 2, 4).

Other, similar fungal genera are known: *Achaetomium*, *Ascotricha*, *Farrowia* and *Thielavia* [29].

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Figure 1. *Chaetomium* sp. - hairy ascomata, × 100.

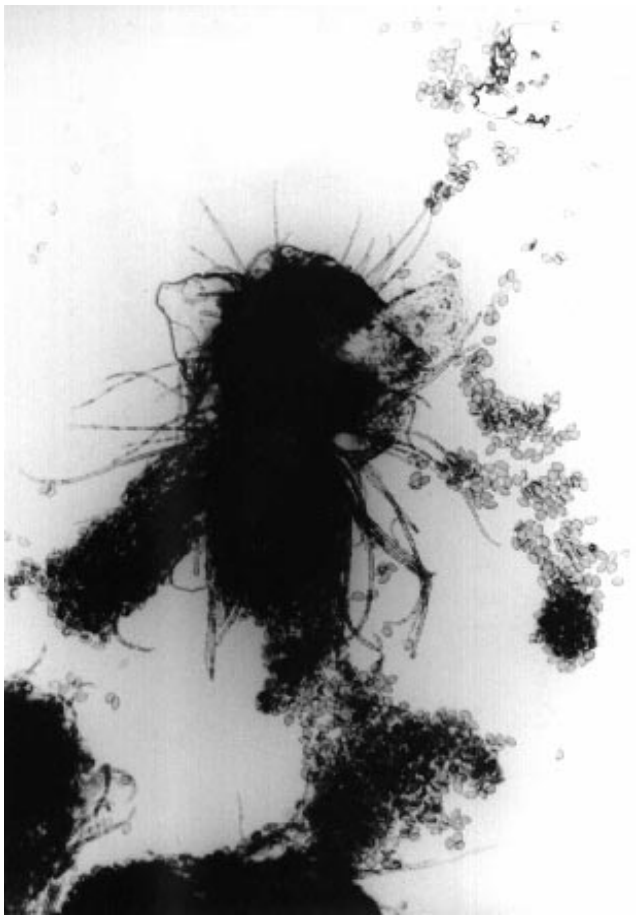

Figure 2. *Chaetomium* sp. - ascomata and ascospores, × 200.

According to the optimal growth temperature when cultivated on malt agar with 2% yeast extract, Millner [16] distinguished several groups of chaetomia: mesophilic (15–35°C), semi-mesophilic (15–37°C), microthermophilic (15–40°C), thermotolerant (15–45°C) and thermophilic (25–55°C). The ascospores are very resistant to environmental conditions. They showed a slight heat resistance in Sabouraud agar medium - survived at 60°C for 60 min, but not 70°C for 10 min in our previous study of soil-borne microfungi [13].

Chaetomium spp. occur worldwide. Most species show high cellulolytic activity, deteriorate foods, feeds, plant drugs for pharmaceutical and cosmetic use, paper, textile, seeds etc. [29]. Protease was also produced by *Ch. globosum* found in ruminants in Southern Iraq [18]. Chaetomia (1–35% of all fungal isolates) were isolated from soil of Canadian pastures [4], from salt marshes in Kuwait [17], from soil in Spain [6] and *Ch. globosum* from strong acid soil with toxic elements and heavy metals of geological origin in Slovakia [28]. *Ch. aureum, Ch. botrychoides, Ch. circinatum, Ch. elatum, Ch. funiculosum, Ch. fusiforme, Ch. globosum, Ch. indicum, Ch. jonesi, Ch. murorum, Ch. perlucidum* and *Ch. piluliferum* were found in soil samples from former Czechoslovakia [24]. In 1998, there were strains of *Ch. botrychoides, Ch. cochlioides, Ch. funiculosum, Ch. fusiforme, Ch. globosum, Ch. indicum, Ch. olivaceum, Ch. piluliferum, Ch. spirale* and *Ch. trilaterale* mentioned in the check-list of non-vascular and vascular plants of Slovakia [14]. *Chaetomium* spp. were isolated also from hair of small mammalians, feathers and bird's nests in former Yugoslavia and the Czech Republic [9, 10].

In indoor environments, isolates of chaetomia were reported from kitchens, bathrooms, wall paper, mattresses, carpets and window frames as the third most frequent ones among the present microfungi [22]. *Chaetomium* spp. were found in the air of dwellings in London, Central Scotland and USA [3, 11, 15], *Ch. globosum* in Dutch buildings [23], *Ch. botrychoides* in apartments in Saudi Arabia [25]. Chaetomia present in the hospital environment caused a nosocomial infection of 4 patients after bone-marrow transplantation [31].

