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Antifungal properties of silver nanoparticles against indoor mould growth

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HIGHLIGHTS

- Growth of common indoor fungi is inhibited in the presence of silver nanoparticles.
- Silver nanoparticles cause morphological changes in fungi.
- Fungi reproductive structures are affected by silver nanoparticles.
- Silver nanoparticles might stimulate growth of fungal species.

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ABSTRACT

The presence of moulds in indoor environments causes serious diseases and acute or chronic toxicological syndromes. In order to inhibit or prevent the growth of microorganisms on building materials, the disruption of their vital processes or the reduction of reproduction is required. The development of novel techniques that impair the growth of microorganisms on building materials is usually based on silver nanoparticles (AgNPs). It makes them an alternative to other biocides. AgNPs have proven antibacterial activity and became promising in relation to fungi. The aim of the study was to assess growth and morphology of mycelia of typical indoor fungal species: *Penicillium brevicompactum*, *Aspergillus fumigatus*, *Cladosporium cladosporoides*, *Chaetomium globosum* and *Stachybotrys chartarum* as well as *Mortierella alpina*, cultured on agar media. The antifungal activity of AgNPs was also tested in relation to *C. globosum* and *S. chartarum* grown on the surface of gypsum drywall. It was found that the presence of AgNPs in concentrations of 30–200 mg/l significantly decreased the growth of fungi. However, in the case of *M. alpina*, AgNPs stimulated its growth. Moreover, strong changes in moulds morphology and colour were observed after administration of AgNPs. Parameters of conidiophores/sporangiophores varied depending on mould region and changed significantly after treatment with AgNPs. The experiments have shown antifungal properties of AgNPs against common indoor mould species. Their application to building materials could effectively protect indoor environments from mould development. However, consideration must be given to the fact that the growth of some fungal strains might be stimulated by AgNPs.

1. Introduction

Present-day urban society spends approximately 85–90% of its time inside buildings. A strong trend in modern construction is energy saving; thus, the demand for new technologies to significantly reduce heat exchange between interior parts of buildings and the exterior environment (Chamakura et al., 2011). Current building materials, when exposed to high relative humidity in areas of poor or inappropriate lightning, heating or ventilation, are frequently found to be excellent substrates for mould formation. This leads to indoor air quality problems and is directly associated with "sick building syndrome" (SBS) and "building related illness" (BRI). People who reside in fungi affected indoor environments might experience their health getting worse and/or feel discomfort after spending long amounts of time indoors (The Environmental Protection Agency — EPA). The SBS has been linked mostly to the presence of allergens, antigens, β-1,3-glucans, mycotoxins and microbial volatile organic compounds (MVOCs) (Crook and Burton, 2010; Shoemaker and House, 2005; Tuomi et al., 2000). Due to prolonged exposure of human organism to these factors, the respiratory, circulatory, and nervous systems are affected. Other negative impacts are skin irritation and non-specific hypersensitivity reactions and the development of cancer (Shoemaker and House, 2005).

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Aspergillus fumigatus is the most common fungal species that occupies indoor environments and causes allergic bronchi-pulmonary aspergillosis. This disease leads to the fatal destruction of lung tissue (Latge, 1999; Bush and Portnoy, 2001). Another species, *Penicillium brevicompactum*, produces chemical compounds like mycophenolic acid, asperphenamate, brevianamide A, and roquefortine C, a tanzawaid acid analogue that was reported to be strong allergens. They may cause hypersensitivity pneumonitis and allergic alveolitis, especially in susceptible individuals. Moreover, other infections such as keratitis, penicilliosis, and otomycosis were noted (Bush and Portnoy, 2001; Cooley et al., 2004; Shen et al., 1996). Mycelium of *Cladosporium cladosporoides* that commonly grows on damp indoor surfaces and causes allergic reactions or asthma symptoms due to the production of mycotoxins such as asperentin, cladosporic acid, sterigmatocystin and diacetoxyscirpenol (DAS) (Bush and Portnoy, 2001; Tuomi et al., 2000). Residents of buildings highly affected with *Stachybotrys chartarum* manifested adverse health effects due to the many bioactive compounds released by these types of moulds: macrocylic trichothecenes, related trichoverroids (Andersen et al., 2002; Bennett and Klich, 2003; Kuhn and Ghannoum, 2003), phenylspirodrimanes (spirolactones and spirolactams) and cyclosporins (potent immunosuppressive agents) (Nielsen et al., 2002; Nielsen, 2003). Some of these metabolites could cause respiratory, dermatological, eye and constitutional symptoms (Gravesen et al., 1994), or they have suppressant effects on the immune system Jarvis and Miller (2005). Over 40 chaetoglobosins were isolated and characterized from the *Chaetomium globosum* species present on the paper (cellulose) surface of gypsum boards. Many of them demonstrated acute toxicity to mammals and strong cytotoxicity to cells. *C. globosum* produces two mycotoxins (chaetoglobosins A and C) that belong to the group of cytochalasins, known as actin binding compounds (Bloch, 1973; Fogle et al., 2007; Griffin et al., 1982).

Mortierellales constitutes one of the largest groups of zygomycetes. Most of the *Mortierella* species are known as soil born fungi, while we have been able to isolate *Mortierella alpina* from basements of the Cracovian Market Square. *M. alpina* has biotechnological significance as the producer of polyunsaturated fatty acids (PUFA); it might contain more than 25% of lipids in its biomass. *Mortierella isabellina* has been applied in various regio- and stereospecific biotransformations. *Mortierella wolfii* is reported as an animal pathogen causing bovine mycotic abortion, pneumonia and systemic mycosis (Certik and Shimizu, 1999; Domsch et al., 1980; Dyal and Narine, 2005; Jefferys et al., 1953). Information provided by the Occupational Safety & Health Administration of the United States Department of Labor states that the *Mortierella* species might cause certain health effects such as: allergies, irritation, hypersensitivity pneumonitis, and dermatitis. Thus, the potential health risks associated with the presence of mould fungi inside buildings is a major concern for proprietors and, building administrators, as well as insurance companies.

Currently available anti-fungal agents dedicated to indoor applications must be non-toxic for humans and other animals, non-volatile, odourless, and hypoallergenic. Additionally, the chemistry of such agents should provide long-term protection, especially in environments that promote fungal growth (e.g. high humidity) (Clausen and Yang, 2007; Jarvis and Miller, 2005; Shoemaker and House, 2005). The presence of the fungal species leads to biological corrosion of the building materials. In order to inhibit or prevent the growth of microorganisms on these building materials, the use of relevant biocides is needed. However, many microorganisms become resistant to the hitherto applied chemicals. Therefore, there is a great need to develop new, more efficient antimicrobial agents for application in building material preservation. Silver nanoparticles (AgNPs) seemed to demonstrate strong antimicrobial properties. They started being widely used during the development of novel construction materials particularly, those materials that are excellent substrates for microorganism growth and development within specific environmental conditions. Recently, a few AgNPs containing products available on the market were used as antimicrobial agents to prevent bacterial growth, but little is known about their efficiency in respect to fungal growth (Chamakura et al., 2011; Choi et al., 2008; Kim et al., 2007 and Martínez-Castañón et al., 2008).

