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Effect of tea tree essential oil on microorganisms 2. Evaluation of fungal reaction to tea tree oil under different conditions

D. Peèiulytë

Institute of Botany,
Þaliøjø eþerø 49, LT-2021 Vilnius,
Lithuania

Antifungal activity of *Melaleuca alternifolia* (tea tree) oil was determined mixing the oil with the culture medium (MEA, Malt Extract Agar) and seeding a fungus suspension or mycelium blocs. We report the fungitoxic activity of tea tree oil used at different concentrations (from 1.2 to 0.05% v/v), against 11 isolates of airborne fungi: *Chaetomium globosum*, *Stachybotrys chartarum* (= *S. atra*) and *Ulocladium atrum* (= *Stemphylium atrum*). Two sets of experiments were conducted: tea tree oil and its volatile fraction were tested against fungi during separate *in vitro* investigations. The results showed a strong fungicidal activity of tea tree oil at 0.75% concentration against all fungal isolates investigated. Lower concentrations (0.25–0.5%) inhibited conidia germination, germ tube elongation and mycelial growth of fungi. The indoor air contamination by fungi and their growth on building materials depend on moisture conditions. Effects of material moisture content (75% and 100% MC) on fungus *U. atrum* resistance to tea tree oil vapor used as a disinfectant was investigated. A correlation of the viable *U. atrum* conidia levels and material moisture content was detected for each fungus exposure to tea tree oil vapor.

Key words: *Melaleuca***,** Myrtaceae, essential oil, anti-fungal activity, airborne fungi, moisture conditions

INTRODUCTION

Public offices, hospitals and flats are places where germs (bacteria, viruses, and spores) in the air are least desirable. Fungal contamination of air-handling units is a widespread phenomenon in buildings [1–3]. 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. Virtually no microorganisms are able to grow below the equilibrium relative humidity (ERH) of a material 65% (water activity, a_w 0.65). The growth of xerophilic fungi is possible above ERH 65–70%, while hydrophilic decay fungi require at least ERH 90–95% for germination and growth on nutrient-rich media [4–7]. On the other hand, dirt and dust accumulated on building materials can serve as nutrients and may enable microorganisms to grow at a lower ERH level than on clean and new materials [4, 8–11]. Wet building materials containing cellulose (*e.g.*, gypsum board, wallpaper, insulation materials and straw) are especially susceptible to colonization by fungal species with a strong cellulolytic activity [12]. Reduction of moisture content of a medium 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. Various industries are now searching for sources of alternative, more natural and environmentally friendly antimicrobials and antibiotics as protection agents. It is known that many biologically active substances of vegetative origin (essential oils, terpenes, kumarines, fluvonoids, etc.) show an inhibiting activity against a number of microorganisms. Biological activity of tea tree oil has been known for centuries; it has become an increasingly popular product in recent years, but its mode of action is not fully understood. Results of our previous studies [13] as well as results obtained by other researchers [14, 15–18] show that *Melaleuca alternifolia* (tea tree) oil exhibit a strong activity against fungi, bacteria and yeasts, which are frequently recorded in the human environment.

This publication confirms and characterizes the antifungal activity of tea tree oil against fungi able to grow on wall-paper under high relative humidity. The aim of this investigation was to compare the methods used to evaluate the fungistatic activity of tea tree oil as well as the spectrum and mechanism of their action under different conditions.

MATERIALS AND METHODS

All assays were done with two commercially available pure tea tree oils to verify the results.

Four isolates of fungus *Stachybotrys chartarum* (Ehrenb. ex Link) Hughes (= *S. atra* Corda), five isolates of *Chaetomium globosum* Kunze and two isolates of *Ulocladium atrum* Preuss (= *Stemphylium atrum* Preuss) were used. These fungi were isolated from buildings with mold problems in Vilnius during our previous study [13] and were identified according to Domsch et al. [19]. *S. chartarum* and *C. globosum* are two important species colonizing wet building materials containing cellulose. Both fungal species can produce toxic metabolites in pure cultures as well as on artificially inoculated building materials [12]. Isolates of the genus *Chaetomium* have been reported to produce secondary metabolites and toxins: chaetoglobosins, chaetonin, and also sterigmatocystin, which are a potent carcinogen [20, 21]. *S. chartarum* has been reported to produce trichotecenes and antranones and is believed to be responsible for mycotoxicoses in humans [22]. *U. atrum* was used as a test-organism to investigate tea tree oil effects on fungi under different conditions in detail. One set of the experiments was done with all three fungi (*Stachybotrys chartarum*, *Chaetomium globosum* and *Ulocladium atrum*), and the other set with *Ulocladium atrum* isolate.

