

Ten Essential Oils for Beech Wood Protection - Efficacy Against Wood-destroying Fungi and Moulds, and Effect on Wood Discoloration

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This work analyses the anti-fungal efficacy and stability of 10 essential oils, as well as their colour stability, in wood. The efficacy of oils against the decay fungi *Coniophora puteana* and *Trametes versicolor* as well as the moulds *Aspergillus niger* and *Penicillium brevicompactum* was evaluated first on filter papers treated with 1, 3.5, 10%, or 100% concentrate, and then on beech wood treated with 10% solutions. Accelerated ageing of treated beech samples was done before mycological tests and consisted of heating, leaching followed by heating, and Xenotest followed by heating. The highest growth inhibition of moulds and *C. puteana* was caused by thyme, oregano, sweet flag, and clove oils, while the savory and birch oils were less effective. These oils are potentially useful for wood protection against brown-rot fungi and moulds, mostly in interior conditions. The essential oils had only a negligible effect against the white-rot fungus *T. versicolor*, which was more apparent after previous ageing of wood. Some essential oils with a yellow tone (birch, oregano, sweet flag, savory, and tea tree oils) significantly changed ($p < 0.05$) the natural colour of beech wood, but the new colours were relatively stable and underwent only mild changes after accelerated ageing in Xenotest.

Keywords: Wood protection; Essential oils; Wood-destroying fungi; Moulds; Colour

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INTRODUCTION

Many wood preservatives that are effective against decay and mould attacks (containing heavy metals such as Cr, As, and Sn) have been prohibited in many countries in recent years (Reinprecht 2010) due to their negative effects on human health (risks of cancer, foetus defects, liver and kidney damage, neuropathic changes, asthma, and various allergic reactions) as well as ecological problems during service-life, particularly during the disposal of such products. However, common types of wood in a natural state are degraded by microorganisms in a relatively short time. Therefore, researchers are looking for new compounds and wood protection methods with little or no negative ecological and human health impacts. New ways of wood protection against pests can be necessary after possible prohibition of presently used biocides. For example, modified wood is already widely used, e.g., thermally treated wood (Militz 2002; Schwarze and Spycher 2005; Hill 2006), furfurylated wood (Lande *et al.* 2006), acetylated wood (Westin *et al.* 2004; Hill 2006), natural chitosan treated wood (Schmidt and Müller 1995;

Chittenden *et al.* 2004; Maoz and Morrell 2004), or wood treated with lignins against brown-rot (Chirkova *et al.* 2011).

Biological methods for protection of wood, also on a commercial basis, include using the antagonistic effect of some microscopic fungi and bacteria against more dangerous wood-destroying fungi (Kim and Morrell 1998; Score *et al.* 1998; Tiralová *et al.* 2007). It is more common to use natural products from plants or extractives from very durable wood species *e.g.*, tannins, flavonoids, or terpenoids (Huang *et al.* 2004; Amusant *et al.* 2005; Asamoah and Antwi-Boasiako 2007), extracts from chili with the effective compound capsaicin (Singh *et al.* 2006), or propolis with an antibiotic effect (Budija *et al.* 2008). The cited works have shown the potential of natural substances for wood protection against decay fungi, moulds, or insects.

An interesting method of wood protection is the use of essential oils and their effective compounds from plants having known anti-fungal, insecticidal, and anti-bacterial effects. This method has many advantages: (1) Plants can be produced in large amounts as agricultural products (renewable resource); (2) Some oils can combine anti-fungal, insecticidal, and anti-bacterial effects; (3) Essential oils are health-friendly (many of them are used in medicine, aromatherapy, and cosmetics), and they cause only small problems in terms of liquidation of treated products after their service-life. Some disadvantages of using essential oils for wood protection are their high volatility and non-stable concentrations of effective compounds (Batish *et al.* 2008).