The objectives of the present study were to estimate the antifungal activity of silver nanoparticles in agar substrates and on gypsum wallboards. Morphological changes as well as the growth rate were described in the presence of increasing concentrations of AgNPs. Moreover, the diameters of conidiophores/sporangiophores were determined at the central part and edge of the mould colonies.

2. Materials and methods

2.1. Characterization of silver nanoparticles (AgNPs)

A solution of silver nanoparticles was obtained from NANOPAC Company (Krakow, Poland). Experimental concentrations of AgNPs were prepared from a stock solution (2000 mg/l) diluted with deionized water. Hydrodynamic sizes of AgNPs were determined at 25 °C using 173° dynamic light scattering approach (DLS, ZetaSizer Nano ZS). Visible/UV absorption spectra were recorded using a Hewlett–Packard HP 8452A diode-array spectrophotometer in 1 cm optical path cuvettes. The solution of Ag nanoparticles was analysed with the use of a Dimension Icon AFM (Bruker, Santa Barbara, CA) working in the PeakForce Tapping (PFT) mode with standard silicon cantilevers for measurements in the air (nominal spring constant of 0.4 N/m). Silver colloids were diluted 100 times and sonicated for 20 min prior to deposition. Silicon wafers were purified in "piranha" solution (a mixture of H_2SO_4 and H_2O_2 at a 1:3 ratio) and cleaned in water. The average size of nanoparticles reached 55 ± 5 nm. AgNP working solutions were added to the fungi culturing medium (PDA) or sprayed over gypsum drywall.

2.2. Sampling and identification of fungal species

Fungal species were isolated from affected basements of the Cracovian Market Square on Sabouroud medium containing Rose Bengal and purified by the sub-culturing on Potato Dextrose Agar (PDA, BD BBL™, Maryland, USA) or Czapek-Dox Broth (BD BBL™, Maryland, USA) prepared according to the manufacturer's protocol. Fungal species were identified on the basis of microscopy examinations and culture studies as previously described by Domsch et al. (1980). Identification was verified by sequencing their DNA at the ribosomal internal transcribed spacer (rDNA-ITS) region (Innis et al., 2012). DNA isolation was performed based on a modified method proposed by Kjøller and Rosendahl (2000). The nuclear ITS region of fungal DNA was amplified with the use of primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1990). Then, pure DNA was bidirectionally sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (cat. 4337455, Applied Biosystems, USA). Reaction products were further purified with the use of EDTA–EtOH protocol. Finally, ITS sequences were analysed in 3130*xl* Genetic Analyser (ABi, USA). BLAST search (Altschul et al., 1997) was performed against sequences deposited in GenBank and the closest matches were selected for phylogenetic analysis.

2.3. Inoculum preparation

Suspension of spores obtained from *A. fumigatus*, *P. brevicompactum*, *C. cladosporoides*, *C. globosum* and *S. chartarum* or fragments of *M. alpina* mycelia were used as the inoculants. First, the fungal strains were cultured in Petri dishes on PDA substrate at 23 ± 1 °C for 14 days. Using a sterile inoculating loop, fungal spores were transferred from the stock culture to a 10 ml sterile physiological salt solution (0.85% NaCl, 0.03% Tween 80). The spore suspension was filtered through a 32 μm pore size nylon mesh (Sefar, Switzerland) to remove mycelia fragments, centrifuged at 13,523 *g* (Eppendorf centrifuge 5424, Germany), and supernatant was discarded. The spores were finally suspended in 50 ml of sterile physiological salt solution, but before inoculation, the number of spores was adjusted to 10^7 spores/ml by using a

counting chamber. The spore suspensions were used immediately afterwards for experiments as well as to test their viability according to ISO 846:1997(E) (www.iso.org/iso). The viability test was carried out at 23 ± 1 °C for 3 days, and spore germination inspected stereomicroscopically.

2.4. Experimental design

Two experimental groups were established depending on the fungal culture substrate in use: culture medium — fungi were cultured on standard agar substrate and gypsum drywall — fungi were cultured on boards of gypsum drywalls. The choice of gypsum substrate relates to their use in building construction, especially to construct interior walls and ceilings, whereas agar is a standard fungal culture substrate.

2.4.1. Culture medium

A. fumigatus, *P. brevicompactum*, *C. cladosporoides*, *M. alpina*, *C. globosum* and *S. chartarum* were used in this study. AgNP solution was separately autoclaved and added to a PDA to obtain the final concentrations of 30, 60, and 100 mg/l. The concentrations applied were defined on the basis of preliminary studies taking into account data available elsewhere (Kim et al., 2007; Choi et al., 2008; Ruparelia et al., 2008; Panáček et al., 2009). AgNPs were absent in the control group. The substrates were inoculated with 0.1 ml of freshly prepared spore suspensions (*A. fumigatus*, *P. brevicompactum*, *C. cladosporoides*, *C. globosum*, *S. chartarum*) or 5 mm fragments of *M. alpina* mycelium dissected from a core part of a fungal culture. All experimental specimens were incubated for 4 weeks at 23 ± 1 °C in darkness and 70% relative humidity. Colony growth was recorded weekly. After 4 weeks of incubation colony size was calculated as a percentage of the area covered by fungal mycelium in relation to the Petri dish area (standard plastic Petri dishes Ø90 mm). Additionally, conidiophore/sporangiophore diameters were measured. Mycelium from the centre and an edge of the colony were collected and stained with aniline blue. Twenty slides per experimental sample were prepared, and diameters of 100 conidiophores/sporangiophores per slide were measured with Image-Pro Plus (Media Cybernetics, version 4.0) software. Images were obtained by using a digital camera TK-C1380, Digital ½ Inch CCD.

2.4.2. Gypsum drywall

C. globosum and *S. chartarum* were used in this study since both species are common in mould-contaminated indoor environments (Bennett and Klich, 2003; Brasel et al., 2005; Fogle et al., 2007; Kuhn and Ghannoum, 2003; Nielsen et al., 2002; Shoemaker and House, 2005). Autoclaved boards of gypsum drywall (50 × 100 × 20 mm) were transferred to sterile plastic containers (Ø15 cm). Then, 6 ml of 60, 100 and 200 mg/l AgNP solution or distilled water (control) was sprayed onto the gypsum surface. The concentrations applied were higher than those used in PDA cultures, since preliminary studies performed on paper substrates soaked with AgNPs revealed fungi were more resistant to nanoparticles. Gypsum substrates were inoculated with 1 ml of freshly prepared spore suspensions of both species. All experimental specimens were incubated for 9 months at 23 ± 1 °C in darkness and 70% relative humidity. After 9 months, the total area of the mycelium was calculated in relation to the area of the gypsum wallboard and expressed in percentage.