A. Assay of the tea tree oil fungitoxic activity against fungi *Stachybotrys chartarum, Chaetomium globosum* and *Ulocladium atrum.*

Effects of the tea tree oil on conidia germination

All three fungi were induced to sporulate by growth on malt extract agar (MEA). Conidia (or spore) suspensions were prepared in distilled water (DW) and were passed through four layers of muslin to remove mycelia debris. Each suspension was diluted to 2×10^5 spores/ml. The oil suspension was prepared according to Letessier et al. [23] by adding oil to DW containing 0.001% Tween-80 to obtain oil concentrations of 0, 0.75, 1.5 and 2.25%. A 15 µl droplet of the spore suspension was mixed with a 30 µl droplet of oil suspension on a glass slide (after this, initial oil concentrations on slides were 0, 0.25, 0.5 and 0.75%, respectively). The glass slide was placed on a glass rod in a Petri dish containing moist filter paper. The Petri dishes were sealed with parafilm (semi-permeable tape) to limit evaporation of the volatile compounds and were left for 24–48 h at 25 °C. Each slide was then stained with lactophenol cotton blue containing Tween-80 to stop further germ tube formation and growth. To assess the percentage of germination, the number of germinated spores out of 300 was counted.

Growth of fungi on a medium containing tea tree oil

The fungicidal activity of tea tree oil was studied on malt agar (MA) in 9-cm diameter Petri dishes. Essential oil (sterile) was added to 150 ml flasks of MA immediately prior to use, to obtain final concentrations of 0, 0.25, 0.5 and 0.75%. The prepared medium was poured into dishes (15 ml/dish). Colonized MA plugs, 7 mm in diameter, were cut from margins of actively growing cultures and placed in the center of a dish, with the mycelium facing to the medium. The dishes containing MA without oil inoculated with appropriate fungi strain were used as controls. The dishes were wrapped with parafilm.

Effect of tea tree oil vapor on mycelial growth

In that set of tests, MA without tea tree oil was poured into Petri dishes and, after agar had been cooled, the dishes were inoculated with 7-mm diameter agar blocks. Malt extract agar containing tea tree oil of a concentration investigated, was poured into the opposite plate and the lids of the plates were sealed with parafilm. Plates in which malt extract agar without tea tree oil (in opposite part) was poured were used as control. Plates were incubated for 7–9 days at 25 °C in the dark. Radial growth of the cultures was recorded. Five replicates for each combination of isolate and tea tree oil concentration were used. The mean colony diameter was evaluated.

B. Assay of tea tree oil volatile components against the test fungus *Ulocladium atrum*

Effects of tea tree oil vapor on conidia germination

Conidia suspensions of *U. atrum* were prepared as described above. A droplet (15 µl) of the spore suspension was mixed with a 30 µl of WA on a glass slide. Two separate sets were conducted: slides were suspended on glass rods which were placed a) on the WA agar containing 0, 0.25, 0.5 or 0.75% of the tea tree oil or b) slides were suspended on the rods in dishes containing pure tea tree oil an equivalent quantity. The dishes were sealed up with parafilm and left for 48 h at 25 °C, then glass slides were stained with lactophenol cotton blue and the germination percentage of conidium was evaluated. Glass slides incubated in sealed dishes without tea tree oil were used as controls.

Effect of tea tree oil vapor on test fungus conidia germination and germ tube formation on filter paper containing different moisture levels

There are different ways to describe materials' moisture conditions. In, practice, the moisture-holding properties of materials are usually measured as the moisture content (MC). Materials take up water from a humid atmosphere. Flannigan [24] found that MC for building material wall-paper at a relative humidity 80% of atmosphere is 11.3%. Moisture content of materials increases in water-damaged buildings. Wet building materials hold MC required for fungal growth. Filter paper (5 mm in diameter) was used in this investigation. Effects of tea tree oil on fungus *U. atrum* conidia germination on filter paper containing 75 and 100% MC was studied. A preliminary study was done to determine the water quantity needed to moisten paper samples to 75 or 100% MC. Conidial suspension of 10^{12} conidia/ml was used in the experiments. The suspension aliquots, which were spread on filter paper samples, were equivalent to water quantities determined n the preliminary study. Filter paper samples inoculated with aliquot suspensions were suspended on glass rods in Petri dishes containing 5 μl of pure tea tree oil/dish. Inoculated filter paper samples incubated in the dishes without tea tree oil were used as control. The dishes were incubated at 25 °C for 18–24 h. To assess the percentage of germination, the number of germinated spores out of 100 was counted.

