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Comparison of anti-*Aspergillus* activity of *Origanum vulgare* L. essential oil and commercial biocide based on silver ions and hydrogen peroxide

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Abstract – The antifungal activities of *Origanum vulgare* essential oil (EO) and of a biocide based on silver and hydrogen peroxide (Sanosil S003) against seven *Aspergillus* species isolated from different substrata (stone, brick, silk and paper) of cultural heritage objects in Serbia were evaluated. Microdilution, agar dilution and microatmosphere methods were used to determine minimal fungistatic and minimal fungicidal concentrations (MIC and MFC), and light microscopy to determine structural abnormalities. MIC and MFC values for *O. vulgare* EO ranged from 0.2 to 5 mg mL^{-1} and for Sanosil S003 from 5 to 250 mg mL^{-1}. *Aspergillus* sp. *sect. fumigati* was the most susceptible isolate, where MIC and MFC values were achieved at 0.5 mg mL^{-1} for *O. vulgare* EO, while MIC and MFC values for Sanosil S003 were achieved at 5 and 10 mg mL^{-1}, respectively. Morpho-physiological changes were documented in all isolates, including lack of sporulation, depigmentation of conidiogenous apparatus and conidia, and presence of aberrant fungal structures. *O. vulgare* EO exhibited stronger anti-*Aspergillus* activity than Sanosil S003, as demonstrated by the higher MIC and MFC values and fewer morpho-physiological changes observed in the tested Sanosil S003 concentrations. *O. vulgare* EO could be an excellent alternative to commercial biocides, with high potential in the field of cultural heritage conservation.

Keywords: antifungal activity**,** *Aspergllus* spp., cultural heritage, essential oil, *Origanum vulgare*, Sanosil S003

Abbreviations: CYA – Czapek yeast extract agar, EO – essential oil, MEA – malt extract agar, MEB – malt extract broth, MIC – minimal inhibitory concentration, MFC – minimal fungicidal concentration

Introduction

Aspergillus is a genus of fungi, including more than 180 recognized species with worldwide distribution. Several species have attracted attention as human and animal pathogens or because of their ability to produce various mycotoxins (Samson et al. 2010). Species of this genus are considered one of the most frequent infestation agents of cultural heritage objects (Florian 2002, Hu et al. 2013) and are capable of producing masses of airborne conidia and can grow and reproduce on many different carbon sources, including a variety of materials of which cultural heritage artifacts are composed (paper, textile, frescoes etc.) (Garg et al. 1995, Florian 2002). They are also involved in the degradation of a broad range of organic substrata (Goldman and Osmani 2007) due to their proteolytic, cellulolytic and amylolytic activity (Borrego et al. 2010, 2012a, Eida et al. 2011). A vast number of microfungi, including *Aspergillus* species, are able to produce different organic and inorganic acids during their metabolic activities on monument surfaces (Farooq et al. 2015). This leads to biodeterioration of stone substrata (such as sandstone, limestone, marble, mortar, brick etc.). Furthermore, *Aspergilli* are able to produce different pigments and contribute to the formation of biofilms, which diminish the esthetic value of cultural heritage artifacts and accelerate their biodeterioration (Vivar et al. 2013). Prevention of mold growth is nowadays a significant challenge for restorers, conservators and architects since chemical treatments must be non-destructive and non-toxic (Sterflinger 2010).

In the field of the safeguarding of the cultural heritage, both synthetic and natural biocides are applied. Although substances with biocidal activities extracted from plants are commonly used in medicine and food preservation, their

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use in the control of mold growth on cultural heritage objects is little reported (De Saravia et al. 2008). Oregano (*Origanum vulgare* L., Lamiaceae) is a widely known aromatic plant that has been used in agricultural, pharmaceutical and cosmetic industries (Şahin et al. 2004). Essential oil (EO) of this plant is recognized as an excellent natural source of substances with biological activities, such as terpens and phenolic compounds (Sivropoulou et al. 1996). The antimicrobial, antioxidative and cytotoxic activities of *O. vulgare* EO have already been demonstrated by Sivropoulou et al. (1996) and Şahin et al. (2004), while its antifungal activity has been documented in numerous studies (e.g. Adam et al. 1998, Clef et al. 2013, Delić et al. 2013) along with several studies concerning anti-*Aspergillus* activity (e.g. Viuda Martos et al. 2007, Carmo et al. 2008, Souza et al. 2010).

Commercial biocides include numerous organic compounds, metals, oxidizing agents and various synthetic products that have been widely used in the field of cultural heritage conservation (Warscheid and Braams 2000). Silver and its compounds are known to be one of the most effective antimicrobial agents (Russel 1994). Hydrogen peroxide is an oxidizing agent capable of transforming numerous organic molecules (Petri et al. 2011). It has been documented that this compound exhibits antibacterial and antifungal properties (Baldry 1983). There are several studies concerning the antifungal activity of biocides based on silver ions and hydrogen peroxide (Tasić 2009, Abdel-Mageed et al. 2012) but, to our knowledge, very few regarding *Aspergillus* species (e.g. Nabizadeh et al. 2008).

The aim of this study was to evaluate the *in vitro* antifungal activity of *Origano vulgare* EO, a natural product, and also of a synthetic biocide based on silver ions and hydrogen peroxide, on selected *Aspergillus* species isolated from cultural heritage objects in Serbia.

Materials and methods

Essential oil

Origanum vulgare L. EO (Frey+Lau, Ulzburg, Germany), a commercial sample from the collection of the Institute for Medicinal Plant Research "Dr Josif Pančić" in Belgrade, was used in the experiment. The chemical composition of the tested EO was reported by Stupar et al. (2014). The main components were carvarcrol (64.06%), linalool (17.56%), *p*-cymene (4.44%) and thymol (3.86%). According to the manufacturer's guidelines, the quality of the tested EOs corresponds to European Pharmacopeia 6 (2007).