2.5. Scanning electron microscopy (SEM)

SEM examination was performed for fungi cultured onto the gypsum board only, since substrates composed of the gypsum were dry enough to omit critical point drying procedure. Thus, the paper layer of the board was gently removed from gypsum and 1 cm^2 sampled using a razor blade. The specimens were then positioned onto microscope holders equipped with carbon adhesive tape (SPI Supplies, USA) and left overnight in a vacuum chamber to evacuate excess air and water vapours from the specimens. Finally, the samples were sputter coated (JFC-1100F, Tokyo, Japan) with a thin layer of gold and examined by means of a JEOL JSM 5410 scanning electron microscope (JEOL, Tokyo, Japan).

2.6. Statistical analysis

All experiments were carried out in at least four replicates. Statistica version 10 Software (StatSoft, USA) was used to determine statistically significant differences. Multivariate analysis of variance (MANOVA) was applied to reveal differences in mycelium outgrowth as well as in the diameters of conidiophores/sporangiophores among the agar group, whereas one-way analysis of variance (ANOVA) was performed to compare the growth rate of fungi mycelium cultured on gypsum boards. Statistical differences were considered significant at $p \leq 0.05$.

3. Results

Six fungal species — *Aspergillus fumigatus*, Penicillium *brevicompactum*, Cladosporium *cladosporoides*, Mortierella *alpina*, Chaetomium *globosum* and Stachybotrys *chartarum* were selected for the study of antifungal activity of AgNPs. Morphological characterization of the selected fungal strains was performed and the different responses of each strain depended upon the applied concentration of AgNPs with time.

3.1. Inhibition of the mould growth by silver nanoparticles on the culture medium

3.1.1. Aspergillus fumigatus

A. fumigatus cultured on standard agar medium formed velvet-like, turquoise-grey mycelium covering the surface of Petri dishes within 7 days of incubation. Mature mycelium was green pigmented, echinulate. However, in the presence of 30 and 60 mg/l of AgNPs, the green pigment of the mycelium became pale yellow, and colourless when treated with 100 mg/l AgNPs. It is noteworthy that the edges of mycelia treated with the highest concentration of AgNPs changed its colour to grey (Table 1). The growth pattern, which was consistent for each strain under various concentrations of silver nanoparticles (AgNPs), was observed as a function of time. There was a significant decrease of the mycelium growth when *A. fumigatus* was treated with AgNPs (Fig. 1). After one week of treatment, the mycelia dimensions reached 38%, 17% and 16% of that calculated for the control (mycelium stopped growing at 7.7 ± 0.03 cm in diameter) when cultured with 30, 60 and 100 mg/l AgNPs, respectively (Fig. 1). After four weeks of incubation, further growth of fungi treated with the concentrations mentioned above was noted; however, mycelia of those groups were 90%, 62%, and 54% of the control, respectively (Table 1). No significant changes in conidiophore diameters were observed in relation to the control groups, when fungi were treated with increasing concentrations of AgNPs (Fig. 2). It concerned conidiophores collected from the middle parts but those collected from the peripheral parts of mycelia revealed significantly higher conidiophore diameters when treated with increasing concentrations of AgNPs (Fig. 2).

3.1.2. Penicillium brevicompactum

P. brevicompactum cultures grown on PDA formed fluffy, cotton-like white colonies, which at the end of the experiment became greyish-green with globose conidia. However, when grown on the media containing AgNPs, the mycelia were primarily pale green in colour but changed into greyish-white at the end of the experiment. After one week, the mycelia cultured in control conditions were 2.1 ± 0.03 cm in diameter (Fig. 1). After four weeks of culture, mycelium growth was still noted in AgNP conditions, but at the end of the experiment, *P. brevicompactum* covered only 21% (30 mg/l), 8% (60 mg/l), and 5% (100 mg/l) of substrate, whereas control mycelia reached 64% of the Petri dish total area (Table 1). Conidia were not present at the edge of the mycelia when treated with

Table 1
The summary of silver nanoparticle (AgNP) effects on mycelia colour and rate growth of six fungi species cultured on standard PDA medium for four weeks and two fungi species grown on gypsum drywalls for nine months. The mycelium covered 100% of total Petri dish area *after 14 days, **after 10 days and ***after 5 days of culture.

Effects of AgNPs on fungi cultured on PDA medium					
Species	Mycelium	Control	30 mg/l	60 mg/l	100 mg/l
Aspergillus fumigatus	Colour	Turquoise-grey	Pale yellow	Pale yellow	Colourless with grey edges
	Growth (% of total area)	100%*	90%	62%	54%
Penicillium brevicompactum	Colour	Greyish-green	Greyish-white	Greyish-white	Greyish-white
	Growth (% of total area)	64%	21%	8%	5%
Cladosporium cladosporoides	Colour	Olivaceous-green	Olivaceous-black	Olivaceous-black	Olivaceous-black
	Growth (% of total area)	74%	33%	18%	16%
Mortierella alpina	Colour	White to light grey	Orange to brown	Orange to brown	Orange to brown
	Growth (% of total area)	100%*	80%**	100%**	100%***
Chaetomium globosum	Colour	Dark brown	Light brown	Light brown	Light brown
	Growth (% of total area)	100%*	36%	21%	10%
Stachybotrys chartarum	Colour	Dark olivaceous	Blackish-brown	Blackish-brown	Blackish-brown
	Growth (% of total area)	85%	18%	10%	5.5%
Effects of AgNPs on fungi cultured on gypsum drywalls					
Species	Mycelium	Control	60 mg/l	100 mg/l	200 mg/l
Chaetomium globosum	Colour	Black	Dark olive-brown	Dark olive-brown	Dark olive-brown
	Growth (% of total area)	68%	54%	36%	5%
Stachybotrys chartarum	Colour	Black	Olivaceous-green	Olivaceous-green	Olivaceous-green
	Growth (% of total area)	97%	79%	52%	30%

AgNPs. Measurements of conidiophores revealed, however, a significant increase in their diameters in relation to increasing concentration of AgNPs. It concerned conidiophores from both parts of mycelia — in the middle and at the edge (Fig. 2).