Some known toxic metabolites are produced by chaetomia. *Ch. globosum, Ch. gracile, Ch. homopilatum* and *Ch. virescens* produce cytotoxic mycotoxins chaetoglobosins, sterigmatocystin and o-methylsterigmatocystin. Mutagenic molicelins, antibacterial and cytotoxic chaetocins and dye cochliodinol are produced mainly by *Ch. globosum* [20, 27, 30].

Chaetomium spp. are able to cause mycotic infection of patients with impaired immunity [7, 26]. Imidazoles, 5-fluorocytosin and amphotericin B exert fungistatic effects on both clinical and environmental isolates [8].

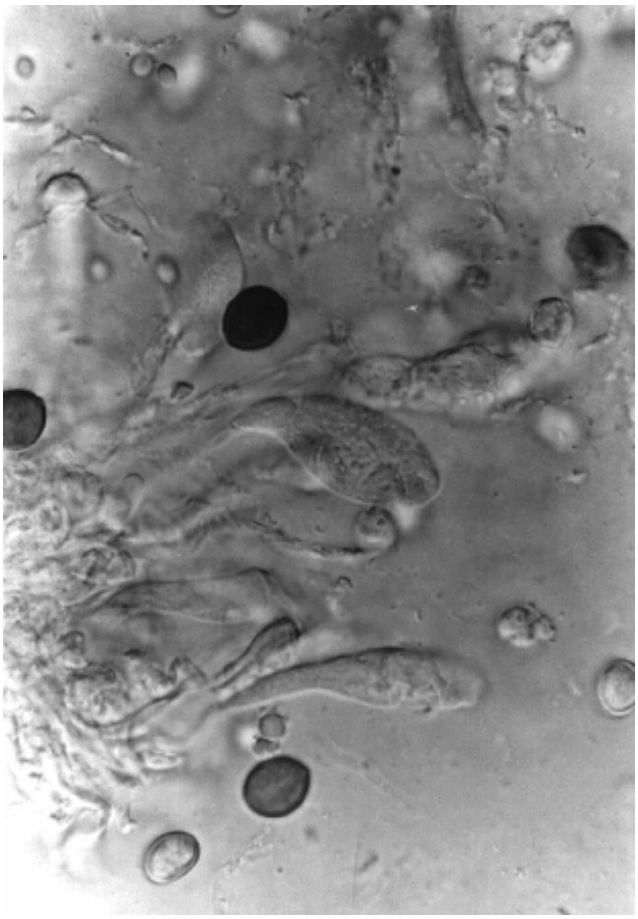

Figure 3. *Chaetomium* sp. - clavate asci, × 1000.

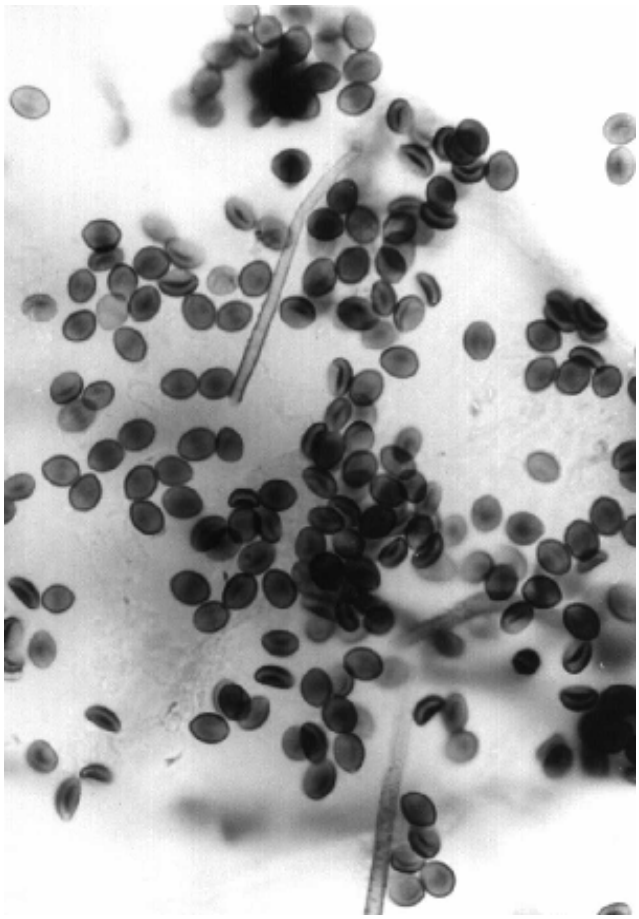

Figure 4. *Chaetomium* sp. - ascospores, × 1000.