Commercial biocide

The commercial biocide Sanosil S003 (Sanosil Ltd.) was obtained, as a water solution of final concentration 2.7% (silver nitrate, 0.2%; and hydrogen peroxide, 2.5%), from the Institute for Protection of Cultural Monuments in Serbia.

Aspergillus isolates

Seven *Aspergillus* species used in this study were isolated from different substrata of cultural heritage objects in Serbia: *Aspergillus* sp. 1 sect. *Flavi* (isolated from stone sculpture in the Museum of Contemporary Art, Belgrade); *Aspergillus* sp. 2 sect. *Flavi* (brick wall of Arača Church, Novi Bečej); *Aspergillus* sp. sect. *Circumdati* and *Aspergillus* sp. sect. *Nigri* (silk icon from Central Institute for Conservation in Belgrade); *Aspergillus* sp. sect. *Terrei* and *Aspergillus* sp. sect. *Fumigati* (archive paper from Central Institute for Conservation in Belgrade); *Aspergillus* sp. sect. *Nidulantes* (stone wall, Church of the Stara Pavlica Monastery, Raška).

All tested isolates were identified to section level using identification keys: Raper and Fennell (1965) and Samson et al. (2010). Isolated fungi were deposited in the Mycotheca of the Department for Algology, Mycology and Lichenology, Institute of Botany, Faculty of Biology, University of Belgrade. Isolates were maintained on malt extract agar (MEA), and Czapek yeast extract agar (CYA), stored at 4 °C and subcultured once a month.

Antifungal assays

The antifungal activities of the selected EO and the biocide Sanosil S003 were investigated using three different methods: microdilution, agar dilution and microatmosphere methods. Microdilution and microatmosphere methods were used for testing the antifungal activity of EO, while the biocide Sanosil S003 was tested using microdilution and agar dilution methods.

Microatmosphere method

The test was performed in sterile Petri plates (85 mm dia.) containing 20 mL of MEA (Maruzzella and Sicurella 1960). Tested *Aspergilli* were inoculated at the center of the MEA medium, using a sterile needle under a stereomicroscope (Stemi DV4, Zeiss), after which the Petri plates were turned over. A sterilized filter paper disc, soaked with various concentrations of *O. vulgare* EO, was placed in the center of the Petri plate lid interior. Concentrations of tested oil ranged from 0.1 to 5 mg mL^{-1}. Plates were incubated for 21 days at 25 °C, during which the fungal growth was monitored weekly. After cultivation period, minimal inhibitory concentrations (MICs), defined as the lowest concentration of added EO with no visible fungal growth on MEA, were determined. Minimal fungicidal concentrations (MFCs) were determined by re-inoculation of treated inoculums onto sterile MEA. The lowest concentrations of EO giving no visible growth after re-inoculation were regarded as MFCs.

Agar dilution method

The modified mycelia growth assay with MEA was used to investigate the antifungal activity of the biocide Sanosil S003 (Ishii 1995). The stock solution of biocide (2.7%) was further diluted in melted MEA in Petri plates to make final concentrations of the biocide ranging from 10 to 250 mg mL^{-1}. The fungi were inoculated at the center of the MEA. Plates were incubated for 21 days at 25 °C. MIC and MFC values were determined in the same manner as described above.

Microdilution method

To determine the antifungal activity of *O. vulgare* EO and the commercial biocide, the modified microdilution technique was used (Hanel and Raether 1998, Daouk et al. 1995). Conidial suspensions of selected *Aspergilli* were prepared by washing conidia from the surface of the 7 days old MEA slants with sterile saline (NaCl 0.85%, HemofarmhospitaLogica) containing 0.1% Tween 20 (Sinex Laboratory). Using a hemocytometer (Reichert, Warner-Lambert Technologies) conidia were counted on a 1 mm^2 surface and the concentration of conidia in the suspensions were calculated per formula:

$$\text{number of conidia} / mm^2 \times 10^4 \times \text{dilution}$$

The conidia suspension was adjusted to a concentration of approximately 1.0×10^7 in a final volume of 100 µL per each well. The inocula were stored at –20 °C for further use. Dilutions of all *Aspergilli* inocula were transferred onto solid MEA to verify contamination absence and in order to check the validity of the inocula.

Determination of the MICs was performed by a serial dilution technique using 96-well microtiter plates. Different volumes of investigated EO and biocide Sanosil S003 were dissolved in malt extract broth (MEB) medium with *Aspergilli* inoculums (10 µL) to make the same final concentrations, as those used in microatmosphere and agar dilution methods. The microtiter plates were incubated for 72 h at 28 °C. The lowest concentrations of tested biocides without visible growth under a binocular microscope were defined as the concentrations that completely inhibited *Aspergilli* growth (MICs). The minimum fungicidal concentrations (MFCs) were determined by serial subcultivation of inocula (2 µL) into microtiter plates containing 100 µL of MEB medium. The lowest concentration with no visible growth was defined as the MFC, indicating 99.5% killing of the original inoculum.

Microscopic analysis

After the incubation period, a sample of mycelium was taken from the periphery of a colony grown on MEA enclosed with evaporated EO (microatmosphere method) or on MEA enriched with different concentrations of Sanosil S003 (agar dilution method). A sample of mycelium was also taken from microwell of a microtiter plate. The samples were dyed and fixed with glycerol and observed under a light microscope (Zeiss Axio Imager M.1, with AxioVision Release 4.6 software) to examine structural abnormalities. Samples from the control plate without oil were also stained and observed. Additional observation was done 7 days after the incubation period in order to assess sustainability of morphological changes.