3.1.3. Cladosporium cladosporoides

Mycelia from the control group were floccose and powdery due to the abundant production of conidia. Colonies were olivaceous-green and olivaceous-brown in colour. However, significant changes in mycelium morphology were noted when *C. cladosporoides* was cultured in the presence of increasing concentrations of AgNPs. Colonies became dark, with glossy blackish-brown colour, olivaceous-black and/or metallic blue (Table 1). They formed velvet-like, wrinkled mycelia with halo-like zones around the fungal colonies (Fig. 3). Silver nanoparticles effectively limited the growth of *C. cladosporoides* (Fig. 1). The diameter of the mycelium from the control group of fungi reached 3.2 ± 0.03 cm after one week of culture. Significantly lower values of mycelium dimensions were noted in the presence of AgNPs. After four weeks of culture, mycelia from the control groups of fungi covered 74% of substrate areas, whereas 33%, 18% and 16% were noted for mycelia grown in the presence of 30, 60 and 100 mg/l AgNPs, respectively (Table 1). Only the addition of 30 mg/l of AgNPs caused a significant increase in conidiophore diameter in relation to that measured for the control fungi when the middle part of the mycelium was taken into consideration. However, the opposite effect was observed when conidiophores were probed from the edges of the cultures. There, significant decreases of conidiophore diameter were noted when fungi grew onto the substrate contaminated with 30 mg/l AgNPs (Fig. 2).

3.1.4. Mortierella alpina

Typical growth of *M. alpina* mycelium cultured on the agar substrate was zonate, rosette-like, and cottony in morphology with an alliaceous-like odour. The colour of the colony was generally white to light-grey or yellowish-white. Young mycelia were coenocytic, but became septated in older cultures. A rosette-like, concentric pattern of *M. alpina* growth was not observed, except, in colonies treated with increasing concentrations of AgNPs. The mycelia grew fast, forming rather fluffy and aerial structures instead of compact colonies. Moreover, the presence of AgNPs stimulated the production of orange pigments that diffused into the agar substrate as well as dark brown droplets deposited onto the surface of the mycelium. Generally, the colour of the mycelium was white; however, when observed from the reverse side of culture dishes, the colonies were orange to brown in colour (Fig. 4). The presence of AgNPs in agar substrates significantly increased fungi growth. The size of the mycelium calculated for the control groups, reached 4.2 ± 0.3 cm after one week of incubation and stopped after two weeks when the size of the mycelium was 7.5 ± 0.3 cm in diameter (Fig. 1). White aerial mycelium grown on PDA containing 30 and 60 mg/l AgNPs covered 80% and 100% the Petri dish area after 10 days of culture. At a concentration of 100 mg/l of AgNPs, the plates were covered with mycelium at 100% after 5 days of incubation. *M alpine* cultured on control PDA medium, however, covered the surface of Petri dishes after 14 days of incubation (Table 1).

The sizes of sporangiophores obtained from the middle part of the *M. alpina* mycelium revealed significantly higher diameter values when cultured with 60 mg/l and 100 mg/l AgNPs in relation to the control group of fungi. That increase of sporangiophore diameter was found to be dependent upon the AgNPs concentration. However, when sporangiophores were probed from the edges of mycelia, their diameters were significantly larger only when 60 mg/l of AgNPs was also present in the agar substrate (Fig. 2).

3.1.5. Chaetomium globosum

C. globosum is a fast grower; a diameter of mycelium of 45 ± 2 mm was reached within one week (50% of a dish area). The addition of AgNPs caused a significant reduction of *C. globosum* growth (Fig. 1). Control colonies of *C. globosum* were dark brown with terminal hairs and spores that were dark olive-brown in colour. As a result of the presence of AgNP in culture media, the colour of colonies became light brown covered with reddish spores. AgNPs significantly limited fungal growth (Fig. 1). At the end of the experiment, 36%, 21% and 10% of the Petri dish area was covered with mycelium when 30, 60 and 100 mg/l concentrations of AgNPs were applied (Table 1). Changes in the diameters of *C. globosum* conidiophores were found to be AgNP concentration dependent. The diameter of the conidiophores sampled from the central part of the mycelium increased, whereas those from the edge of the colonies decreased significantly with increasing concentrations of AgNPs (Fig. 2).

3.1.6. Stachybotrys chartarum

S. chartarum cultures grown on media supplemented with AgNPs changed the mycelium colour from dark olivaceous (control) to blackish-brown. Conidia from control fungi were dark olivaceous-grey, smooth and elliptic, but when *S. chartarum* was treated with AgNPs, the conidia became greenish and echinulate. The presence of