Statistical analysis. For comparison of germination and mycelial growth, inhibition percentages were calculated (% inhibition = (control – treatment) / control × 100) [25]. The statistical analysis of results was done by the Dospekhov [26] method. In the text, data are presented as the mean ± S.D. All data were subjected to analysis of variance and separated with Student's t test (<5%).

RESULTS AND DISCUSSION

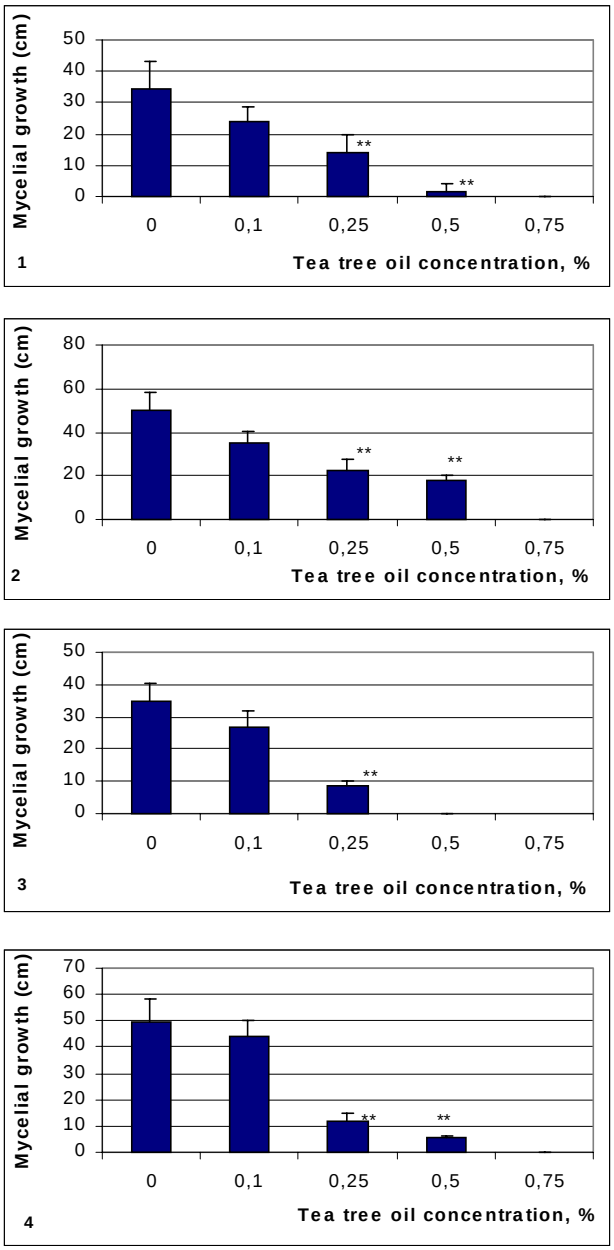
Tea tree oil (TTO) is used as a topical antiseptic. Nenoff et al. [27] evaluated TTO against 26 strains of various pathogenic dermatophyte species, 54 yeast, among them 32 strains of *Candida albicans* and other *Candida* sp., as well as 22 different *Malassezia furfur* strains. They found TTO to inhibit the growth of all clinical isolates. Most of tea tree oil investigations during the last two decades were conducted on clinical isolate tests [14–17, 28]. Only a few works have been done with filamentous fungi as test organisms [18, 29]. The antiseptic action of tea tree oil is one hundred times more powerful than carbolic acid, and it is non-poisonous to human [30]. Tea tree oil was used in the treatment of yeast *Candida* [16, 18, 31] and all sorts of infections, for ringworm, sunburn, acne, athlete's foot, toothache and pyorrhea [28, 30]. A variety of methods to ascertain the antifungal activity of TTO and its volatile components against microorganisms, among them filamentous fungi, isolated from the indoor environment have been employed in a previous study in our laboratory [13]. The minimum inhibitory concentration (MIC) of tea tree oil against filamentous fungi was measured and TTO at 250–750 micrograms/ml was found to be able to inhibit their growth. In this work we present data of the continued research on TTO activity against filamentous fungi. Eleven isolates of three fungal species (*Chaetomium globosum, Stachybotrys chartarum* and *Ulocladium atrum*) were tested against tea tree oil and its volatile components for fungicidal activity studies. We looked at the effect of tea tree oil against fungi able to produce mycotoxins and wanted to determine if fungitoxic oil properties may be useful for the control of these fungi in the indoor air and on building materials. All results are presented as a mean from three replicate experiments with each fungal strain and two tea tree oil samples.