Results

Fungal susceptibility to essential oil

High fungistatic and fungicidal activity of *O. vulgare* EO was demonstrated with low MIC and MFC values. MIC values ranged between 0.2 and 2.5 mg mL^{-1} and MFC values varied between 0.5 and 5 mg mL^{-1}, obtained in both methods used (Fig. 1). *Aspergillus* sp. sect. *Fumigati* was the most susceptible isolate to EO treatments and fungicidal effect was achieved at 0.5 mg mL^{-1}. The most resistant isolates were *Aspergillus* sp. sect. *Nigri* and *Aspergillus* sp. sect. *Terrei*. For these isolates, fungicidal effect in microdilution method was achieved only with the highest tested EO concentration (5 mg mL^{-1}). Furthermore, lower MIC and MFC values were obtained in microatmosphere method for most isolates.

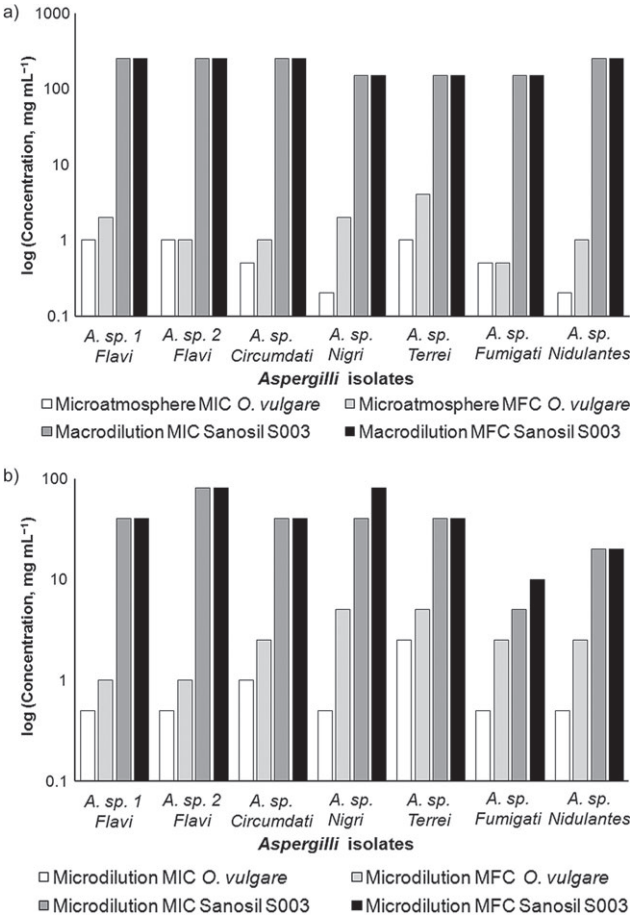

Fig. 1. Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) values of *Origanum vulgare* essential oil and Sanosil S003 on tested *Aspergillus* isolates obtained by: a) microatmosphere and agar dilution methods, b) microdilution method. Values in y axis are shown in logarithmic scale.

Fungal susceptibility to commercial biocide

The biocide Sanosil S003 exhibited moderate antifungal activity, significantly lower than *O. vulgare* EO. MIC values from 5 to 250 mg mL^{-1} and MFC values from 10 to 250 mg mL^{-1} were obtained in both methods used (Fig. 1). *Aspergillus* sp. sect. *Fumigati* was the most susceptible isolate to biocide treatments and fungicidal effect was achieved at 10 mg mL^{-1}. In the agar dilution method, fungicidal effect for both isolates from *Flavi* section and for *Aspergillus* sp. sect. *Circumdati* was obtained only with the highest tested biocide concentration (250 mg mL^{-1}). Lower MIC and MFC values were obtained in the microdilution method for most isolates.

Tab. 1. Observed morpho-physiological changes in tested *Aspergillus* isolates at different concentrations of *Origanum vulgare* essential oil and Sanosil S003, in microdilution method (after 72 h) and in microatmosphere and agar dilution method (after 21 day). Legend: ma – microatmosphere method, md – microdilution method, ad – agar dilution method, (+) – growth without morpho-physiological changes, (–) – no growth, DP – depigmentation of conidiogenous apparatus, LS – lack of sporulation, AC – aberrant conidiogenous apparatus development

Isolates	Concentrations of *O. vulgare* EO (mg mL^{-1})								Concentrations of biocide Sanosil S003 (mg mL^{-1})							
	0.1		0.2		0.5		1		5		10		20		40	
	ma	md	ma	md	ma	md	ma	md	ad	md	ad	md	ad	md	ad	md
Aspergillus sp. 1 sect. *Flavi*	+	DP, LS	+	DP, LS	DP	–	–	–	+	+	+	+	+	DP, LS	+	–
Aspergillus sp. 2 sect. *Flavi*	DP	DP, LS	DP	DP, LS	DP	–	–	–	+	+	+	+	+	+	+	DP, LS
Aspergillus sp. sect. *Circumdati*	+	DP, LS	DP, LS	DP, LS	–	DP, LS	–	–	+	DP	+	DP	+	DP, LS	+	–
Aspergillus sp. sect. *Nigri*	DP, AC	LS	–	DP, LS	–	–	–	–	+	+	+	+	+	+	+	–
Aspergillus sp. sect. *Terrei*	+	DP, LS	DP, LS	DP, LS	DP	DP, LS	–	DP, LS	+	DP, LS	+	DP, LS	+	DP, LS	+	–
Aspergillus sp. sect. *Fumigati*	+	DP, LS	DP, LS	DP, LS	–	–	–	–	+	–	+	–	+	–	+	–
Aspergillus sp. sect. *Nidulantes*	+	DP, LS	–	DP, LS	–	–	–	–	+	DP, LS	+	DP, LS	+	–	+	–