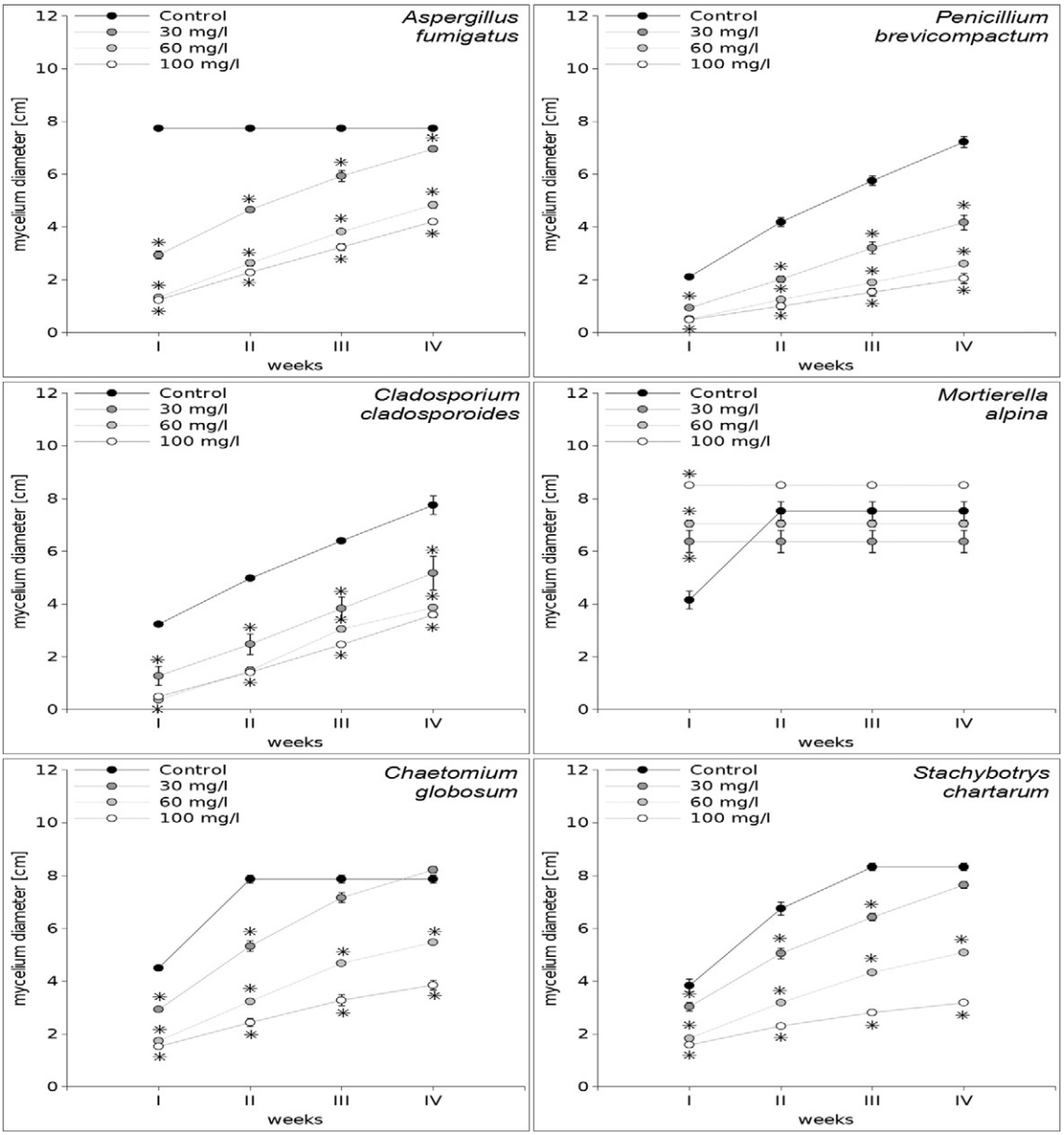

Fig. 1. Diameters of mycelia of six fungal species *A. fumigatus*, *P. brevicompactum*, *C. cladosporoides*, *M. alpina*, *C. globosum* and *S. chartarum* cultured onto PDA substrate in the presence of 30 mg/l, 60 mg/l and 100 mg/l AgNPs as well as in the control conditions (no AgNPs in the substrate). The sizes of mycelia were measured weekly during four weeks of fungi culture. * — statistically significant differences in relation to the control experimental group at $p \leq 0.05$.

AgNPs significantly limited the growth of the species as well (Fig. 1). The control plate area was covered by 85% after twenty-eight days of incubation, while only 18% (30 mg/l), 10% (60 mg/l) and 5.5% (100 mg/l) of the area was covered by mycelium when AgNPs were present in culture media (Table 1). Conidiophores taken from either central parts or edges of cultures have shown significantly smaller diameters in AgNP culture media in relation to those measured for the control group of fungi. The decrease in diameter was AgNP concentration dependent (Fig. 2).

3.2. Inhibition of the mould growth by silver nanoparticles on the gypsum drywalls

3.2.1. C. globosum

The black colour of *C. globosum* mycelia was noted in the gypsum group of fungi cultured in control conditions. However, the presence of AgNPs resulted in greyish-green or dark olive-brown colonies. Similarly, the difference in colours between control and experimental groups was observed in lemon-shaped ascospores of fungi. Those obtained from the control group were dark olivaceous-brown, whereas ascospores collected from AgNP treated mycelia became pale olive-green. After nine months of fungi cultured on gypsum wallboards untreated with AgNPs, the mycelium of *C. globosum* covered 68% of substrate area. When AgNPs were sprayed onto the gypsum surface in the amount of 60 mg/l, the area covered by mycelium was reduced to 54% of the gypsum board. Statistically significant differences, however, were only found after mycelium was cultured in AgNPs conditions — 100 mg/l of particle decreased growth of fungi to 36% of the total gypsum board area, whereas 200 mg/l of AgNPs reduced mycelium to 5% (Tables 1 and 2; Fig. 5).

3.2.2. S. chartarum

Mycelia from the control group of fungi were black, covered by a powdery bloom of dark brown/black conidial masses. However, when grown in AgNPs conditions, the colour changed to olivaceous-green or blackish brown. Also, a significant reduction of olivaceous-grey conidia layers on the surface of mycelium was noted. After nine months of