Effects on conidia germination. Tea tree oil at the concentrations tested inhibited conidial germination and induced germination prolongation from 18–24 h in control to 24–48 h in DW containing tea tree oil. TTO inhibited the germination of all isolates of three fungi, but it had little effect on *U. atrum* isolate conidia germination (Table). The number of germinated conidia decreased with increasing the oil concentration. At a 0.75% TTO concentration only 2.4, 5.6 and 14.8% of *C. globosum* spores, *S. chartarum* and *U. atrum* conidia, respectively, germinated. The average germination for controls was 76.5 ± 6.3% (standard error (SE)) for *C.globosum*,

Table. **Fungal conidia germination (%) on water agar medium containing different tea tree oil concentrations. Significant differences from control are shown at *P = 0.05 and **P = 0.01**

Tea tree oil concentration	*Chaetomium globosum*[a] (n = 24)	***Stachybotrys chartarum***[a] (n = 30)	*Ulocladium atrum*[b] (n = 12)
0	76.5 ± 6.3	84. 7 ± 8.7	94.7 ± 9.9
0.25	42.3 ± 3.8**	56.2 ± 10.2*	83.5 ± 5.7
0.5	21.5 ± 5.1**	32.5 ± 4.2**	54.8 ± 4.6*
0.75	2.4 ± 0.2**	5.6 ± 0.8**	14.8 ± 3.4**
1.0	0	0	1.3 ± 0.2**

a – conidial germination was evaluated after 24 h; b – after 18 h of incubation.



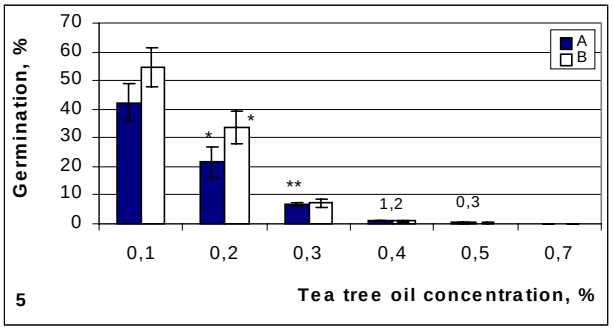
Figs. 1–4. Effects of tea tree oil on mycelial growth of *Chaetomium globosum* (n = 24) (Figs. 1, 3) and *Stachybotrys chartarum* (n = 30) (Figs. 2, 4). Figures 3 and 4 relate to volatile components of tea tree oil. All values are the mean of three replicate experiments with a different number of isolates. Significant differences are shown at *P = 0.05 and **P = 0.01

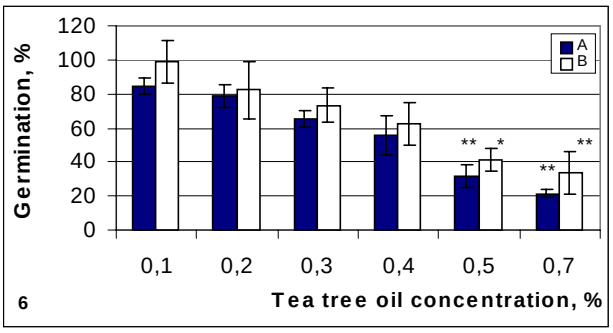
84.7 ± 8.72% SE for *S. chartarum* and 94.7 ± 9.9% SE for *U. atrum*. At the highest tea tree oil concentration used (1%), conidia of *C. globosum* and *S chartarum* did not germinate. The average germination for *U. atrum* was reduced to 1.3 ± 0.2% SE. Germination was less inhibited at 0.2% tea tree oil concentration, but fungi *C. globosum* and *S. chartarum* were inhibited more than *U. atrum* (P = 0.02). Tea tree oil used at 0.25 and 0.5% concentrations resulted in significant (P = 0.01) reductions in the germination of *C. globosum* and *S. chartarum*. Germination of *U. atrum* conidia at a 0.25% oil concentration was reduced to 83.5 ± 5.7% SE (P = 0.05), while at a 0.5% oil concentration the germination was reduced more significantly (P = 0.01) to 54.8 ± 4.6% SE.