The main task of this work was an *in vitro* study of the respiratory toxic potential of chloroform extracts of secondary metabolites of indoor chaetomia isolated from Slovak and Danish buildings using chicken tracheal organ cultures.

MATERIALS AND METHODS

Fungal isolates. In total, 17 isolates were used to produce metabolites tested: 4 *Chaetomium* spp. and 1 *Ch. globosum* isolated from dust and filters from the air-conditioning systems in office buildings, 2 *Chaetomium* spp. isolated from a hospital air-condition system, 1 *Chaetomium* spp. from a crack in a mouldy wall in a dwelling in Slovakia, 3 *Chaetomium* spp. and 3 *Ch. globosum* isolated from dust samples from mouldy dwellings in Denmark and, finaly, 3 strains of Ch. globosum (CCM 8156, CCMF 232 and CCMF 275) from the Czechoslovak Collection of Microorganisms (Brno, Czech Republic).

Fungi were cultivated on Sabouraud agar (Imuna Co., Šarišské Michaľany, Slovakia) slants at 25°C for 8 days. The biomass from 3 tubes per each strain was mixed in 500 ml Erlenmeyer flasks with 200 ml of liquid medium containing 2% of yeast extract and 10% of sucrose. The system was stationary cultivated at 25°C for 10 days.

Metabolites. Two types of chloroform extracts were used in the experiments:

1) Chloroform extracts of cultivation medium (exo-metabolites): After filtration of the biomass, the medium was twice extracted with 200 ml of chloroform, the pooled extracts were dried with anhydrous Na_2SO_4 and concentrated to dryness in a water bath.

2) Chloroform extracts of biomass (endometabolites): Biomass filter cakes were also extracted twice with 200 ml of chloroform in a blender (Stomacher Lab-Blender 400, Seward Medical UAC House, London, England) for 10 min and the dry extracts were obtained as mentioned above. Each residue was redissolved in dimethylsulfoxide (Me_2SO) to the concentration 1 mg/ml and kept at 4°C in a medicine flask with a teflon plug.

Toxicity *in vitro*. The ability of the crude extracts of exo- and endometabolites of chaetomia to stop ciliary beating in chick tracheal organ cultures was evaluated [12, 19]. One-day-old chickens (State Research and Productional Co., Častá Hatchery, Slovakia) were decapitated, tracheas were removed within 3 min, washed twice in the minimal essential medium according to Eagle with Earl's salts – E-MEM (Institute for Sera and Vaccines, Prague, Czech Republic) and cut into 0.1 mm slices. Tracheal rings (20–30) were placed into a Petri dish (diameter 60 mm) with

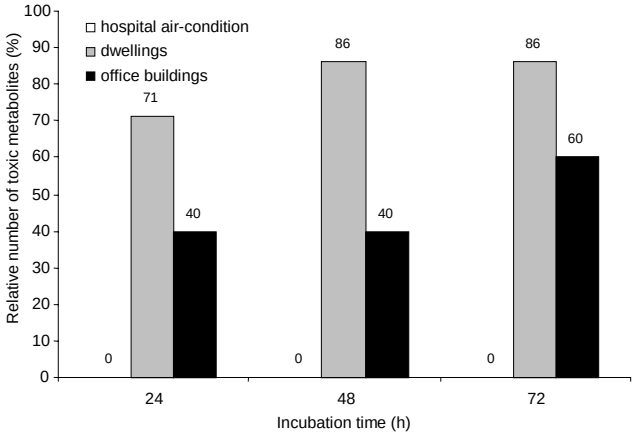

Figure 5. *In vitro* ciliostatic activity of endometabolites of *Chaetomium* spp. isolated from different indoor environments.

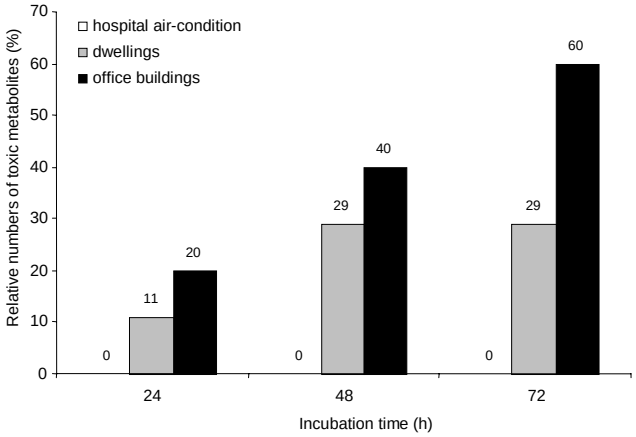

Figure 6. *In vitro* ciliostatic activity of exometabolites of *Chaetomium* spp. isolated from different indoor environments.