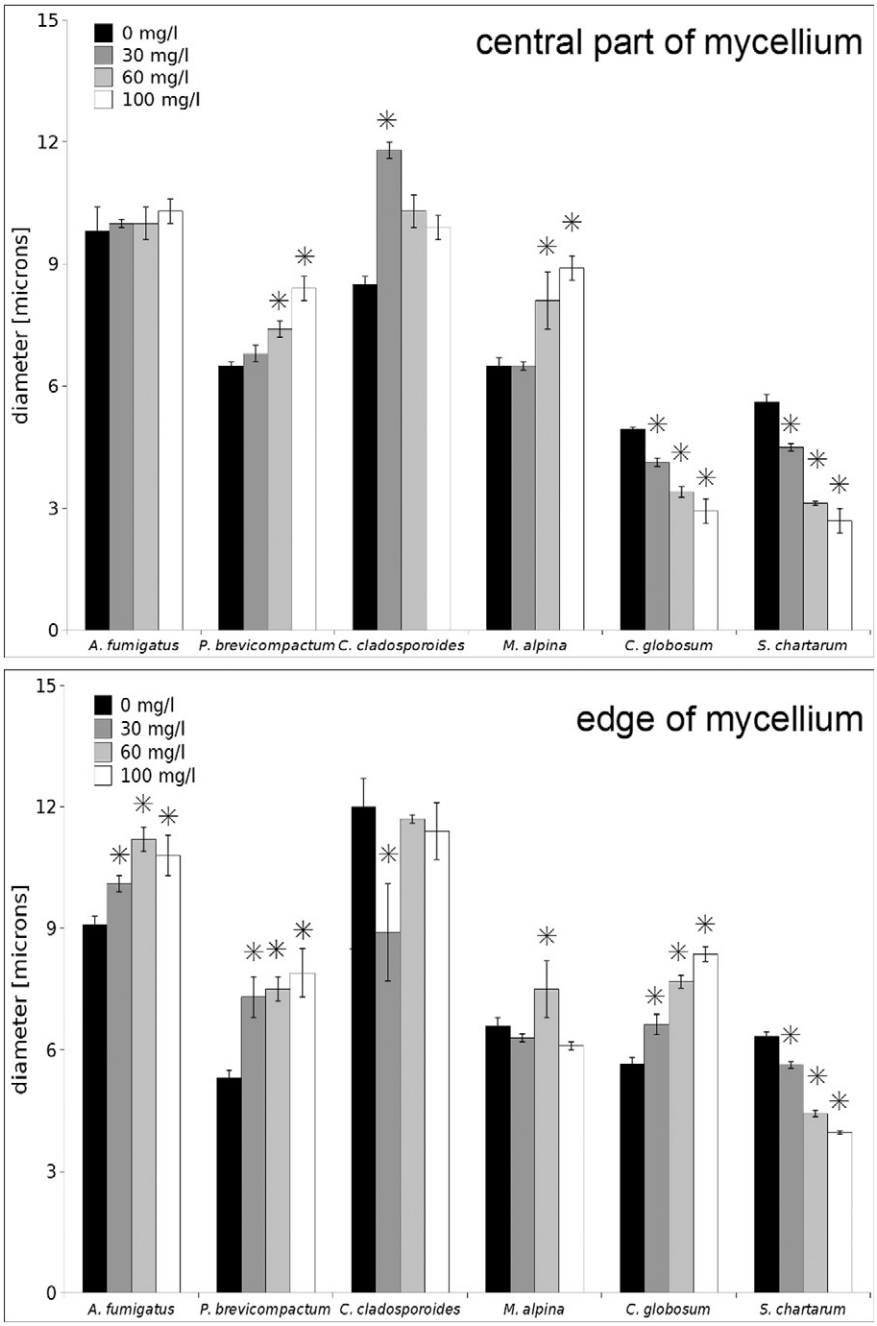

Fig. 2. Diameters of conidiophores of six fungal species *A. fumigatus*, *P. brevicompactum*, *C. cladosporoides*, *M. alpine*, *C. globosum* and *S. chartarum* cultured onto PDA substrate in the presence of 30 mg/l, 60 mg/l, and 100 mg/l AgNPs as well as in the control conditions (no AgNPs in the substrate). The upper panel shows conidiophore/sporangiophore sizes taken from the middle part of mycelium, whereas lower panel indicates diameters of those collected from the edge of mycelium. * — statistically significant differences in relation to the control experimental group at $p \leq 0.05$.

culture, the control group of gypsum boards was covered by 97% of mycelium of *S. chartarum* (Table 2; Fig. 5). Similar to *C. globosum*, 60 mg/l of AgNPs was not sufficient to impair the growth of *S. chartarum*. In those conditions, 79% of the total gypsum area was covered by fungi. Significantly reduced growth of fungi was noted in 100 mg/l and 200 mg/l concentrations of AgNPs, leading to 52% and 30% of the gypsum boards covered by the mycelium (Tables 1 and 2; Fig. 5).

4. Discussion

Silver has been in use since ancient times in the form of metallic silver, and silver nitrate, and silver sulfadiazine for the treatment of burns, wounds and several bacterial infections. Silver and its compounds are known as effective antimicrobial agents (Jung et al., 2008; Rai et al., 2009). In recent years, there has been substantial interest in the application of silver nanoparticles (AgNPs) in various fields. AgNPs have been applied in intercalation materials for electrical batteries, optical sensors, catalysts in chemical reactions, biosensors and bioactive materials, medical and pharmaceutical nanoengineering for delivery of therapeutic agents and for antimicrobial agents (Balan et al., 2007; Luo et al., 2006; Qureshi et al., 2011; Rybak et al., 2010; Thanh and Green, 2010; Tolaymat et al., 2010). The main interest related to innovative products based on silver nanoparticles is concentrated on the management of diseases related to skin/wound infections (Ilić et al., 2009; Paladini et al., 2014; Silva et al., 2014) and the design of antimicrobial agents and drug delivery vehicles (Kim et al., 2007; Ge et al., 2014;

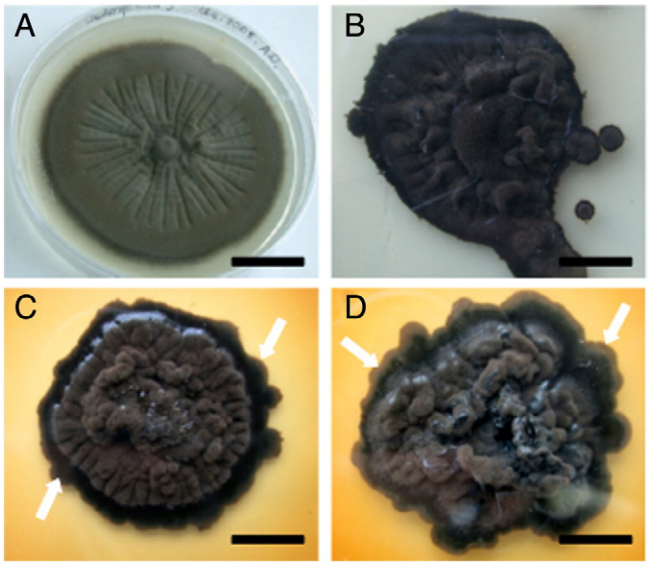

Fig. 3. The representative colony of *C. cladosporoides* grown onto the PDA substrate without AgNPs (A) as well as in the presence of 30 mg/l (B), 60 mg/l (C) and 100 mg/l (D) of AgNPs. The arrows indicate halo-like zones, typical for mycelia cultured onto media with high concentration of AgNPs. Scale bar: 2 cm (A) and 1 cm (B–D).

Narayanan and Park, 2014; Ramage et al., 2014; Kumar and Poornachandra, 2015). AgNP as a metal possesses unique physicochemical characteristics, including a high ratio of surface area to mass, sizes in the range of nanometres (10^{-9} m), high electrical and thermal conductivity, chemical stability, catalytic activity and nonlinear optical behaviour (Balan et al., 2007; Tran et al., 2013). Heavy metals including silver, even in small concentrations can induce structural and functional changes and, thereby destroy the cell of a microorganism. At relatively high concentrations, heavy metals act as a general protoplasmic poison, inducing denaturation of proteins and nucleic acid. The antibacterial effect and possible mechanisms of AgNP actions involved in the deactivation of bacterial strains are known, often mistakenly interpreted as a basis for antifungal activity (Choi et al., 2008; Martínez-Castañón et al., 2008; Rai et al., 2009; Xiu et al., 2011). However, little is known regarding the effects and mechanisms of their antifungal activity. It is most likely that, the size of the particle enhances its antimicrobial activity. Nanoparticles have a high surface to volume ratio which changes their properties when compared to non-nanoscale forms of the same material. Moreover, nanoparticles are able to penetrate biological membranes and cell walls more effectively, leading to cell death (Marambio-Jones and Hoek, 2010; Xiu et al., 2011). The aggregation of nanoparticles drastically decreases their accessibility, resulting in insufficient functionality against microorganisms (Badawy et al., 2010, 2012). For this reason,

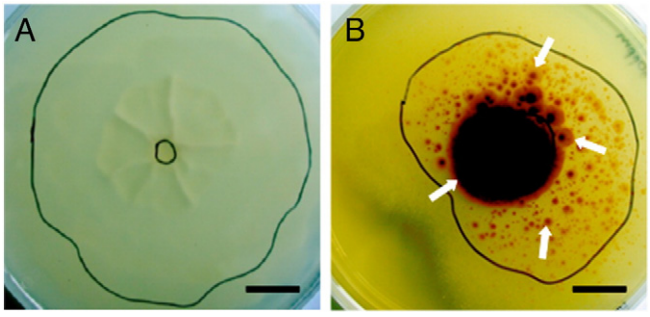

Fig. 4. The reverse sides of the culture dishes with mycelium of *M. alpina* grown on control PDA substrate without addition of AgNPs (A) and substrate supplemented with 100 mg/l of AgNPs (B). Black outline indicates the central part of mycelia, whereas arrows point pigment formation in the presence of AgNPs. Scale bar: 1 cm.