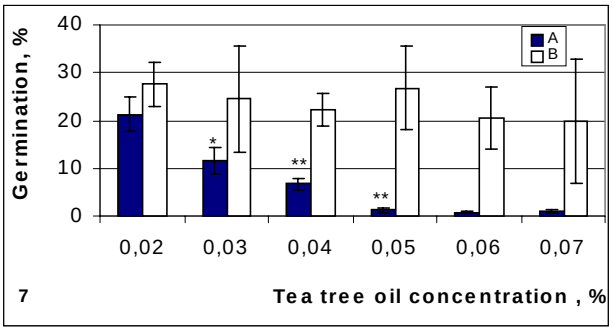
Effects on mycelial growth. Tea tree oil and its vapor effects on *C. globosum* and *S. chartarum* mycelial growth were investigated on MEA. The two fungi have a different colony morphology and growth rate on MEA. The differential inhibition of these two fungi by TTO and its vapor might help explain differences in the response to synergistic action of all tea tree oil components and oil vapor fraction. Although tea tree oil is inhibitory to fungi, the isolates tested differed in their tolerance to this chemical (Figs. 1–4). The average mycelial growth for controls was 34.6 ± 5.6 SE for *C. globosum* (Fig. 1) and 49.8 ± 8.4 SE for *S. chartarum* (Fig. 2). The inhibitory effects of tea tree oil were different on conidia germination and micelial growth of *C. globosum* and *S. chartarum*. In the current study, mycelial growth (Figs. 1–4) was inhibited stronger than conidia germination for *S. chartarum* and spore germination for *C. globosum* (Table). TTO inhibited the radial growth of both fungi. However, all tea tree oil concentrations used inhibited the radial growth of *C. globosum* (Fig. 1) more than of *S. chartarum* (Fig. 2) (P = 0.05). Growth reduction at 0.25 and 0.5% TTO concentrations was significant for both fungi (P = 0.01). The mycelial growth of both fungi was completely inhibited by tea tree oil used at a 0.75% concentration. Tea tree oil inhibited mycelial growth of *C. globosum* stronger than of *S. chartarum* at 0.25 and 0.5% oil concentrations (P < 0.01). The mycelial growth of *S. chartarum* at 0.25 and 0.5% tea tree oil concentrations was reduced significantly as compared with control (P = 0.01), but growth difference at those oil concentrations was not significant (P > 0.05). The fungus *C. globosum* was more sensitive to the increase of TTO concentration in the medium. Tea tree oil at a 0.75% concentration in the growth medium, determined by micelial growth inhibition percentage, was fungicidal for both fungi tested in this experiment.

Data obtained in the study of tea tree oil activity against fungal isolates were compared with those obtained in the study of TTO vapor antifungal activity. Seven days after exposure, the volatile components diffusing from a medium containing 0.75% of tea tree oil completely inhibited the mycelial growth of *C. globosum* at 0.5 and 0.75% oil concentrations (Fig. 3) and the growth of *S. chartarum* at a 0.75% oil concentration (Fig. 4). The average mycelial growth for controls (sealed up plates without tea tree oil addition into the medium) was the same as in the oil action by contact tests (34.6 ± 5.6 SE for *C. globosum* and 49.8 ± 8.4 SE for *S. chartarum*). In this way, fungi were not in direct contact with the

