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Effect of tea tree essential oil on microorganisms. A comparative study of tea tree oil antimicrobial effects

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The biological effect of tea tree (*Melaleuca alternifolia*) oil on airborne microorganisms isolated from the indoor environment was evaluated. Two sets of experiments were conducted: measurements of airborne microorganism concentration were carried out in three flats in compartment houses with mold problems, and the sensitivity of the prevalent species of microorganisms to tea tree essential oil (EO) was investigated in an *in vitro* study. Tea tree EO at a concentration of 0.25% strongly suppressed the growth of the bacteria and yeast tested and inhibited fungal conidia (or spore) germination and germ tube growth. Minimum Inhibitory Concentration (MIC) of tea tree oil to 19 species (17 fungi, yeast and 2 yeast-like fungi) of microorganisms was determined. The mean MICs were 0.75–1.0, 0.35–0.5, and 0.5% to fungi, yeast-like fungi and yeast, respectively. Microorganisms ranked according to resistance to tea tree action as follows: fungi > yeast-like fungi > yeast.

Key words: antifungal, antibacterial, essential oil, tea tree, airborne microorganisms

INTRODUCTION

The last decade has been characterized by a significant increase of the database on bioaerosols in indoor environments. Development of new sampling techniques and analytical methods, as well as advances in human exposure determination have allowed a more precise identification of the sources of microbial contamination, evaluation of the quality of indoor air and the assessment of potential hazards [1, 2]. People living in houses with moldy walls and furnishings, damp indoor air and bad odors are not only frustrated because of subjective discomfort but, in some cases, their health has been seriously affected [3]. Fungal contamination of air-handling units is a widespread phenomenon in buildings with central heating, ventilation, and air-conditioning systems and is a potential source of contamination for occupied spaces [4, 5]. Control of microorganisms in indoor environments has traditionally focused on source control, ventilation, and air cleaning. Source control emphasizes the reduction or elimination of moisture to limit fungal growth. There are different ways to describe the moisture conditions of materials. Reduction of moisture content of source on which microorganisms develop is not achievable in air-conditioning systems during cooling. By design, air-conditioning systems cause moisture to condense from air. Therefore, other methods are needed to reduce fungal contamination. Recently, there has been renewed interest in the use of germicidal UV irradiation to disinfect indoor environments for control of infectious diseases in hospitals, other health care facilities, and public shelter [1]. It is well documented that essential oils can also help and give us some support and protection. Essential oils are known to possess a variety of biological properties, including antimicrobial activity [7–10]. They represent very complex mixtures of compounds, mainly monoterpenes and sesquiterpenes [11].

The first scientific paper on tea tree oil was published as far back as 1923. Dr. A. R. Penfold, a chemist from Sweden, tested tea tree essential oil for its antiseptic properties [12]. He found it to be 13 times stronger in killing bacteria than carbolic acid, the universal standard antiseptic in the early 1900s. In the 1930s tea tree oil was used as an antiseptic for dressing wounds, for oral hygiene and as a disinfectant in hand soap. It was found to be 60 times more effective in killing typhoid bacilli than other disinfectants used in soap at that time. In the last 5 to 10 years there have been a lot of studies documenting the efficacy of tea tree oil in treating a variety of conditions including acne, burns, thrush (yeast), Candida, bacterial and fungal infections [13].

There are different kinds of tea trees, but the one with most potent medical oil is called *Melaleuca alternifolia*. The colorless or pale yellow oil of the tea tree has a pungent aroma resembling that of eucalyptus. It is composed of over 100 different compounds, 79 of which have been identified. Several compounds like viridflorene have never been found in nature before. Fifty to 60 percent of the oil is composed of terpenes (pinenes, terpinene, and cymene [14]. Cineole, which is responsible for the camphoraceous odor, comprises about 6 to 8 percent of the oil. The antibacterial, antifungal and antiviral properties of the oil come of a combination of many of the different compounds working together [15]. Generally, essential oil action is the result of the combined effect of both their active and inactive compounds. Inactive compounds might influence resumption, rate of reactions and bioavailability of the active compounds [16]. Inactive compounds might serve as growth substrate to microorganisms.

In the last 5 to 10 years there have been a lot of studies documenting the efficacy of tea tree oil in treating microorganisms infections in a variety of conditions [9, 16–18]. It has been suggested that volatile oils could act as antioxidants and can stabilize the membranes by decreasing their permeability, and they also have an ability to bind free acids [19].

