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SUCCEPTIBILITY OF SOME FUNGI TO *Boswellia carteri* Birdw. ESSENTIAL OIL

ABSTRACT: Antifungal activity of commercial sample of *Boswellia carteri* essential oil against selected micromycetes was evaluated *in vitro* using a microatmosphere method. When compared with biocide Sanosil S003, used as positive control, the tested essential oil showed moderate antifungal activity. The most susceptible fungi to oil treatment were *Stachybotrys chartarum* and *Trichotecium roseum*. For both fungi, mycelia growth inhibition of 85% was recorded at oil concentration of 100 μL mL^{-1}. The tested essential oil caused inhibition of *S. chartarum* sporulation as well as depigmentation of conidia, which is very significant since melanin contributes to virulence, survival and endurance of pathogenic fungi spores. *Aspergillus niger* was the least susceptible isolate to essential oil treatment. Mycelial growth of this fungus was not inhibited by any oil concentrations used in the experiment.

KEYWORDS: antifungal activity, *Boswellia carteri* Birdw., essential oil, micromycetes

INTRODUCTION

The genus *Boswellia* (order Sapindales; family Burseraceae) consists of 19 species, mostly distributed in tropical regions [Niebler and Buettner 2015]. *Boswellia carteri* Birdw. (syn. *Boswellia sacra* Flueck.), commonly known as frankincense or olibanum tree, is a deciduous middle sized tree which inhabits arid woodland and eroding slopes in Oman, southern Yemen and northern Somalia [Thulin 1998]. Although major botanical and scientific references currently regard two scientific names for frankincense, *B. carteri* and *B. sacra*, as being synonymous [Woolley *et al*., 2012], according to Niebler and Buettner [2015], *B. sacra* refers to frankincense population originating from Oman and Yemen, while the scientific name *B. carteri* is related to the plants of Somalian

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origin. Since the essential oil (EO) used in this study originated from Somalian plants, the name *Boswellia carteri* Birdw. was chosen. Frankincense resin has been widely used in folk medicine for the treatment of rheumatic and other inflammatory diseases, and ulcerative colitis [Prajapati *et al*., 2003]. High assortment of biological activity of *B. carteri* resin and essential oil is related to their chemical composition and presence of active ingredients such as α-pinene, linalool, and 1-octanol [Li *et al*., 2016].

The aim of this study was to estimate antifungal potential of *B. carteri* EO against selected mould species. Literature reports regarding the antifungal properties of *B. carteri* EO are scarce. However, El-Nagerabi *et al*. [2013] indicated significant antifungal properties of *B. carteri* EO against aflatoxin-producing *Aspergillus* species belonging to section *Flavi*. Also, Prakash *et al*. [2014] confirmed antifungal activity of frankincense against toxigenic *Aspergillus* species.

MATERIALS AND METHODS

Essential oil

The *Boswellia carteri* EO used in the study was a commercial sample obtained from Herba, d.o.o, Belgrade, Serbia (serial number: 8606103256300), as a product imported from France. The frankincense resin originated from Somalia, and was hydrodistilled in France in order to yield high quality EO.

Biocide

Biocide, Sanosil S003 (Sanosil Ltd.), used as a positive control in antifungal assay, was obtained from the Institute for Protection of Cultural Monuments in Serbia, as a water solution of the final concentration 2.7% (silver nitrate 0.2%, and hydrogen peroxide 2.5%).

Tested fungi

Fungi used in antifungal assay (*Aspergilus melleus* Yukawa (BEOFB 351m), *Aspergillus niger* Tiegh (BEOFB 342m), *Emericella nidulans* (Eidam) Vuill. (BEOFB 331m), *Stachybotrys chartarum* (Ehrenb.) S. Hughes (BEOFB 1410m), and *Trichotecium roseum* (Pers.) Link (BEOFB 1510m)) belong to the fungal collection of the Department for Algology, Mycology and Lichenology, Institute of Botany, Faculty of Biology, University of Belgrade. Fungal isolates were maintained on malt extract agar (MEA), potato dextrose agar (PDA), stored at 4 °C and subcultured once a month. All tested fungi are human, animal or plant pathogens.