oil and any effect on mycelium growth could be attributed to vapor activity. The water content evaporated from the medium did not affect mycelial growth of fungi in this investigation. Fungal growth was slightly affected by volatile components diffusing from a medium containing 0.1% of tea tree oil. The growth increased significantly (P = 0.01) under tea tree oil vapor diffused from a medium containing 0.25 and 0.5% of tea tree oil. The growth of both fungi was more strongly affected by vapor than by the tea tree oil as a whole present in the growth medium. These differences were not large but significant (P = 0.05) and agreed with results obtained by Letessier et al. [23], which suggested a higher inhibition of radial growth by vapor than by complete oil, detected during Hyssop (*Hyssopus officinalis*) activity against *Pyrenophora avenae* and *Pyricularia oryzae*. The inhibitory activity of tea tree oil incorporated into agar was in fact due to volatile components that accumulated above the medium. Carlton et al. [32] suggested that separate components of an essential oil show different modes of action, which complement each other in the whole oil. They reported that complete oil would probably present a greater barrier to fungus adaptation than would a relatively simple mixture. We have not studied activity of separate compounds of TTO against filamentous fungi, therefore it is difficult to explain the higher fungicidal activity of tea tree oil vapor than TTO as a whole in this investigation. New experiments are needed to elucidate the mode of action of tea tree oil and its volatile components. The active ingredient in tea tree oil is terpinen-4-ol mostly responsible for antimicrobial activity [15] and is believed to be effective as an antifungal, antibacterial and antiviral agent.

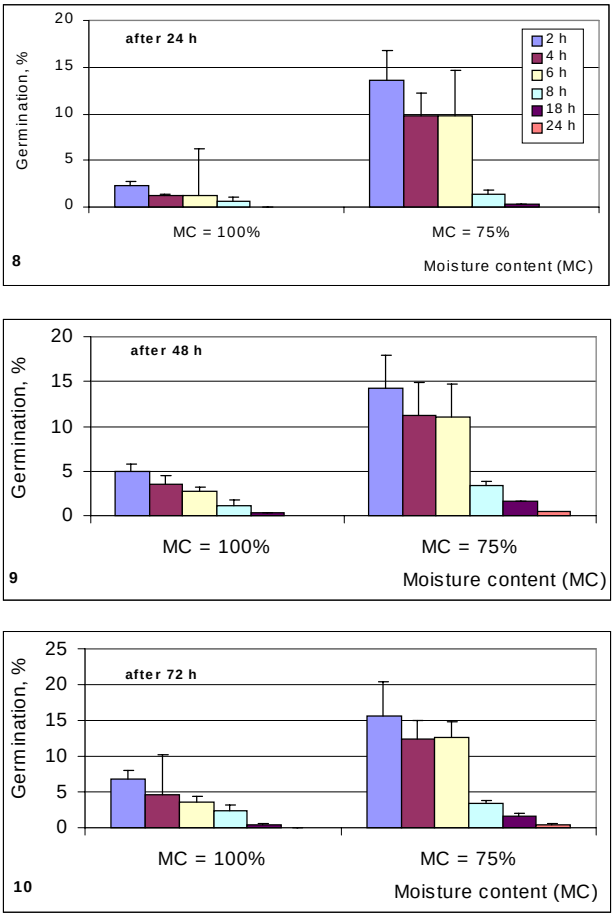






Figs. 5–7. Effect of tea tree oil and its vapor on conidia germination of *Ulocladium atrum* isolates (n = 12) (A – the dishes sealed with parafilm, B – the dishes not sealed on). Figure 5 refers to tea tree and Figures 6 and 7 to vapor of tea tree oil (Fig. 6 – volatile components diffused from agar medium, Fig. 7 – volatile compounds diffused from pure oil). All values are the mean of twelve replicates. Significant differences are shown at *P = 0.05 and **P = 0.01. Tea tree oil was dissolved in distilled water containing 0.001% Twenn-80

Effects of the tea tree oil on *U. atrum* conidia germination. Conidial germination of *U. atrum* isolates on MEA, containing different tea tree oil concentrations, and its conidia germination under the action of tea tree oil volatile components was studied *in vitro*. Germination percentages determined during two separate experiments are shown in Fig. 5–7. The average germination for *U. atrum* control was 94.7 ± 9.9% SE. Two variants of each experiment were conducted: in one set Petri dishes were sealed up with Parafilm (Figs. 5–7, A) and in the other set dishes were left unsealed (Figs. 5–7, B). In this experiment, addition of TTO into MEA inhibited *U. atrum* conidia germination significantly (P = 0.01) ≥ at a 0.3% oil concentration (Fig. 5). At 0.1% oil concentration, the percentage of germination was almost the same in the sealed and unsealed dishes, while at a 0.2% oil concentration these differences were significant (P = 0.05). The strongest antifungal effect of TTO against *U. atrum* was achieved when oil vapor diffusing from pure tea tree oil was used (Fig. 7). An equivalent germination inhibition effect was achieved by the action of TTO at a 10-fold lower concentrations than in the two other experiments; the results are presented in Fig. 5 and 6. One can see from Fig. 7 that the inhibitory substances of tea tree oil could disappear via evaporation following a prolonged incubation, allowing the conidia to resume germination. Strong and significant differences among conidia germination in the sealed and unsealed dishes (P = 0.01) were detected (Fig. 7). Mean germination values at all TTO concentrations in the unsealed dishes were almost similar (20–26%) and the standard errors were very high. TTO vapor diffusing from the pure oil at a 0.05–0.07% concentration inhibited *U. atrum* conidia germination up to 100%. Only 1.2, 0.8 and 1.01% of conidia germina-