The aim of the current investigation was to evaluate the antimicrobial activity of the commercially available tea tree oil and its volatile compounds and the possibility to use tea tree oil as a preventive means against microorganisms in indoor environments.

MATERIALS AND METHODS

The bacterial and fungal residential concentrations were investigated in three flats in compartment houses with mold problems. Sampling was performed in rooms where the inhabitants spent most of their time, by the gravitational (sediment) methodology described by Šveistytė [20] in detail. All three premises were equipped with a water-based central heating system and ventilated naturally, without the use of any ventilating or air-conditioning devices. Microbe collection from the indoor air was made on alternate days for all month. Species predominant in the indoor environment were used as test organisms (Table 1). A few species of *Chaetomium, Cladosporium, Stachybotrys, Phoma* and *Trichoderma*, which were commonly found to grow on building materials, were also included as test fungi. For comparative purposes, two samples of commercially available tea tree oil were evaluated using a similar methodology. Cell viability was determined by serial dilution and plating onto nutrient agar [21–23]. Two different methods were used in the evaluation. Inhibition of test organisms was determined by measuring the Minimum Inhibitory Concentration (MIC) in broth or agar dilution tests and by the technique of diffusion into agar. The microorganisms were incubated on nutrient media for 1–2 weeks at 25 °C. The spores or conidia were scraped into sterile distilled water to make culture suspensions, which were adjusted to 10^6 conidia per milliliter (standard suspensions). Those suspensions were used in all variants of the investigation.

Table 1. **List of species and strains and frequency of occurrence in the air of three indoor environments**

Microorganisms	Strains	Range of the frequency of occurrence (%)
Chaetomium globosum Kunze	BG-23	26.8–33.4
Cladosporium cladosporioides (Fresen.) G. A. de Vries	Wg-14	53.4–69.9
Aspergillus niger Tiegh.	BN-3	96.8–100
	OG-25	
Aspergillus versicolor (Vuill.) Tirab.	BG-12	36.5–64.2
	BG-3	
Aureobasidium pullulans (de Bary) G. Arnaud	OG-8	56.0–84.6
	OG-53	
Paecilomyces variotii Bainier	OG-7	84.1–100
Penicillium chrysogenum Thom	BG-31	67.3–82.5
Phoma glomerata (Corda) Wollenw. et Hochapfel	VG-2	23.6–45.7
Phoma sp.	BG-17	
Rhizopus stolonifer (Ehrenb. ex Fr.) Vuill.	OG-24	34.2–41.7
	DG-16	
Stachybotrys chartarum (Ehrenb. ex Link) Hughes	WG-5	36.7–49.8
Trichoderma viride Pers.	BG-19	23.4–35.4
Ulocladium atrum Preus	BG-55	25.2–36.9
Candida lipolytica (F. C. Harrison) Diddens et Lodder (= *Mycotorula lypolytica* Harrison)	BM-8	3.6–5.4
Geotricum candidum Link: Fr.	BM-11	16.8–19.4

Method 1: disc diffusion assay

• A sample of 100 µl of each culture suspension was spread evenly over the surface of a 20 ml malt extract agar (MEA, Oxoid), or Sabouraud agar (SA) plates for fungi and yeast, respectively.

• After the inoculated suspensions dried, a 15 mm paper disc was placed in the center of the plate, and 50 µl of tea tree oil (at concentrations 0.1, 0.15, 0.25, 0.3, 0.35, 0.5, 0.75, 1.0 or 1.5 % v/v) was placed on the paper disc.

• After the oils had soaked into each disc, the plates were inverted and incubated at 25 °C (yeast for 24 h and fungi for 48–72 h).

• After incubation, the plates were observed for a zone of no growth around each disc. This was called the zone of growth inhibition by tea tree oil.

The method is easy to perform and was useful for initial screening of tea tree oil concentrations for antimicrobial activity. Diffusion of oil components through agar is limited by their lack of water solubility [24], and inconsistent results and a lack of correlation with MIC have been reported [15, 25]. To ensure contact between the test organism and tea tree oil for the duration of the assay, we used an emulsifying agent, Tween 80, at a concentration of 0.01–0.1%. Higher concentrations of Tween 80 in the test medium can the decline antifungal activity of tea tree oil [23, 26].