Morpho-physiological changes

Morpho-physiological changes, such as depigmentation of conidiogenous apparatus and conidia, lack of sporulation and presence of aberrant fungal structures, in addition to slower mycelial growth, were observed at EO concentrations lower than MICs obtained for each isolate (Tab. 1). Aberrant formation of conidial heads, including changes in shape and color, was observed in *Aspergillus* sp. sect. *Nigri* colonies grown at EO concentration of 0.1 mg mL^{-1} (Fig. 2, Tab. 1). Vesicles had a squashed appearance and were sometimes depigmented. Metulae were not developed – phialides were directly formed on vesicles in contrast to the biseriate conidial heads observed in control sample. Additionally, hyphae of *Aspergillus* sp. sect. *Nigri* had swellings and apical buddings (On-line Suppl. Fig. 1). Pigmentation loss and shape change in reproductive structures were observed in *Aspergillus* sp. 1 sect. *Flavi* colonies grown at EO concentration of 2.5 mg mL^{-1} (On-line Suppl. Fig. 2).

In biocide concentrations lower than the MICs obtained in the agar dilution and microdilution methods, fewer morpho-physiological changes were documented in the tested isolates (Tab. 1). These included lack of sporulation and depigmentation of conidiogenous apparatus and conidia. No morpho-physiological changes were documented in any of the tested concentrations of the biocide in the agar dilution method, although slower mycelial growth was observed in concentrations lower than the MICs obtained for each isolate.

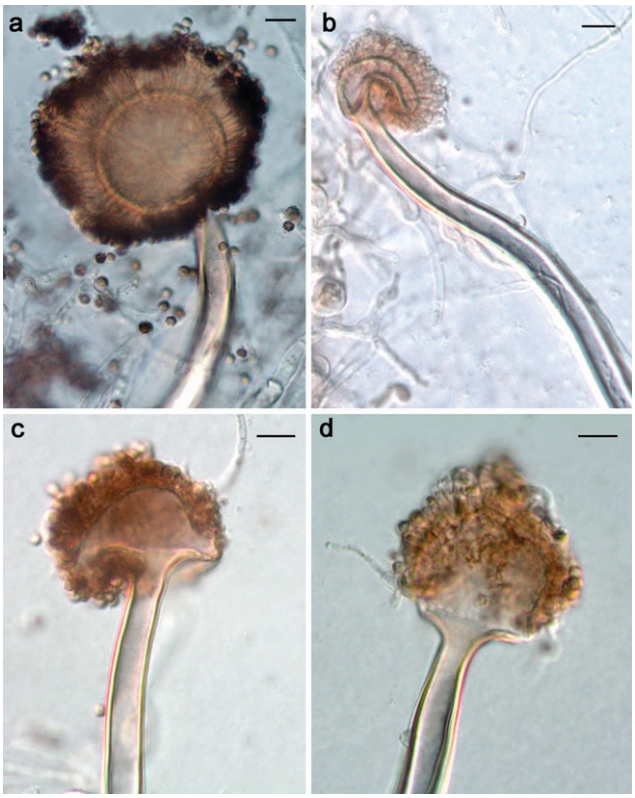

Fig. 2. Morphological changes of *Aspergillus* sp. sect. *Nigri* reproductive structures observed in microatmosphere method (essential oil in concentration of 0.1 mg mL^{-1}): a) normal conidiogenous apparatus (control); b–d) aberrant conidiogenous apparatus. Scale bars = 10 μm.

Discussion

The results of the microdilution and microatmosphere methods showed that various concentrations of *O. vulgare* EO exhibited relatively strong fungistatic and fungicidal activity against the tested *Aspergillus* isolates. The antifungal potential of this EO was demonstrated in several other studies. Antifungal activity of *O. vulgare* ssp. *vulgare* EO against 15 fungal isolates was documented, including *A. flavus* and *A. variecolor* (Şahin et al. 2004) and strong antifungal activity of the same EO against *A. niger* and *A. flavus* was shown by Viuda-Martos et al. (2007). The latter authors showed that *O. vulgare* EO exhibited stronger antifungal activity than the EOs of *Thymus vulgaris* and *Syzigium aromaticum*. The antifungal activity of *O. vulgare* EO against 6 *Aspergillus* species was demonstrated by Carmo et al. (2008), who noted that some concentrations of EO exhibited stronger antifungal activity than the tested antifungicals (amphotericine B and ketoconasole). Some authors documented the antifungal activity of *O. vulgare* EO against several fungi isolated from different substrates of cultural heritage objects, including *A. niger* and *A. ochraceus* (Stupar et al. 2014). These authors pointed out that *O. vulgare* EO exhibited antifungal activity similar to that of the tested biocide benzalkonium chloride but stronger compared than the EOs of *Rosmarinus officinalis* and *Lavandula angustifolia*.

The strong antifungal activity of *O. vulgare* essential oil can be attributed to its high content of some phenolic compounds, mostly carvacrol and thymol (Viuda-Martos et al. 2007, Clef et al. 2013). Although the thymol content in the EO used in this study was relatively low (3.86%), carvacrol was the main component (64.06%). Phenolic compounds, in appropriate concentrations, are reported to be effective against some microorganisms. Their mechanism of antimicrobial activity is related to disruption of microbial cell membrane and precipitation of cellular proteins (Burt 2004). It is also suggested that presence of an aromatic nucleus with OH group is responsible for making hydrogen bonds with active sites of target enzymes (Velluti et al. 2003). Therefore, it is possible to suppose that these groups are responsible for antimicrobial activity.