Figs. 8–10. Percentage germination of *Ulocladium atrum* conidia under two different moisture conditions, due to exposure to tea tree oil vapor time (2, 4, 6, 8, 18, 24 h) and period (18, 48 and 72 h) after which the exposure to vapor was discontinued. Vapor diffused from the 5 ml pure oil/plate. All values are the mean from the twelve replicates (n = 12). Significant differences are shown as *P = 0.05 and **P = 0.01. *U. atrum* conidia percentage germination for controls was 98.2 ± 8.7% for both 75 and 100% MC

ted when vapor diffused from 0.05, 0.06 and 0.07% pure tea tree oil concentrations, respectively, in the sealed Petri dishes.

Isolates of *U. atrum* were investigated using an assay, which compared the activity of TTO against fungus conidia spread on filter paper (FP) with a different moisture content. These assays showed that conidia on FR samples with a higher moisture content were significantly more susceptible to tea tree oil vapor than those inoculated on a less moistened substrate (Figs. 8–10). We wanted to determine the exposure period to TTO vapor effects on the *U. atrum* conidia ability to survive and reproduce when removed from the presence of tea tree oil. Viable conidia values were determined to reveal the extent to which treated conidia were able to survive and reproduce. Conidia were spread and incubated on filter paper samples containing one of the two tested moisture contents (MC = 75 and 100%). Filter paper samples were inoculated with *U. atrum* suspension (10^{12} conidia/ml). Inoculated FP samples were suspended in Petri dishes containing 5 µl of pure (100% concentration) tea tree oil and sealed with Parafilm. After incubation at 18 °C for 2, 4, 6, 8, 18 and 24 hours, the dishes were unsealed and filter paper samples were examined under a binocular microscope. Conidial germination percentage was determined 0, 18, 48 and 72 hours after the dishes were unsealed. The effect of the exposure period to TTO vapor on conidia germination under a 5 µl tea tree oil concentration was determined. Different germination rates of conidia on FP samples containing different MC were recorded. *U. atrum* conidia did not germinate on filter paper containing 15% of water, which is reported in wall-paper under a 80% relative humidity of the indoor environment [24]. No fungus can germinate when the substrate MC is less than 60% [7]. After a 24-h incubation germinated 98.2 ± 8.7% SE of *U. atum* conidia on control FP samples (in dishes without TTO vapor) contained both moisture contents. The concentration of germinated *U. atrum* conidia was different on FP samples and depended on the period of exposure to TTO vapor and on moisture conditions (Figs. 8–10). A significant decrease in the germination percentage after TTO vapor action was observed on FP samples of 100% MC. However, 48 h and 72 h after the dishes were unsealed, the conidial percentage germination increased. The germination increase and the time of exposure of the FP samples inoculated with conidia to TTO vapor were inversely proportional. TTO vapor diffusing from a relatively low (5 µl) oil content very strongly inhibited *U. atrum* conidia germination on 100% moistened FP samples. Conidia germination was less reduced on FP samples moistened to 75%. During observation of conidia on FP samples under a binocular microscope we could notice a collapse of the most *U. atrum* conidia, which did not germinate under TTO vapor and 100% moisture conditions. The percentage of collapsed conidia was evaluated on FP samples after different exposu-