Table 2
The percentage of the gypsum drywall area covered by mycelia of *C. globosum* and *S. chartarum* cultured in the control (without AgNPs) conditions as well as in the presence of AgNPs in 60 mg/l, 100 mg/l, and 200 mgl concentrations. The values presented as the means ± standard deviations at the end of the experiment (after 90 days of fungi growth).

Gypsum drywalls	AgNP concentration [mg/l]			
	control	60	100	200
C. globosum	68 ± 8%	54 ± 3%	37 ± 11%*	5 ± 2%*
S. chartarum	97 ± 2%	79 ± 13%	52 ± 12%*	30 ± 9%*

* Statistically significant differences between experimental and control groups of fungi at $p \leq 0.05$.

homogeneous distribution of nanoparticles over building materials is required to guarantee better contact and reaction with microorganisms.

Fungi, bacteria, and algae may mediate the removal of heavy metals by bioaccumulation, biosorption and chemical transformation. Fungi can be involved in toxic metal immobilization via adsorption, bioaccumulation and metal micro-precipitation (Gadd, 2010; Haas et al., 1998; Tobin et al., 1994). Specific mechanisms could be employed for certain metals in particular fungal species. It is also possible that more than one mechanism is involved in reducing the toxicity of a metal (Pócsi, 2011).

Only recently, have bacterial pathogenic strains (*Staphylococcus epidermidis*, *Bacillus megaterium*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Pseudomonas aeruginosa* and *Escherichia coli*) of highly virulent properties and resistance to antibiotics become a main target for AgNPs application to impair the growth of those microorganisms (Son et al., 2006; Ilić et al., 2009; Paladini et al., 2014; Kumar and Poornachandra, 2015). The antibacterial properties of AgCl–TiO_2 nanoparticles against *Chromobacterium violaceum* were purposed for a potential use in active food packaging (Naik and Kowshik, 2014). In the case of fungi species, most of publications refer to tests where antimicrobial activities of AgNPs were examined in relation to clinical isolates of *Candida albicans*, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Aspergillus niger* (Panáček et al., 2009; Narayanan and Park, 2014; Silva et al., 2014). Very recently, an antifungal activity of AgNPs against *Alternaria alternata*, *Alternaria brassicae*, *Alternaria brassicicola*, *Bipolaris oryzae*, *Botrytis cinerea*, *Penicillium digitatum*, *Colletotrichum higginsianum*, *Colletotrichum orbiculare*, *Fusarium oxysporum* and *Pyricularia oryzae* has been described when immobilized on chitin nanofibers. The AgNPs/chitin films inhibited the spore germination of almost all tested pathogens (Ifuku et al., 2015). Also, biosynthesized AgNPs were evaluated in combination with fluconazole to assess their antifungal activity against human pathogenic *C. albicans* or plant fungi *Phoma glomerata*, *Phoma herbarium*, *Fusarium semitectum*, and *Trichoderma* sp. It has been shown that the antifungal activity of fluconazole increased in the presence of AgNPs (Gajbhiye et al., 2009). Some tests on the growth inhibitory activity of AgNPs were performed against eighteen plant pathogenic fungi cultured in vitro on different substrates. Those results revealed the ability of AgNPs to impair the growth of pathogens, however, the effects varied depending on the concentration and type of AgNPs applied (Kim et al., 2012).

The application of selected fungal species *P. brevicompactum*, *A. fumigatus*, *C. cladosporoides*, *S. chartarum*, *C. globosum* often led to biological corrosion of the building materials. This study showed that concentrations of AgNPs (from 30 to 200 mg/l) significantly reduced the growth rate of tested fungal species on the PDA medium and gypsum wallboard. Fungal morphology was altered by silver nanoparticles and changes in mycelial density and morphology were observed (Table 1). Formation of very dense mycelia or mycelial 'bushes' might be an effect of the penetration of hyphae into metal containing media (Gadd, 2007). During the experiment, each fungal strain responded differently to the presence of silver nanoparticles. Fungi are able to deal with high concentrations of toxic metals by employing different pathways through changes in metal speciation. It might lead to increased or decreased mobility, solubility, bioavailability and toxicity of metals. These include redox

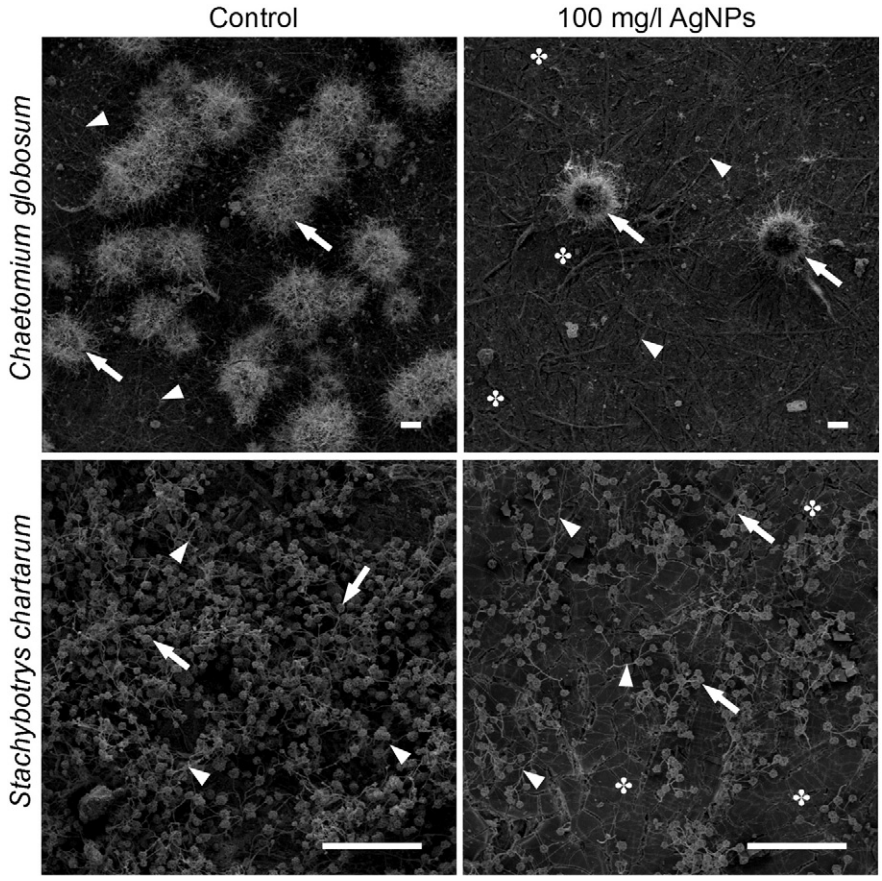

Fig. 5. Fruiting bodies and conidiophores (arrows) of *Chaetomium globosum* and *Stachybotrys chartarum*, respectively, present on the surface of the gypsum wallboard when fungi were treated with 100 mg/l of AgNPs (right panel) or untreated (control; left panel). Hyphae of fungi covering and penetrating the paper surface are well visible (arrowheads). ✣ indicates places when the paper surface is degraded due to the presence of fungi. Scale bar: 280 μm.

transformation, precipitation and production of cell wall metal-binding proteins such as metallothioneins, as well as cysteine-rich proteins called hydrophobins, extracellular polymeric substances (EPS), and pigments (Gadd, 2007; Pócsi, 2011; Puglisi et al., 2012). Overall, fungi could interact with metals by several different physico-chemical and biological mechanisms leading to their bioaccumulation, biosorption, micro-precipitation and chemical transformation.