It has been shown that EOs are able to cause morpho-physiological changes in *Aspergillus* species including loss of sporulation and pigmentation and aberrant development of both hyphae and reproductive structures (De Billerbeck et al. 2001). Morpho-physiological changes were documented in all tested isolates in our study, such as lack of sporulation, depigmentation of conidiogenous apparatus and conidia, terminal and intercalar swellings and apical buddings. As a result of *O. vulgare* EO activity, morpho-physiological changes in *A. flavus* were showed by Souza et al. (2010). Authors reported loss of cytoplasm content, depigmentation and distorted development of hyphae with swellings and apical budding. Furthermore, the absence of conidiation was noted. Some authors used four different EOs in an antimicrobial vapor assay and documented morpho-physiological alterations of several fungal isolates, including *A. niger* (Ferdeş and Ungueranu 2012). They reported absence of phialides on vesicles and reduced conidial formation. Degenerative changes in hyphal morphology were reported as well. Sporulation is an essential part of the fungal life cycle and melanin is responsible for the survival and endurance of fungal spores (Wheeler and Bell 1988). Depigmentation of spores and conidial heads is probably due to the inhibition of melanin synthesis. Melanin is an important virulence factor for pathogenic fungi (Tsai et al. 1999) and, therefore, its loss can significantly reduce pathogenicity. This may be of great importance when *Aspergillus* species are considered, since some of them are well known human and animal pathogens (e.g. *A. fumigatus*), and producers of mycotoxins (e.g. *A. flavus*, *A. parasiticus*, *A. ochraceus*) (Samson et al. 2010). It is reported that application of EOs may result in retraction of cytoplasm and interaction of EO components with fungal cell wall (Carmo et al. 2008). There also may be interference in enzymatic reactions of cell wall synthesis, which affects fungal growth and morphogenesis (De Billerbeck et al. 2001, Souza et al. 2010). The fact that structural changes were observed in all fungal isolates tested in this study indicates that *O. vulgare* EO can affect the morphological development of different *Aspergillus* species and probably other fungi as well.

Results of the microdilution and agar dilution method showed that different concentrations of the biocide Sanosil S003 exhibited moderate fungistatic and fungicidal activity. Antifungal studies of biocides based on silver ions and hydrogen peroxide are scarce and there are only few studies concerning anti-*Aspergillus* activity. According to the manufacturer (Sanosil Ltd), antifungal properties of the biocide have been proven against numerous microorganisms, including *A. niger* as one of the tested fungal species, but there is no information about MIC or MFC values. It was shown that the biocide Sanosil Super 25 in a concentration of 40 ppm destroys the blastospores of *Candida albicans* (Tasić 2009). The same biocide also inhibits mycelial growth and sclerotal formation of *Botrytis cinerea* and *Sclerotinia sclerotiorum* (Abdel-Mageed et al. 2012). Antifungal activity of the biocide based on silver and hydrogen peroxide (Nanosil) was investigated by Nabizadeh et al. (2008). According to these authors, bacterial species were more sensitive to tested biocide than fungal species, where *A. niger* was the most resistant tested microorganism in the study.

Antifungal activity of Sanosil S003 could be ascribed to synergistic activity of silver ions and hydrogen peroxide. Hydrogen peroxide is an inorganic compound that belongs to the group of reactive oxygen species (ROS). This molecule is relatively stable under physiological conditions and can oxidize lipids, proteins, DNA and other organic molecules (Bienert et al. 2007). It is known that silver ions are highly reactive and can interfere with numerous biological processes in microorganisms, including structural and functional alterations of cell membrane and cell wall (Jo et al. 2009, Rai et al. 2012). Silver ions can inhibit replication by binding to DNA molecule (Landsdown 2002, Castellano et al. 2007). Moreover, silver ions have the capacity to react with thiol groups of proteins which leads to the inactivation of enzymes (Feng et al. 2000) and they can also cause pro-

tein inactivation by binding to their functional groups (Sondi and Salopek-Sondi 2004). In this way, silver ions block aquaporin-mediated water diffusion in *Saccharomyces cerevisiae* cells (Bienert et al. 2007). Since silver ions can interfere with structural components of the cell, cellular metabolism and reproduction, it is clear that they possess strong antifungal activity. It has been shown that combination of silver ions and hydrogen peroxide exhibits a synergistic mode of action that can sometimes be a thousand-fold higher than the sum of the separate agents (Pedahzur et al. 2000, Tasić 2009).

The aim of this study was to compare the antifungal activity of *O. vulgare* EO, which is a mixture of various organic compounds, and Sanosil S003, a commercial product that is a solution of inorganic compounds. According to the results presented in this study, it is evident that *O. vulgare* EO exhibits stronger antifungal activity than Sanosil S003 on the tested *Aspergillus* isolates. In some cases, the MIC and MFC values obtained with Sanosil S003 were several hundred times higher than those obtained with *O. vulgare* EO. Weaker antifungal activity of Sanosil S003 was confirmed with fewer observed morpho-physiological changes in the microdilution method, while none were observed in the agar dilution method.

The global commercial supply of conventional biocides is well established. However, biocides containing EOs are typically used in small niche product applications due to limited efficacy and therefore are only sold in small amounts (Browne et al. 2012). Additionally, EOs have only recently been recognized as a special class of biocides by EU biocide legislation (since 2011). EOs have seldom been tested *in vitro* against fungi isolated from cultural heritage objects. Examples include isolates from the documentary heritage (Borrego et al. 2012a), royal tomb paintings (Sakr et al. 2012), stone and wooden artifacts (Stupar et al. 2014). Reports regarding the implementation of natural products in the field of conservation of cultural heritage are scarce. However, Rakotonirayni et al. (2005) demonstrated preventive and curative action of linalool, as a main component of some EOs, on inoculated books in a glass test chamber. Gatenby and Townley (2003) showed the efficiency of *Melaleuca alternifolia* EO, in vapor form, in reducing spore number in Castle Hill Museum (Sydney) storage rooms. According to Chung et al. (2001), volatile extracts of some EOs have no negative effects on organic materials and as such can be used as an alternative source in control of biodeterioration of cultural heritage. Also, plant products with antimicrobial activities, such as EOs, have little negative impact on the environment or human health (Borrego et al. 2012b). Since *O. vulgare* EO demonstrated stronger antifungal activity than a commercial biocide against *Aspergillus* species isolated from cultural heritage objects the high potential of EOs as alternative agents in conservation of cultural heritage is indicated. Further studies are required to develop appropriate methods to apply EOs in the conservation of cultural heritage objects.