2 ml of culture medium, i.e. E-MEM with 1% of 3% glutamine, 2.5% of 7.5% $NaHCO_3$ with phenol red (Institute for Sera and Vaccines), 10% of bovine fetal serum, 100 µg streptomycin, 100 U penicillin and 20 µg crude extract per 1 ml. Reference media were prepared with 1% Me_2SO instead of extracts as well as controls with pure rich medium. The cultivation of organ models was carried out at 37°C and 5% CO_2.

The ciliary movement was observed on 5–7 tracheal rings microscopically (×250 magnification; Olympus BX50) after 0, 24, 48 and 72 h of incubation.

RESULTS

The *in vitro* ciliostatic activity of the exo- and endometabolites of 14 indoor isolates of chaetomia from Slovakia and Denmark, and 3 *Ch. globosum* strains from the CCM deposit is shown in Table 1. Endometabolites of 10 chaetomia stopped the tracheal ciliary beating in the biological model used: metabolites of 7 isolates (41% of all tested), among them *2 Ch. globosum*, in 24 h; 9 isolates (53%) with 4 *Ch. globosum*, including *Ch. globosum* CCMF 275, in 48 h; and 10 isolates (59%), including the same *Ch. globosum* strains as previously, in 72 h. Exometabolites of 6 chaetomia showed some ciliostatic activity under experimental conditions: metabolites of 2 isolates (12%) with 1 *Ch. globosum* in 24 h; 5 isolates (29%) with 3 *Ch*. globosum, including CCM 8156, in 48 h; and 6 isolates (35%) with 4 *Ch. globosum*, including the same CCM strain as above, in 72 h.

Chaetomium sp. originated in the hospital and office air-condition systems (2 isolates of each), 1 *Ch. globosum* from dwellings and CCMF 232 did not produce ciliostatically active metabolites at all. During the experiment, no ciliostatic activity was observed in the biological systems with endometabolites of *Ch. globosum* CCM 8156 and CCMF 232 and with exometabolites of 4 *Chaetomium* spp. and 1 *Ch. globosum* isolated from the dwellings and CCMF 275, respectively. On the other hand, both exo- and endometabolites of another *Chaetomium* sp. isolated from the office building, endometabolites of 4 chaetomia from the dwellings, 1 *Ch. globosum* from the office and another one from the dwelling, together with exometabolites of the last *Ch. globosum* with office origin were the strongest toxic samples studied in this experiment – they destroyed ciliary movement in 24 h. The metabolites of all other chaetomia tested showed limited ciliostatic effect on chicken's tracheal epithelium. 60% of the office isolates produced ciliostatically active endo- as well as exometabolites. The isolates from the hospital air-conditioning tested did not produce toxic metabolites in this experiment (Fig. 5, 6).

When evaluated according to their geographical origin, the majority of chaetomia from Danish samples – 5 isolates (83%), in comparison with 4 (50%) indoor isolates from Slovakia, produced endometabolites ciliostatically active for at least 72 h. Number of strains with toxic exometabolites was relatively similar - 2 (33%) Danish and 3 (37%) Slovak isolates (Fig. 7, 8).

DISCUSSION

Many of environmental microscopic fungi have already been identified as infectious agents in immunocompromised patients. But there is a persistent lack of information sufficiently explaining possible pathological effects of common indoor micromycetes and their toxic metabolites on the healthy human organism. To fill this gap, relevant *in vitro* studies are required. As the self-clearance of upper airways provided by ciliated epithelium and mucous production represents an important part of the defence mechanism eliminating airborne injurants, we have studied the ability of metabolites of 14 indoor and 3 chaetomia strains deposited in the CCM to debilitate this reaction of macroorganism by breaking down the ciliary movement in chick's trachea *in vitro*.

Chaetomium spp. strains are known producers of antibiotics (chaetocins, chaetomin, cochliodiol), cytotoxic chaetoglobosins A–K, hydroxylamines and nitrites, and cytotoxic, antibiotic, carcinogenic, mutagenic, hepato-

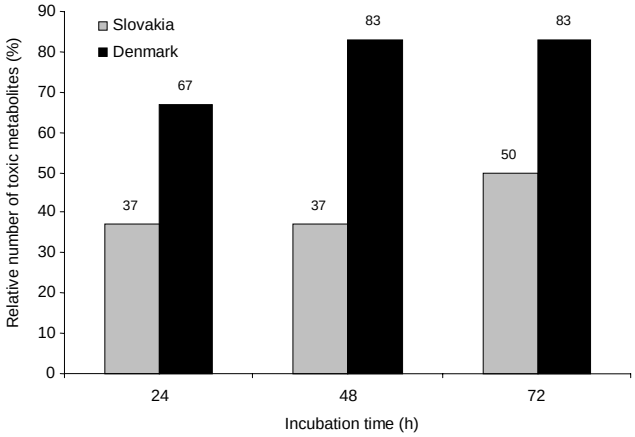

Figure 7. *In vitro* ciliostatic activity of endometabolites of indoor isolates of *Chaetomium* spp. according to their geographic origin.