Microatmosphere method

For studying the effect of the volatile fractions of the *B. carteri* EO, modified microatmosphere method, described by Maruzzella and Sicurella [1960], was used. The assay was performed in sterile Petri dishes (85mm, Ø) containing MEA (20ml). After inoculation of tested fungal isolates in the center of MEA, Petri plates were overturned. Sterilized filter paper (1cm^2, surface area) sodden with *B. carterii* EO at final concentrations of 5, 25, 50, 75 and 100 µL mL^{-1} were placed in the center of the Petri dish lid. Inoculated Petri dishes were then incubated in incubator (Memmert) at the temperature of 24 ± 1 °C. Colony growth of tested fungi was measured after 7 days. Effect of antifungal activity was expressed in terms of mycelial growth inhibition (MGI) and calculated according to Pandey et al. (1982) formula:

$$MGI\ (\%) = 100\ (DC - DT)/DC$$

DC = average diameter of fungal colony in control (mm);
DT = average diameter of fungal colony in treatment (mm).

Agar dilution method

To investigate the antifungal activity of the biocide Sanosil S003, agar dilution method, with MEA as medium, was used [Ishii 1995]. The stock solution of biocide (2.7%) was further diluted in melted MEA in Petri dishes to achieve final concentrations of 1, 5, 10, 20, 50 and 100 µL mL^{-1}. The tested fungi were then transferred to the center of MEA, and Petri dishes were incubated for 7 days (24 ± 1 ^{o}C). MGI of the biocide was determined in the same manner as for the micro-atmosphere method.

Microscopic analysis

After the incubation period, mycelium samples were taken from the margin of a colony grown on MEA enclosed with evaporated *B. carteri* EO (microatmosphere method) or on MEA enriched with different concentrations of Sanosil S003 (agar dilution method). The samples were dyed and fixed with Lactophenol cotton blue and observed under a light microscope (Zeiss Axio Imager M.1, with AxioVision Release 4.6 software) to examine the occurrence of morphological abnormalities. Samples from the control plates were also stained and observed.

Statistical analysis

One-way ANOVA (Microsoft office Excel 2007) was performed for mycelial growth assay, and p value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The tested fungi exposed to *Boswellia carteri* EO displayed different susceptibility. The least susceptible species was *Aspergillus niger,* with MGI not documented for any of the EO concentrations used in the experiment. On the other hand, *Stachybotrys chartarum* and *Trichotecium roseum* were the most sensitive fungal isolates ($p < 0.05$), with the highest documented MGI (85 ± 1.88% and 85.0 ± 1.24%, respectively) at the concentration of 100 µL mL^{-1} (Figure 1a). Biocide Sanosil S003, used as positive control, exhibited stronger antifungal activity compared with *B. carteri* EO. Although the tested fungi showed different susceptibility to biocide, in case presented here it can also be concluded that *S. chartarum* was the most sensitive fungi. Sanosil S003 at concentration of 5 µL mL^{-1} caused 100% of MGI for this mould. On the other hand, *A. melleus* and *Emericella nidulans* were the least sensitive fungi in biocide treatment ($p < 0.05$) (Figure 1b).