Conclusions

The results of this study show the efficacies of *O. vulgare* EO and of the commercial biocide Sanosil S003. The anti-*Aspergillus* potential of *O. vulgare* EO was higher than that of Sansoil S003. This claim was substantiated by the observed morpho-physiological changes in all tested isolates. This study presents *O. vulgare* EO as an excellent alternative to commercial biocides and suggests it can be usefully applied in the field of the conservation of cultural heritage.

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References

Abdel-Mageed, M. H., Mohamed, F. G., Soltan, H. H., Hafez, M. S. Abdel-Rahman, F. A., 2012: Controlling the grey mold and white rot diseases on bean pods under storage conditions using some safely chemical compounds. Journal of Biological Chemistry and Environmental Sciences 7, 617–634.

Adam, K., Sivropoulou, A., Kokkini, S., Lanaras, T. Arsenakis, M., 1998: Antifungal activities *of Origanum vulgare* subsp. *hirtum*, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* essential oils against human pathogenic fungi. Journal of Agricultural and Food Chemistry 46, 1739–1745.

Baldry, M. G. C., 1983: The bactericidal, fungicidal and sporicidal properties of hydrogen peroxide and peracetic acid. Journal of Applied Microbiology 54, 417–423.

Bienert, G. P., Møller, A. L., Kristiansen, K. A., Schulz, A., Møller, I. M., Schjoerring, J. K. Jahn, T. P., 2007: Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. Journal of Biological Chemistry 282, 1183–1192.

Borrego, S., Guiamet, P., De Saravia, S. G., Batistini, P., Garcia, M., Lavin, P., Perdomo, I., 2010: The quality of air at archives and the biodeterioration of photographs. International Biodeterioration and Biodegradation 64, 139–145.

Borrego, S., Lavin, P., Perdomo, I., Gómez De Saravia, S., Guiamet, P., 2012a: Determination of indoor air quality in archives and biodeterioration of the documentary heritage. ISRN Microbiology. doi:10.5402/2012/680598

Borrego, S., Valdés, O., Vivar, I., Lavin, P., Guiamet, P., Battistoni, P. Borges, P., 2012b: Essential oils of plants as biocides against microorganisms isolated from Cuban and Argentine documentary heritage. ISRN Microbiology. doi:10.5402/2012/826786

Browne, B. A., Geis, P., Rook, T., 2012: Conventional vs. natural preservatives. CSPA preservative defense task force. Retrieved October 15, 2012 from http://www.dow.com/microbial/pdfs/6974NatPreserve0512.pdf

Burt, S., 2004: Essential oils: their antibacterial properties and potential applications in foods – a review. International Journal of Food Microbiology 94, 223–253.

Carmo, E. S., Lima, E. O., Souza, E. L., 2008: The potential of *Origanum vulgare* L. (Lamiaceae) essential oil in inhibiting

the growth of some food-related *Aspergillus* species. Brazilian Journal of Microbiology 39, 362–367.

Castellano, J. J., Shafii, S. M., Ko, F., Donate, G., Wright, T. E., Mannari, R. J., Payne, W. G., Smith, D. J. et al., 2007: Comparative evaluation of silver-containing antimicrobial dressings and drugs. International Wound Journal 4, 14–22.

Chung, Y. J., Lee, K. S., Han, S. H., Kang Iii, D., Lee, M. H., 2001: The use of fungicide and insecticide from medicinal plants for the conservation of cultural property (in Korean). Conservation studies 22, 5–26.

Clef, M. B., Madrid, I., Meinerz, A. R., Meireles, M. C. A., De Mello, J. R. B., Rodrigues, M. R., Escareno, J. J. H., 2013: Essential oils against *Candida* spp.: in vitro antifungal activity of *Origanum vulgare*. African Journal of Microbiology Research 7, 2245–2250.

Daouk, K. D., Dagher, M. S., Sattout, J. E., 1995: Antifungal activity of the essential oil of *Origanum syriacum* L. Journal of Food Protection 58, 1147–1149.

De Billerbeck, V. G., Roques, C. G., Bessière, J. M., Fonvieille, J. L., Dargent, R., 2001: Effects of *Cymbopogon nardus* (L.) W. Watson essential oil on the growth and morphogenesis of *Aspergillus niger*. Canadian Journal of Microbiology 47, 9–17.

De Saravia, S. G. G., De La Paz Naramjo, J., Guiamet, P., Arenas, P., Borrego, S. F., 2008: Biocide activity of natural extracts against microorganisms affecting archives. Boletin latinoamericano y del caribe de plantas medicinales y aromaticas 7, 25–29.

Delić, D., Skorbonja, J., Karaman, M., Matavulj, M., Bogavac, M., 2013: Antifungal activity of essential oils of *Origanum vulgare* and *Rosmarinus officinalis* against three *Candida albicans* strains. Journal of Natural Sciences, Matica Srpska Novi Sad 124, 203–211.