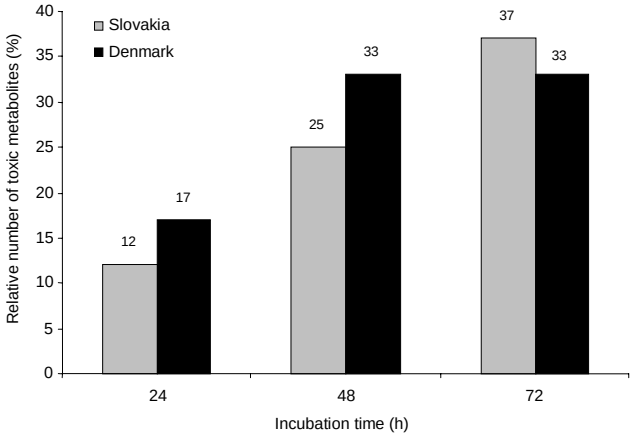

Figure 8. *In vitro* ciliostatic activity of exometabolites of indoor isolates of *Chaetomium* spp. according to their geographic origin.

and nephrotoxic mycotoxin sterigmatocystin as well [2]. Of all those compounds, only the ciliostatic activity of sterigmatocystin has been studied so far: 20 µg/ml stopped the tracheal ciliary movement in 1-d-old chickens *in vitro* in 24 h and even 0.03 µg/ml in 48 h [12].

Toxins produced by some indoor chaetomia have been characterized and 6 isolates from wall paper produced 5–7 mg/cm^2 of chaetoglobosins A and C [20]. The toxic activity of crude chloroform extracts of the metabolites of fungi isolated from raw flax and cotton studied by a bioassay was described in our previous paper. One flax-borne isolate of *Chaetomium* spp. tested produced no metabolites able to stop chick tracheal cilia even in the 6-day-experiment [21].

Based on the present study, the *in vitro* toxicity of crude chloroform extracts of endometabolites of *Chaetomium* sp. was higher than those of exometabolites which could be caused by synergism of toxins and other chemicals extractable from the cells, e.g. cell wall fatty acids. The metabolites of fungal isolates from the dwellings were the most active ciliostatically, followed by the metabolites of chaetomia related to office buildings, while hospital isolates did not produce any active metabolites at all in the experiment. Finally, relatively more chaetomia which originated from Denmark produced toxic metabolites when compared with the isolates coming from Slovakia. Regarding all our findings, a precise toxin's profile, even

Table 1. *In vitro* ciliostatic activity of chaetomial metabolites on chick trachea.

Fungi	*Endometabolites*			*Exometabolites*		
	24h	48h	72h	24h	48h	72h
Chaetomium sp. F	-	-	-	+	+	+
Chaetomium sp. FD	-	-	-	+	+	+
Chaetomium sp. FD	-	-	-	+	+	+
Chaetomium sp. FD	-	-	-	+	+	+
Chaetomium sp. H	+	+	+	+	+	+
Chaetomium sp. H	+	+	+	+	+	+
Chaetomium sp. O	+	+	-	+	-	-
Chaetomium sp. O	-	-	-	-	-	-
Chaetomium sp. O	+	+	+	+	+	+
Chaetomium sp. O	+	+	+	+	+	+
Ch. globosum FD	-	-	-	+	-	-
Ch. globosum FD	+	-	-	-	-	-
Ch. globosum FD	+	+	+	+	+	+
Ch. globosum O	-	-	-	+	+	-
Ch. globosum CCMF 275	+	-	-	+	+	+
Ch. globosum CCM 8156	+	+	+	+	-	-
Ch. globosum CCMF 232	+	+	+	+	+	+
Toxic metabolites	41%	53%	59%	12%	29%	35%

Notes: + = cilia moved; - = ciliary movement stopped; O = isolate from office buildings; F = isolate from dwellings (flats); H = indoor hospital isolate; D = Danish origin.

combined with genomic analysis of *Chaetomium* spp. tested, could be very helpful to clarify all their details.

It can be concluded that a possible toxic effect of *Chaetomium* spp. strains on the respiratory tract should not be underestimated in occupants of damp mouldy buildings.

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