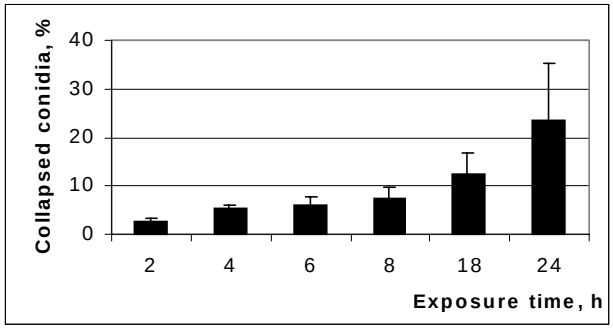


Fig. 11. Percentage of collapsed *Ulocladium atrum* conidia induced by tea tree oil vapor in 100% moisture conditions. All values are a mean from nine replicates

re to TTO vapor (Fig. 11). Although the standard error was high, we could observe a significant increase of collapsed conidia percentage with increasing exposure to TTO vapor. *U. atrum* conidia did not collapse on control FP samples containing 100% of moisture. Hence we may conclude that conidial collapse could be due only to the TTO vapor activity. Cox et al. [33] detected that inhibitory effects of tea tree oil are consistent with effects related to the partitioning of its monoterpene components into cell membranes. The decline in viability and the inhibition of respiration was accompanied by increased cell membrane permeability. The ability of tea tree oil to inhibit respiration and increase membrane permeability in microbial cells suggests that its lethal actions are primarily the result of inhibition of membrane-located metabolic events and a loss of chemiostatic control. Perhaps a higher water content conditioned monoterpene penetration from TTO vapor to the conidia and their killing rate. The results obtained suggest that under higher moisture conditions TTO vapor activity against *U. atrum* isolates increases. It has been stated that moisture conditions on the surface are critical for development of fungal growth in a material, because fungi grow on the surface of a substrate and cannot utilize moisture accumulated inside the material [10, 34]. In the present study, a good correlation between the conidia germination and water content in filter paper indicated that fungi colonized the surface of the material studied. In addition, the results emphasize that tea tree oil vapor can be used as a tool inhibiting conidia germination and growth.

In conclusion, this study hs shown that the effect of tea tree oil and its vapor is impressive. Tea tree oil as well as other oils can be used in disinfecting solutions and cleansers. It might become popular, because it does not cause undesirable side effects when properly used and, most imporantly, does not induce fungal tolerance. We are currently trying to develop a method for applying tea tree oil as vapor at a low concentration for disinfection of the indoor environment. Application of oil vapor at a continuous low concentration should prevent also the tainting of fresh products during storage. Given the antifungal activity of tea tree oil shown in this study, particularly against the pathogens *Stachybotrys chartarum* and *Chetomium globosum*, tea tree oil can be potentially used not only in hygiene but also for indoor environment disinfection.

The results of this study imply the necessity to continue tea tree oil antifungal activity investigations, because many of the questions have not been answered; moreover, new goals arise for the future study.

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D. Peèiulytë

ARBATMEDÞIO ALIEJAUS POVEIKIS MIKROORGANIZMØ VYSTYMUISI 2. ARBATMEDÞIO ALIEJAUS FUNGICIDINIØ SAVYBIØ RAIÐKOS SKIRTINGOMIS SÀLYGOMIS ÁVERTINIMAS

Santrauka

Ištirta eterinio *Melaleuca alternifolia* (arbatmedþio) aliejaus fungicidiniø savybiø raiðka mikromicetø pasëlio ant mitybinës (AM, alaus misos) terpës su aliejaus priedu atþvilgiu. Straipsnyje apþvelgiamas skirtingø arbatmedþio aliejaus koncentracijø poveikis vienuolikai mikromicetø *Chaetomium globosum*, *Stachybotrys chartarum* (= *S. atra*) ir *Ulocladium atra* (= *Stemphylium atra*) rûðiø padermiø. Tyrimà atlikome dviem etapais: atskirais bandymais nustatëme arbatmedþio aliejaus ir jo lakiø junginiø fungicidiniø savybiø poveiká grybams. Patalpø oro uþterðtumas grybø pradais bei jø vystymasis ant medþiagø pavirðiaus priklauso nuo drëgmës. Iðtyrëme arbatmedþio aliejaus aktyvumà mikromiceto *Ulocladium atrum*, auginamo ant skirtingo drëgnumo (75 ir 100%) substrato, atþvilgiu. Apibendrinome santykinio substrato drëgnumo ir aliejaus vienalaikio poveikio mikromiceto vystymuisi rezultatus.