Eida, M. F., Nagaoka, T., Wasaki, J., Kouno, K., 2011: Evaluation of cellulolytic and hemicellulolytic abilities of fungi isolated from coffee residue and sawdust composts. Microbes and Environments 26, 220–227.

European Pharmacopeia 6.0. 2007. Council of Europe. Strasbourg.

Farooq, M., Hassan, M., Gull, F., 2015: Mycobial deterioration of stone monuments of Dharmarajika, Taxila. Journal of Microbiology and Experimentation. 2, 36. doi:10.15406/jmen.2015.02.00036

Feng, Q. L., Wu, J., Chen, G. Q., Cui, F. Z., Kim, T. N., Kim, J. O., 2000: Mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. Journal of Biomedical Materials Research 52, 662–668.

Ferdeş, M., Ungureanu, C., 2012: Antimicrobial activity of essential oils against four food-borne fungal strains. UPB Scientific Bulletin. Series B 74, 87–98.

Florian, M. L. E., 2002: Fungal facts: solving fungal problems in heritage collections. Archetype publications. London.

Garg, K. L., Jain, K. K., Mishra, A. K., 1995: Role of fungi in the deterioration of wall paintings. Science of the Total Environment 167, 255-271.

Gatenby, S., Townley, P., 2003: Preliminary research into the use of the essential oil of *Melaleuca alternifolia* (tea tree oil) in museum conservation. AICCM Bulletin 28, 67–70.

Goldman, G. H., Osmani, S. A. (eds.), 2007: The Aspergilli: genomics, medical aspects, biotechnology, and research methods. CRC press. Boca Raton.

Hanel, H., Raether, W., 1998: A more sophisticated method of determining the fungicidal effect of water-insoluble preparations with a cell harvester, using miconazole as an example. Mycoses 3, 148–154.

Hu, H., Ding, S., Katayama, Y., Kusumi, A., Li, S. X., De Vries, R. P., Wang, J., Yu, X. Z., Gu, J. D., 2013: Occurrence of *Aspergillus allahabadii* on sandstone at Bayon temple, Angkor Thom, Cambodia. International Biodeterioration & Biodegradation 76, 112–117.

Ishii, H., 1995: Monitoring of fungicide resistance in fungi: biological to biochemical approaches. In: Singh, S. U., Singh, P. R. (eds.), Molecular methods in plant pathology, 483–495. Lewis Publisher, Boca Raton, London, Tokyo.

Jo, Y. K., Kim, B., Jung, G., 2009: Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. Plant Disease 93, 1037–1043.

Landsdown, A. B. G., 2002: Silver I: its antibacterial properties and mechanism of action. Journal of Wound Care 11, 125–138.

Maruzzella, J. C., Sicurella, N. A., 1960: Antibacterial activity of essential oil vapors. Journal of American Pharmacists Association 49, 692–694.

Nabizadeh, R., Samadi, N., Sadeghpour, Z., Beikzadeh, M., 2008: Feasibility study of using complex of hydrogen peroxide and silver for disinfecting swimming pool water and its environment. Iranian Journal of Environmental Health Science and Engineering 5, 235–242.

Pedahzur, R., Katzenelson, D., Barnea, N., Lev, O., Shuval, H., Fattal, B., Ulitzur, S., 2000: The efficacy of long-lasting residual drinking water disinfectants based on hydrogen peroxide and silver. Water Science and Technology 42, 293–8.

Petri, B. G., Watts, R. J., Teel, A. L., Huling, S. G., Brown, R. A., 2011: Fundamentals of ISCO using hydrogen peroxide. In: Siegrist, R. L., Crimi, M., Simpkin, T. J. (eds.), In situ chemical oxidation for groundwater remediation, 33–88. Springer, New York.

Rai, M. K., Deshmukh, S. D., Ingle, A. P., Gade, A. K., 2012: Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. Journal of applied microbiology 112, 841–852.

Rakotonirainy, M. S., Lavédrine, B., 2005: Screening for antifungal activity of essential oils and related compounds to control the biocontamination in libraries and archives storage areas. International Biodeterioration and Biodegradation 55, 141–147.

Raper, B. K., Fennel, D. I., 1965: The Genus *Aspergillus*. The Williams and Wilkins Company. Baltimore, Maryland.

Russell, A. D., Path, F. R. C., Sl, F. P., Hugo, W. B., 1994: Antimicrobial activity and action of silver. Progress in Medicinal Chemistry 31, 351.

Şahin, F., Güllüce, M., Daferera, D., Sökmen, A., Sökmen, M., Polissiou, M., Agar, G., Özer, H., 2004: Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. vulgare in the Eastern Anatolia region of Turkey. Food Control 15, 549–557.

Sakr, A. A., Ghaly, M. F., Abdel-Haliem, M. E. F., 2012: The efficacy of specific essential oils on yeasts isolated from the royal tomb paintings at Tanis, Egypt. International Journal of Conservation Science 3, 87–92.

Samson, R. A., Houbraken, J., Thrane, U., Frisvad, J. C., Andersen, B., 2010: Food and indoor fungi. 1st edition. CBS-KNAW Fungal Biodiversity Centre. Utrecht. Netherlands.

Sivropoulou, A., Papanikolaou, E., Nikolaou, C., Kokkini, S., Lanaras, T., Arsenakis, M., 1996: Antimicrobial and cytotoxic activities of *Origanum* essential oils. Journal of Agricultural and Food Chemistry 44, 1202–1205.

Sondi, I., Salopek-Sondi, B., 2004: Silver nanoparticles as antimicrobial agent: A case study on *E. coli* as a model for Gram-negative bacteria. Journal of Colloid and Interface Science 275, 177–182.