Our results indicate that silver nanoparticles affect production and germination of conidia. Nano-silver caused colour changes of conidiophores and conidia of *P. brevicompactum*, *A. fumigatus*, *C. cladosporoides*, *S. chartarum*, and *C. globosum*. Colour changes of conidia and conidiophores observed in the above-mentioned fungal strains under AgNP treatment might be an effect of epigenetic changes either in gene expression or cellular phenotype such as the production of structural proteins and/or enzymes involved in the formation of pigments. Generally, toxic metals are potent inhibitors of enzymatic reactions and cause their irreversible inactivation. Laccase is one of the enzymes, which is involved in the process of forming various pigments, including melanin. Addition of 1 mM Ag^{+}, Hg^{2+}, Pb^{2+}, Zn^{2+}, H_2O_2 to the medium reduces activity of this enzyme (Baldrian, 2003). Production of melanin results in a colour change to black, and the development of chlamydospores (Gadd, 2007). Siderophores (iron-sequestering agents) could also play an important role in metal complexation (Francis, 1990; Gadd, 2007). The formation of droplets *A. fumigatus*, *P. brevicompactum*, *M. alpine*, *C. cladosporoides*, *S. chartarum*, and *C. globosum* on the surface was observed in media supplemented with AgNPs. Among other things, the intensive production of droplets might function as a water reservoir for maintaining a constant growth of aerial hyphae (Jennings, 1991) and as a reservoir of metabolic by-product's, metabolite reserves, secondary metabolites or enzymes (Colotelo, 1978). In addition, it was proposed that the droplets could indicate a response to stress due to unfavourable water potentials of cultivation media. In other cases, low-molecular weight organic acids (LMWOAs) might constitute an important pool of ligands, which could result in metal-complex formation. Filamentous fungi secrete considerable quantities of humic and organic acids such as: oxalate, oxalic acid, citric, gluconic, itaconic, and lactic acids (Gadd, 2007; Goldberg and Rokem, 2009; Magnuson and Lasure, 2004; Ogar et al., 2014; Papagianni, 2011). *Aspergillus* spp. produces citric, oxalic, fumaric, pyruvic, and succinic acids. *Penicillium* spp. excretes citric, tartaric, α-ketoglutaric, malic, gluconic, pyruvic and acetic ones (Seifert and Frisvad, 2000). *M. alpina* has the ability to produce arachidonic acid, acetic acid and a whole range of fatty acids (Certik and Shimizu, 1999; Dyal and Narine, 2005). *C. cladosporoides* strains are known from the production of pyruvic, acetic and aspartic acids (Devevre et al., 1996; Domsch et al., 1980). Known metabolites of *S. chartarum* include a number of carboxylic acids and humic-acid-like polymers, as well as pantothenic acid (Kuhn and Ghannoum, 2003). *C. globosum* produces orsellinic and heptelidic acids (Itoh et al., 1980; Seifert and Frisvad, 2000; White et al., 1997). It was presented that cytotoxicity of AgNPs appeared to be affected by humic acid (HA) concentration as well as by the presence of sunlight irradiation. Each fungal strain inhabited different sites with specific types of HA present in the environment. Therefore the potential toxicity of AgNPs in the environment could also vary (Dasari and Hwang, 2010).

The hyphae of *M. alpina* that grew on the medium with the addition of AgNPs formed a fluffy and cottony mycelium. Their growth was more intense compared to the control one. The changes of medium colour were observed; the reverse side of Petri dishes reflected a shade of orange/brown pigment that diffused into the medium. The presence of AgNPs most likely stimulated the production and excretion of extracellular chelating agents such as pigments. It could be a mechanism to

reduce/immobilize toxic Ag^{+} via extracellular chelating, chemical binding and finally, protection of hyphae from ion entrance to the mycelium. Formation of liquid droplets was observed on the surface of *M. alpina* mycelium when the medium was enriched with AgNPs. It is known that *M. alpina* could produce polyunsaturated fatty acids (PUFAs), accounting for up to 50% of its dry weight in the form of triacylglycerols (Wang et al., 2011). Metal-induced toxicity is also related to enhanced peroxidation of lipids. Lipid peroxides are formed due to metal-derived free radicals interactions with polyunsaturated fatty acid residues, which developed a cascade of events that finally produce mutagenic and carcinogenic exocyclic DNA adducts (Weber, 2002; Valko et al., 2005). Chelating compounds may include polyunsaturated fatty acids (PUFAs) such as gamma linolenic acid (GLA), arachidonic acid (AA), and diphenylhexatriene (DPH). *C. cladosporoides* exposed to AgNPs formed shiny blue colonies. This is most like due to silver ions precipitating with other ions that were present in the medium. A metallic blue sheen could be caused by the reaction of silver with chlorides. Silver chloride exposed to light gives a metallic sheen. The reverse side became metallic indigo blue, jade green blue around the mycelium. Presumably, the colour change was caused by the increased production of melanin and tyrosinase enzymes. Those substances are involved in the process of detoxification (Gadd, 2010). The presence of chitin, pigments such as melanin, and extracellular polysaccharides, increases the capacity of cell walls to bind metals (Gadd, 2004, 2007, 2010). Further studies are required to assess the mechanisms of AgNPs immobilization caused by fungal activity.

5. Conclusion

The effects of silver nanoparticles were tested against the most common fungal genera present in indoor environments: *Penicillium*, *Aspergillus*, *Cladosporium*, *Stachybotrys* and *Chaetomium* species. These studies demonstrated that the growth rates of all tested fungal species, except *Mortierella* species, were sensitive to the addition of AgNPs, which caused the limitation of *Chaetomium* and *Stachybotrys* on gypsum products. The response of each fungus was distinct and dependent upon the applied silver concentration and the rate of Ag ions released into the environment. However, possible bioaccumulation of silver in human and mammalian tissues gives rise to concerns about the potential ecotoxicological effects of uncontrolled release of nanoparticles into the environment. The rate of their release, through erosion from matrices, along with future exposure to living organisms, highlights a need for thorough and long-term ecotoxicological tests. Thus, studies on the potential impact of nanoparticles in relation to environment and human and animal health are necessary before the examination of their practical application in daily use products.

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