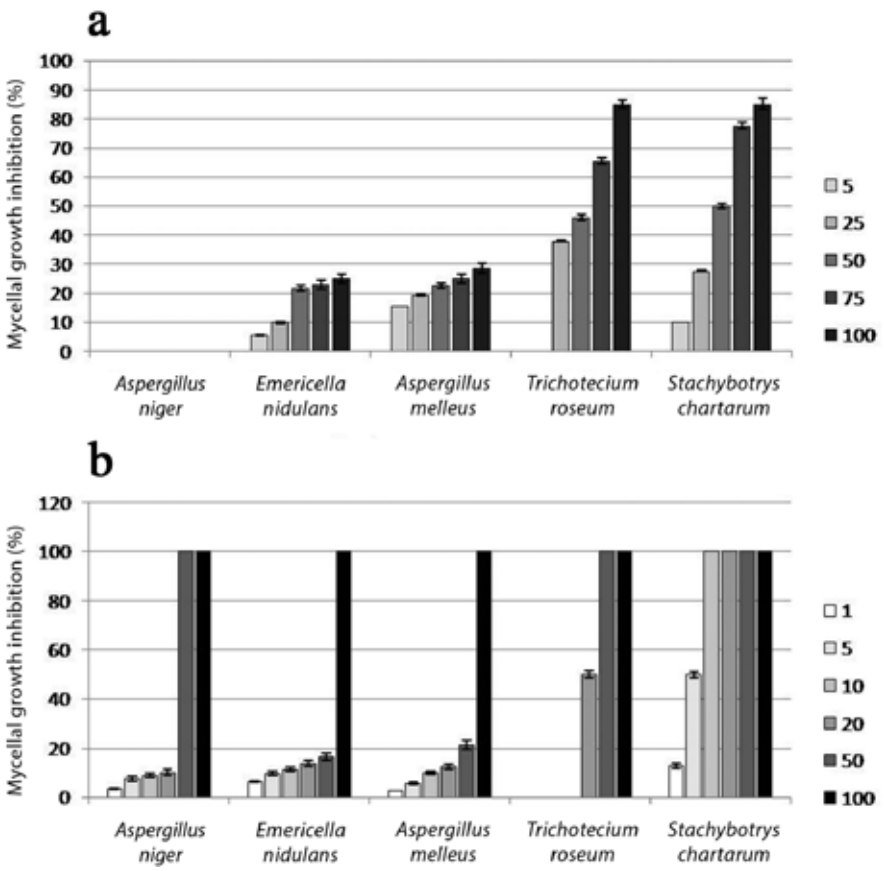

Figure 1. Susceptibility of the tested fungi to (a) *Boswellia carteri* essential oil and (b) biocide Sanosil S003. The concentration of oil and biocide is expressed in µL mL^{-1}.

In addition to MGI, variations in morphological aspects were observed for some fungi grown in essential oil enriched microatmosphere, such as different growth dynamics, absence of sporulation depigmentation, etc. (Figure 2). The highest concentration of *B. carteri* EO (100 µL mL^{-1}) caused scarce sporulation in *A. niger* colonies (Figure 2c). It appears that *B. carteri* EO can prevent *A. niger* to complete its life cycle by interfering with conidia formation. Similar variations in *A. niger* colonies, due to interaction with different EOs, have already been reported. Visible lack of sporulation and pigmentation of *A. niger* colonies grown with essential oil isolated from *Citrus sinensis* (L.) epicarp were reported by Sharma and Tripathi [2008], while Stupar *et al*. [2014] pointed out *Helichrysum italicum* (Roth) G. Don EO sporulation-inhibiting activity against *A. niger*.

Figure 2. Colony growth of (a) *Stachybotrys chartarum*, (b), *Trichotecium roseum* and (c) *Aspergillus niger* in *Boswellia carteri* essential oil enriched microatmosphere.

Likewise, scarce sporulation in *S. chartarum* colonies was recorded in the presence of *B. carteri* EO (75 and 100 µL mL^{-1}) (Figure 2a). Also, *B. carteri* EO caused depigmentation of *S. chartarum* conidia, probably due to inhibition of melanin synthesis during development of hyphae and conidia. Since melanin production by certain pathogenic fungi contributes not only to their virulence [Butler *et al*., 2001], but also to survival and endurance of fungal spores [Wheeler and Bell 1988], demelanization induced by interaction with *B. carteri* EO is a very significant result. Furthermore, *S. chartarum* is a well known producer of toxic secondary metabolites (atranones, dolabellanes, satratoxins, roridins and trichodermin) and exposure to this fungus leads to rashes, mucosal irritation and bleeding [Samson *et al*., 2010]. In recent years, this fungus has attracted attention as a possible causative agent of the so called "sick building syndrome"

[Mahmoudi and Gershwin 2000]. To our knowledge, there are no scientific reports regarding the *S. chartarum* susceptibility to *B. carteri* EO.

In case of *E. nidulans*, morphological alterations included formation of both teleomorphic and anamorphic fungal reproductive structures. Teleomorphic state of *E. nidulans* included formation of cleistotecial ascocarps. It is well known that formation of cleisthotecia involves the coordinated development of two quite different tissue types: ascogenous cells that ultimately give rise to asci and the network of sterile hyphae that surround the asci forming peridium [Sohn and Yohn 2002]. Since the observations obtained with light microscopy revealed the abundant presence of cleisthotecia surrounded by Hülle cells in the highest tested EO concentration (100 μL mL^{-1}) (Figure 3b), it can be assumed that increasing EO concentrations favored the formation of ascocarps. At lower oil concentration only conidiophores bearing the conidial heads were present (asexual state *Aspergillus nidulans*) (Figure 2a).