Method 2: viability of test organisms in medium with tea tree oil

• Standard suspensions of each organism were diluted into double-strength malt extract broth (MEB).

• The suspensions obtained were then used to inoculate an equal volume of each oil concentration (from 0.05 to 1.5% varying by 0.05% intervals).

• The organism/tea tree oil solutions were mixed thoroughly, then incubated at 25 °C in the dark. Yeast was incubated for 24 h, and fungi for 48–72 h, depending on the strain.

• After incubation the organism/oil solutions were diluted and 10 µl of each dilution was spread onto MEA and SA plates.

• Inoculated plates were incubated for 18–72 h and the number of colonies was counted.

Broth dilution methods have generally been developed for use with water-soluble preparations and are not always suitable for use with essential oils of low water solubility. To ensure contact between the test organism and tea tree oil for the duration of the assay, it was necessary to use an emulsifying agent. A more suitable dispersing agent for tests carried out in broth is bacteriological agar at a concentration of 0.15–0.2% (w/v) [23, 25]. Agar dilution methods, in which various concentrations of the test substance are added to the agar medium before inoculation with the test organisms, overcome the turbidity problem, but emulsifiers are still needed to obtain a homogeneous mixture.

RESULTS AND DISCUSSION

The data obtained from our *in vitro* studies brought a new information connected with the biological activity of tea tree oil and its volatile compounds towards microorganisms common in the indoor environments. The indoor environment of dwellings is colonized by a number of various species of microorganisms. Their quantity in the air depends on the physiological properties of individual species, as well as on the type of activities of the occupants [27]. Fungal propagules and bacterial cells are important coherents to infectious diseases such as allergic rhinitis, asthma and hypersensitivity pneumonitis [1, 28, 29]. Fungal contamination of air-handling units is a widespread phenomenon in buildings with central heating, ventilation, and air-conditioning systems and is a potential source of contamination for occupied spaces. Control of microorganisms in the indoor environments has traditionally focused on source control, ventilation, and air cleaning. Disinfecting compounds often are toxic. In screaning for new less toxic compounds we as other researches have evaluated tea tree oil known for its broad antifungal properties [30]. This investigation was conducted with test microorganisms isolated from three flats in multi-family buildings with mold problems during a central heating season (in January 2002). During the preliminary phase of this study, we found dramatically high contamination of the indoor environments by fungi. The aim of this study was to highlight the potential of tea tree oil as a novel less toxic compound with potent *in-vitro* fungicidal and bactericidal activity against microorganisms common in indoor environments. People living in the flats observed in this investigation did not report allergic reactions or respiratory disease symptoms. However, microorganism growths on building materials increases all time and increases microorganism concentration in the air. Fungi recorded in these indoor environments (*Aspergilus* spp., *Chaetomium* spp, *Penicillium* spp., *Stachybotrys chartarum*, *Rhizopus stolonifer*, *Aureobasidium pullulams*, *Phoma glomerata*) possess allergenic and toxic properties and are known as risk factors of occupational respiratory diseases [28, 29]. The relative frequency of the prevalent genera in the indoor environment (mean of the three flats studied) is presented in Table 1. The isolated fungi belong to the following genera: *Alternaria* (recorded in 34.2% of collections by sediment method), *Aureobasidium* (in 56–84.6%), *Aspergillus* (96.8–100%), *Chaetomium* (26.8–33.4%), *Cladosporium* (53.4–69.9%), *Paecilomyces* (84.1–100%), *Penicillium* (67.3–82.5%), *Phoma* (23.6–45.7%), *Rhizopus* (34.2–41.7%), *Sporotrichum* (12–15.6%), *Stachybotrys* (36.7–49.8%), *Trichoderma* (23.4–35.4%) and

Ulocladium (in 25.2–36.9% of collects) (Table 1). Among the isolated fungi, *Absidia corymbifera, Aspergillus niger, A. versicolor, Aureobasidium pullulans*, *Paecilomyces variotii, Penicillium chrysogenum, Penicillium* spp. and *Rhizopus stolonifer* were predominant, but *Cladosporium spp.*, *Mortierella* spp., *P. carneus, Geotrichum* sp. and *Trichoderma* spp. were also present. Yeast *Candida lipolytica* (= *Mycotorula lypolytica*) was also predominant in the microflora of indoor air. A few strains of the genera *Foma*, *Cladosporium, Chaetomium* and *Trichoderma* were included in this investigation, because they were frequent (41.7%, 69.9%, 33.4% and 35.4%, respectively) on the walls of mould-affected dwellings, especially on the bath-walls. Piecková and Kunová [3] reported that usually walls are colonized by the "first colonizers" (*Aspergillus* Fr.: Fr. sp. and *Penicillium* sp.) and the "second" ones (namely *Cladosporium* Link sp.); the third group of colonizers are *Alternaria* sp., *Phoma* sp. and some *Aspergillus* and *Penicillium* spp. These filamentous fungi are known as able to produce compounds with a very high ciliostatic toxicity [3, 31].