Efficacy of essential oils against wood-destroying fungi, wood-staining fungi, and moulds was searched already in some scientific works. Mohareb *et al.* (2013) isolated essential oils from 18 Egyptian plants. An inhibitory effect against the wood-decaying fungi *Hexagonia apiaria* and *Ganoderma lucidum* was demonstrated for 6 essential oils from *Artemisia monosperma*, *Cupressus sempervirens*, *Citrus limon*, *Thuja occidentalis*, *Schinus molle*, and *Pelargonium graveolens*. After 6-week-mycological tests of oil-treated Scots pine sapwood, the *C. limon* oil had the highest anti-decay effect against *H. apiaria*, while the highest efficacy against the *G. lucidum* was found for oil from *A. monosperma*. Similar interesting anti-fungal effects of essential oils or other natural compounds at testing treated pine wood was achieved by some other researches, *e.g.* by Chittenden and Singh (2011) with geranium oil, eugenol and cinnamaldehyde extracts against the growth of fungi *Oligosporus placenta*, *Coniophora puteana*, *Antrodia xanthan*, *Ophiostoma floccosum*, *Ophiostoma piceae*, *Sphaeropsis sapinea*, *Leptographium procerum*, and *Trichoderma harzianum*. Further work by Li *et al.* (2007) showed important effects of cinnamon oil against the growth of *Ophiostoma piceae*, *Alternaria alternata*, and *Aspergillus niger*. Last but not least, Yang and Clausen (2006) shows effects of seven kinds of oils against the moulds *Aspergillus niger*, *Trichoderma viride*, and *Penicillium chrysogenum*. Interesting results have also been achieved in the work of Dhyani *et al.* (2005), which considered poplar wood treated with tea tree oil against *Poria monticola* and *Polyporus versicolor*, or in work of Kartal *et al.* (2006), who tested wood treated with cinnamic acid, ferulic acid, and cinnamaldehyde and assessed its decay and termite resistance. Voda *et al.* (2003) performed screening efficacy tests of 22 essential oils against the growth of fungi *Trametes versicolor* and *Coniophora puteana*. The effect of eucalyptus oil against moulds from the genus *Aspergillus* and against the growth of the fungus *Phaeoramularia angolensis* was studied by Viela *et al.* (2009) and Dongmo *et al.* (2008). Maoz *et al.* (2007) researched the effect of selected essential oils against the fungus *Gloeophyllum trabeum*, whereas Wang *et al.* (2005) tested the essential oil from *Chamaecyparis formosensis* wood against fungi *Laetiporus sulphureus* and *Trametes*

versicolor. Likewise, Su *et al.* (2013) studied the anti-fungal activity of essential oils from leaves and fruits of *Juniperus formosana* against four decay fungi, noting that better results were achieved with the oil from leaves in which the main active anti-fungal substances were determined to be α -cadinol and elemol.

Generally, the best anti-fungal efficacy of essential oils in the mentioned works was found for oils containing phenolic compounds such as carvacrol (oregano, savory), thymol (thyme), eugenol, oxygenated compounds such as elemol and cinnamaldehyde (cinnamon), and for geranium oil and oils from *Inula viscosa* and *Chamaecyparis formosensis*. On the other hand, the tea tree oil had only a limited efficacy.

Many works have focused on the efficacy of essential oils (or their effective compounds) against bacteria (Cox *et al.* 2001; Mahady *et al.* 2005; Papadopoulos *et al.* 2006; Wong *et al.* 2008). Thus, essential oils are also useful for protecting wood or wood composites against the accumulation and growth of disease-causing and harmful bacteria in interior areas such as kitchens, dining rooms, and hospitals. However, more work in this field of research is required.

The basic tasks and questions for the research of essential oils for wood protection are as follows: (1) To determine the minimum effective concentration of essential oils for wood protection; (2) To select the most effective oils and their compounds (as a function of their efficacy and cost); (3) To achieve stability of oils in wood (by adding stabilisation additives or by creating chemical bonds on wood functional groups) against evaporation, leaching, or UV-radiation in, exterior conditions; (4) To produce effective selected compounds in factories and to test the potential synergistic effect of chemical compounds in oils from naturally produced plants; and (5) To analyse potential synergistic effects between various oils and additives introduced into different wood species against wood-destroying fungi, moulds, and bacteria.

The present work was focused on two basic points: (1) Efficacy of essential oils at protection of beech wood (not durable species) against fungi, together with determination of their stability in wood at artificial ageing; and (2) The anti-fungal effect of the lesser-known sweet flag oil containing *cis*-isoasarol trimethylether. These two aims of work were solved together with determination of the minimum effective concentration of selected 10 essential oils (birch, clove, lavender, oregano, sage, savory, sweet flag, tea tree, thyme, and oil mixture) against the growth and damaging activity of the wood-destroying fungi *Coniophora puteana* and *Trametes versicolor*, and against the growth of the moulds (microscopic fungi) *Aspergillus niger* and *Penicillium brevicompactum*. Anti-fungal efficacy of essential oils was evaluated first on filter papers treated with 1, 3.5, 10, or 100% concentrations of oils, and then on beech wood treated with a 10% concentration of oils by a modified EN 113 (1997). The long-term anti-fungal effect of essential oils, or their stability in treated beech samples, was evaluated on the basis of their anti-fungal efficacy after different accelerated ageing processes (*i.e.*, heat only, leaching in distilled water and heat, and ageing in Xenotest and heat).

An additional aim of this work was to analyse the effect of essential oils on the colour of beech wood and on the stability of new colours of treated beech wood after its accelerated ageing in Xenotest, because the colour stability of wood is a significant factor for its indoor and outdoor applications.

EXPERIMENTAL

Paper and Wood Samples

Filter paper (Whatman® 3 CHR cellulose chromatography paper) with a diameter of 14 mm was used in the first step of the mycological tests, the goal of which was to rate the effect of essential oils against the growth intensity of decay fungi and moulds.

Samples from healthy beech wood (*Fagus sylvatica* L.) with dimensions of 25 × 25 × 3 mm (L × T × R) were used in mycological tests in accordance with modified EN 113 (1997) as well as in the colour tests.