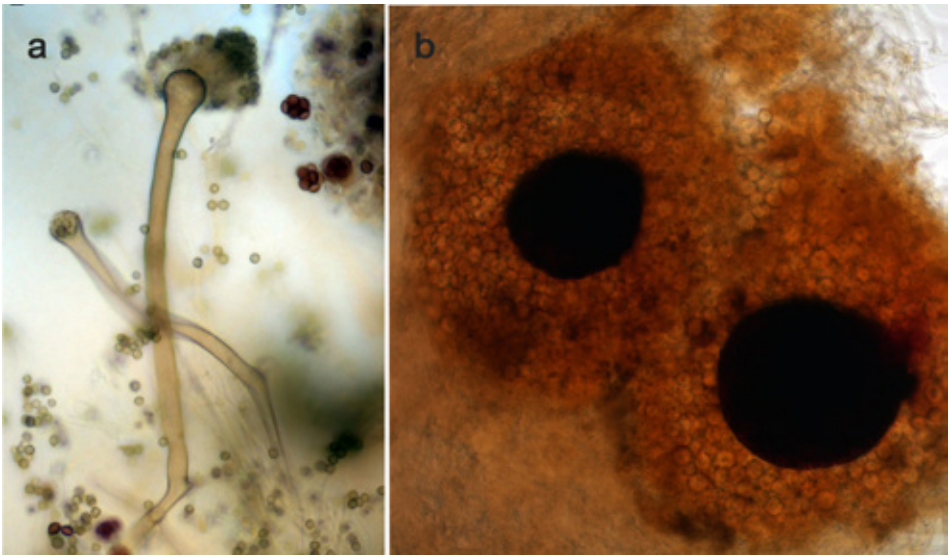

Figure 3. Influence of *Boswellia carteri* essential oil on anamorph/teleomorph occurrence of *Emericella nidulan*: a) anamorphic stage, *Aspergillus nidulans*, dominant in control colony, b) favorized cleisthotecia formation, documented at oil concentration of 100 μL mL^{-1}

The antifungal activity of *B. carteri* EO obtained in this experiment can be considered moderate and significantly lower than antifungal effect of Sanosil S003. However, documented MGI and morphophysiological variations suggested that investigated EO can interfere with fungal metabolism. Application of EOs against fungi may lead to cytoplasm retraction and hyphal wall disintegration [Carmo *et al*., 2008]. Also, EOs components can interfere with enzymatic reactions within the hyphae, and as such affect

fungal growth and morphogenesis [Souza *et al*., 2010]. On the other hand, antifungal activity of biocide Sanosil S003 could be ascribed to synergistic activity of its main components: silver ions and hydrogen peroxide. The main mechanisms of action of this biocide include oxidizing of lipids, proteins and DNA [Bienert *et al*., 2007], as well as functional alterations of cell membrane and hyphal walls [Jo *et al*., 2009], and enzyme inactivation [Feng *et al*., 2000].

In general, essential oils can be good alternative for nowadays commonly applied biocides, due to low mammalian toxicity, susceptibility to biodegradation and consequently low impact on the environment.

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ОСЕТЉИВОСТ МИКРОМИЦЕТА НА РАЗЛИЧИТЕ КОНЦЕНТРАЦИЈЕ ЕТАРСКОГ УЉА *Boswellia carteri* Birdw.

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РЕЗИМЕ: Антифунгална активност комерцијалног препарата етарског уља *Boswellia carteri* испитивана је методом ароматичне коморе. У поређењу са биоцидом Sanosil S003, коришћеним као позитивна контрола, испитивано етарско уље тамјана показало је умерену антифунгалну активност. Најосетљивије микромицете на испитивано етарско уље тамјана биле су *Stachybotrys chartarum* и *Trichotecium roseum*, код којих 85% инхибиције раста мицелије забележено при концентрацији уља 100 μL mL^{-1}. Такође, код врсте *S. chartarum* забележено је смањење интензитета спорулације, као и депигментација конидија. Меланин присутан у конидијама доприноси вурулентности и опстанку патогених врста. Врста *Aspergillus niger* показала је најмању осетљивост на испитивано етарско уље с обзиром да инхибиција раста мицелије ове врсте није забележена при највећој концентрацији уља коришћеном у експерименту.

КЉУЧНЕ РЕЧИ: антифунгална активност, *Boswellia carteri* Birdw., етарска уља, микромицете