Souza, N. A. B., Lima, E. D. O., Guedes, D. N., Pereira, F. D. O., Souza, E. L. D., Sousa, F. B. D., 2010: Efficacy of *Origanum* essential oils for inhibition of potentially pathogenic fungi. Brazilian Journal of Pharmaceutical Sciences 46, 499–508.

Sterflinger, K., 2010: Fungi: their role in deterioration of cultural heritage. Fungal Biology Reviews 24, 47–55.

Stupar, M., Grbić, M. L., Džamić, A., Unković, N., Ristić, M., Jelikić, A., Vukojević, J., 2014: Antifungal activity of selected essential oils and biocide benzalkonium chloride against the fungi isolated from cultural heritage objects. South African Journal of Botany 93, 118–124.

Tasić, S., 2009: Efficiency evaluation of the disinfectant based on silver and hydrogen peroxide. Journal of Agricultural Sciences 54, 269–275.

Tsai, H. F., Wheeler, M. H., Chang, Y. C., Kwonchugi, K. J., 1999: A developmentally regulated gene cluster involved in conidial pigment biosynthesis in *Aspergillus fumigatus*. Journal of Bacteriology 181, 6469–6477.

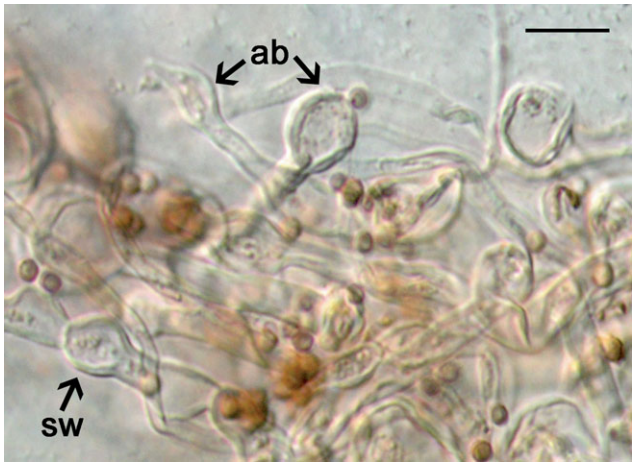
Velluti, A., Sanchis, V., Ramos, A. J., Egido, J., Marin, S., 2003: Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oils on growth and fumonisin B production by *Fusarium proliferatum* in maize grain. International Journal of Food Microbiology 89, 145–154.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., Pérez-Álvarez, J. A., 2007: Antifungal activities of thyme, clove and oregano essential oils. Journal of Food Safety 27, 91–101.

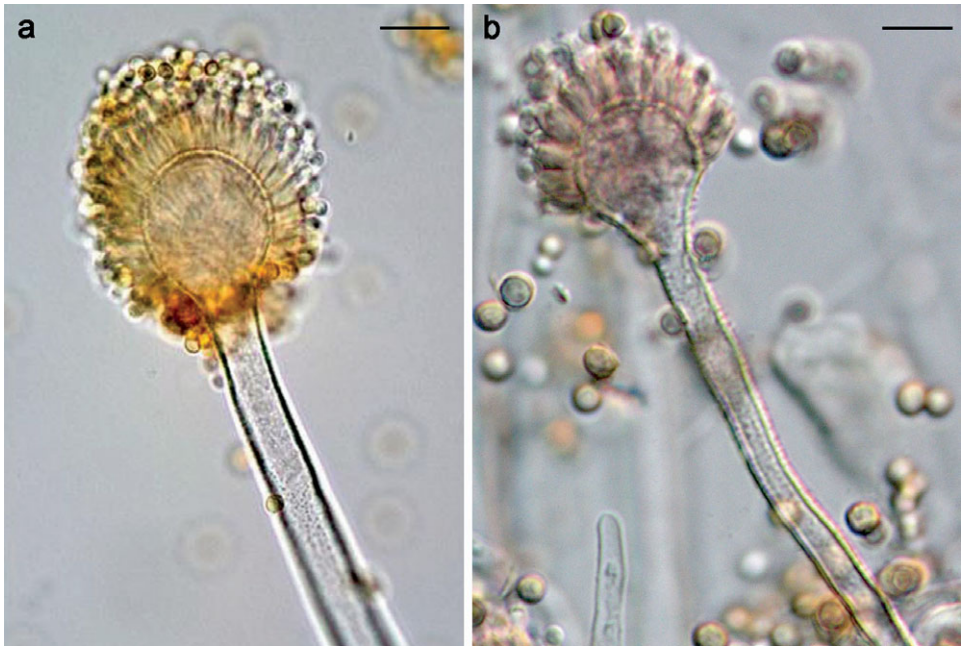
Vivar, I., Borrego, S., Ellis, G., Moreno, D. A., García, A. M., 2013: Fungal biodeterioration of color cinematographic films of the cultural heritage of Cuba. International Biodeterioration & Biodegradation 84, 372–380.

Warscheid, T., Braams, J., 2000: Biodeterioration of stone: a review. International Biodeterioration and Biodegradation 46, 343–368.

Wheeler, M. H., Bell, A. A., 1988: Melanins and their importance in pathogenic fungi. In: Mcginnis, M. R. (ed.), Current topics in medical mycology, 338–387. Springer-Verlag, New York.



On-line Suppl. Fig. 1. Morpho-physiological changes of *Aspergillus niger* hyphae observed in microatmosphere method (essential oil in concentration 0.1 μL mL^{-1}): ab – apical budding; sw – swelling; scale bar = 10 μm.



On-line Suppl. Fig. 2. Morpho-physiological changes of *Aspergillus flavus* reproductive structures observed in microatmosphere method (essential oil in concentration 2.5 μL mL^{-1}): a) normal conidiogenous apparatus (control), b) depigmented conidiogenous apparatus. Scale bars = 10 μm.