Antimicrobial activity tests of tea tree oil in this investigation were made by use of paper disc–agar diffusion and broth dilution techniques. Two tea tree oil samples were very active against yeast *Candida lipolytica* (BM-6) and yeast-like fungi *Aureobasidium pullulans* (OG-8) and *Geotrichum candidum* (BM-11), and showed no antifungal activity at 0.25–0.5% v/v oil concentration (Table 2). MICs against fungi determined by paper disc–agar diffusion were 2–3 times higher that those against yeast and bacteria. The fungal isolates *Penicillium chrysogenum* (BG-31), *Paecilomyces variotii* (OG-7), *Phoma glomerata* (VG-2) were least susceptible to tea tree oil. MICs against those fungi ranged within 1.0–1.5%. Tea tree oil also showed a slight antifungal activity against *Aspergillus niger* (BN-3) and (OG-25), *Stachybotrys chartarum* (WG-5) and *Aspergillus versicolor* (BG-3). A 0.75–1.0% tea tree oil concentration induced an inhibition zone in the culture of these fungi.

The MICs of the tea tree oil were determined for microorganisms predominant in indoor environments by the broth dilution method. MICs for tea tree oil was defined as the lowest concentration that inhibited 100% of the visible growth when compared with the control growth. All assays were done in duplicate (with two differently obtained commercially tea tree oil samples) to verify the results, which are presented as mean of the two oil samples. Yeast isolates were susceptible to tea tree oil at MICs range 0.5–0.25% (Table 3). Unlike the activity detected against the yeast isolates, tea tree oil had essentially no effect against any of the fungus isolates we tested. Tea tree oil had the lowest MIC against *Candida lipolytica* (BM-8) (range, 0.1–0.25%). The second most susceptible group of microorganisms includes yeast-like fungi *Aureobasidium pullulans* (OG-8) and *Geotrichum candidum* (MM-11) with a similar MIC range (0.25–0.3%) (Table 3). The most susceptible of all of the fungal isolates was *Chetomium globosum* (BG-23), with MIC range

Table 2. **MIC values (% v/v) to microorganisms determined by disc-agar diffusion technique. Means from two tea tree oil samples investigated and three replicates of each experiment**

Microorganisms	Strains	MICs
Chaetomium globosum	BG-23	0.50 ± 0.05
Cladosporium cladosporioides	Wg-14	0.50 ± 0.10
Aspergillus niger	BN-3	0.75 ± 0.25
	OG-25	0.75 ± 0.05
Aspergillus versicolor	BG-12	0.75 ± 0.15
	BG-3	0.75 ± 0.25
Aureobasidium pullulans	OG-8	0.35 ± 0.15
	OG-53	0.35 ± 0.05
Paecilomyces variotii	OG-7	1.20 ± 0.25
Penicillium chrysogenum	BG-31	1.0 ± 0.20
Phoma glomerata	VG-2	1.50 ± 0.15
Phoma sp.	BG-17	0.75 ± 0.15
Rhizopus stolonifer	OG-24	0.50 ± 0.15
	DG-16	0.75 ± 0.20
Stachybotrys chartarum	WG-5	0.70 ± 0.25
Trichoderma viride	BG-19	0.55 ± 0.05
Ulocladium atrum	BG-55	0.75 ± 0.15
Candida lipolytica	BM-8	0.50 ± 0.10
Geotricum candida	BM-11	0.35 ± 0.15

Table 3. **MIC values (% v/v) to microorganisms determined by nutrient broth dilution technique. Means from two pine oil samples investigated and three replicates of each experiment**

Microorganisms	Strains	MICs
Chaetomium globosum	BG-23	0.13 ± 0.05
Cladosporium cladosporioides	Wg-14	0.20 ± 0.02
Aspergillus niger	BN-3	0.35 ± 0.05
	OG-25	0.30 ± 0.05
Aspergillus versicolor	BG-12	0.25 ± 0.01
	BG-3	0.30 ± 0.01
Aureobasidium pullulans	OG-8	0.25 ± 0.10
	OG-53	0.20 ± 0.01
Paecilomyces variotii	OG-7	0.70 ± 0.15
Penicillium chrysogenum	BG-31	0.30 ± 0.05
Phoma glomerata	VG-2	0.50 ± 0.15
Phoma sp.	BG-17	0.75 ± 0.25
Rhizopus stolonifer	OG-24	0.45 ± 0.015
	DG-16	0.75 ± 0.15
Stachybotrys chartarum	WG-5	0.35 ± 0.05
Trichoderma viride	BG-19	0.25 ± 0.05
Ulocladium atrum	BG-55	0.30 ± 0.01
Candida lipolytica	BM-8	0.10 ± 0.025
Geotricum candida	BM-11	0.25 ± 0.075

of 0.05–0.15%. Among the fungi tested were isolates (*Paecilomyces variotii* and *Phoma glomerata*) for which the MICs were 1.0–1.2%. Their conidia germination was not inhibited at the tea tree oil concentrations lethal to yeast in this investigation.

The results of this study confirm the excellent *in vitro* efficacy of tea tree oil against the more common microorganisms in indoor environments. *Melaleuca* oil demonstrated the lowest MICs and was most active against yeast and bacteria, with similar MIC and narrow MIC ranges. *Melaleuca* oil also shows similar activity against *Chaetomium globosum* and *Aureobasidium pullulans*. On the other hand, tea tree oil demonstrated lower activity against *Geotrichum candidum*, although still within the efficacy range, and not much higher than the MICs of the very susceptible strain of *Candida lipolytica*. Moreover, the MIC results indicate that *Melaleuca* is fungicidal for all of the fungal species evaluated. In addition to the broad antimicrobial activity of *Melaleuca* oil, the most exciting observation was its remarkably good activity demonstrated against bacteria and yeast strains. Our results agree with those obtained by Varquer and co-workers: *Melaleuca alternifolia* oil demonstrated a poor *in vitro* activity against two filamentous fungi, *Aspergillus fumigatus* and *A. nidulans* [30]. Other researchers have shown that the essential oil of *Melaleuca alternifolia* exibits antifungal activity against a wide range of common postharvest pathogens. Tea tree oil has antibacterial and antifungal properties that have secured it a place in the commercial pharmaceutical market. *Melaleuca* compounds may be a valuable addition to the management of bacteria and fungal infections in the future [18, 30, 32, 33].

In addition, comparison of our data with those previously published [10, 11, 16, 18, 30, 33] demonstrate very similar *in vitro* susceptibility results. Unfortunately, as previously stated by Hammer et al. [34], it is difficult to compare data from different investigators since the chemical composition of the oils may differ.

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D. Pečiulytė

ARBATMEDŽIO ALIEJAUS POVEIKIS ORE ESANČIŲ MIKROORGANIZMŲ VYSTYMUISI. ARBATMEDŽIO ALIEJAUS LYGINAMOJO EFEKTYVUMO PRIEŠ MIKROBUS TYRIMAS

S a n t r a u k a

Tyrėme arbatmedžio aliejaus biologinį aktyvumą mikroorganizmams, išskirtiems iš gyvenamųjų patalpų oro. Tyrimus atlikome dviem etapais: nustatėme mikroorganizmų koncentracijas trijų pelėjimo židinius turinčių butų ore ir laboratorinėmis sąlygomis tyrėme vyraujančių mikroorganizmų rūšių atsparumą arbatmedžio aliejui. Nustatėme minimalias, 19 rūšių mikroorganizmų (17 mikromicetų, 1 mielės ir 2 mieliagrybių) augim[1], stabdančias (inhibuojančias) koncentracijas (MIK), taip pat vidutines arbatmedžio aliejaus MIK, kurių vertės šio tyrimo sąlygomis mikromicetams buvo 0,75–1,0%, mieliagrybiams – 0,35–0,5%, o tirtoms mielėms – 0,15–0,2%. Mikroorganizmų jautrumą arbatmedžio aliejui išreiškia ši seka: mikromicetai > mieliagrybiai > mielės.