All papers and beech samples were sterilised with UV-radiation (800 W for 10 min for papers and 30 min for wood) before the mycological tests. The beech samples were also sterilised before treatment with essential oils using thermal drying at 103 ± 2 °C for 4 h.

Essential Oils

Selected pure essential oils of pharmacopoeia quality were used in the experiments (Table 1). Essential oils were prepared by the steam distillation of flowers and leaves of plants (Note: Only lemon oil by pressing of rind, and birch oil by dry distillation and then by steam distillation.). Their chemical composition was analysed by the producer Nobilis Tilia s.r.o., Czech Republic. Oils were applied in four defined concentrations, *i.e.*, 1%, 3.5%, and 10% (by mass) ethanol solutions and as the original 100% concentrate.

Table 1. Essential Oils used in the Experiments

Common name	Scientific name	Major Effective Components
Birch	<i>Betula pendula</i>	Methyl salicylate (99%)
Clove	<i>Syzygium aromaticum</i>	Eugenol (82%), Caryophyllene (16.5%)
Lavender	<i>Lavandula angustifolia</i>	Linalyl acetate (37.1%), Linalool (33.6%), Terpinen-4-ol (2.6%)
Oregano	<i>Origanum vulgare</i>	Carvacrol (71.8%), Thymol (5%), gamma-Terpinene (4.5%)
Sweet flag	<i>Acorus calamus</i>	Cis-isoasarol trimethylether (78%)
Savory	<i>Satureja hortensis</i>	gamma-Terpinene (41.3%), Carvacrol (31.6%), p-cymol (13.8%)
Sage	<i>Salvia officinalis</i>	alpha-Thujone (26.7%), Camphor (20.2%), 1,8-Cineole (9.6%)
Tea tree	<i>Melaleuca alternifolia</i>	Terpinen-4-ol (42.2%), gamma-Terpinene (20.8%), alpha-Terpinene (9.8%), 1,8-Cineole (3.6%)
Thyme	<i>Thymus vulgaris</i>	Thymol (41.3%), p-cymol (22.6%), gamma-Terpinene (7.7%), Carvacrol (2.9%)
Oil mixture 1:1:1:1 (by mass) of Sage, Thyme, Eucalypt, Lavender, Lemon	<i>Salvia officinalis, Thymus vulgaris, Eucalyptus globulus, Lavandula angustifolia, Citrus limon</i>	alpha-Thujone (5.3%), Thymol (8.2%), 1,8-Cineole (18.3%), Linalool (7.8%), Linalyl acetate (7.4%), Limonene (14.2%), p-cymol (4.5%)
Source of essential oils: Nobilis Tilia s.r.o., Czech Republic		

Wood-destroying Fungi and Moulds

Two species of wood-destroying fungi, the brown-rot fungus *Coniophora puteana* (Schumacher ex Freis) Karsten, strain BAM Ebw. 15, (Bundesanstalt für Materialforschung und -prüfung, D-12205 Berlin) and the white-rot fungus *Trametes versicolor* (Linnaeus ex Freis) Pilat, strain CTB 863 A, (Centre Technique du Bois et de l'Ameublement, 10 Avenue de Saint-Mandé, F-75012 Paris), were used in all mycological tests, *i.e.*, testing the efficacy of oils present in filter paper and in beech samples. Two species of moulds, the *Aspergillus niger* Tiegh. and *Penicillium brevicompactum* Dierckx, were used for testing the efficacy of oils present in filter papers. Used decay fungi and moulds are stored in the Mycology Laboratory of Faculty of Wood Sciences and Technology, Technical University in Zvolen, Slovak Republic.

Treatment of Samples with Essential Oils

Treatment of filter papers

Filter papers were dipped for 20 s in essential oils at 100 kPa and 20 °C. With each type of oil and its defined concentration, 36 filter papers were treated: 9 for testing with *C. puteana*, 9 for testing with *T. versicolor*, 9 for testing with *A. niger*, and 9 for testing with *P. brevicompactum*. A total of 1440 filter paper specimens were studied (10 types of oils × 4 concentrations for each oil × 36 papers).

Treatment of beech samples

Beech samples were dipped for 24 h in essential oils at 100 kPa and 20 °C. With each type of oil at a 10% concentration (the potentially effective concentration from the previous mycological tests with filter papers) that underwent one type of ageing before mycological tests, 12 beech samples were treated: 6 for testing with *C. puteana* and 6 for testing with *T. versicolor*. The same 12 samples were used for colour analyses prior to the mycological tests. A total of 480 beech samples were tested (10 types of oils at one concentration for each oil × 4 ageing processes × 12 samples).

Accelerated Ageing of Beech Samples Treated with Essential Oils

The stability of essential oils in beech wood was determined by the following accelerated ageing processes performed before the mycological tests: No ageing of wood (N, non-aged); heating of wood in a drying oven for 8 h at 103 ± 2 °C (T, thermo); leaching of wood based on a modified EN 84 (1997) (modified cycle because smaller samples were used, with 7 day leaching and four changes of distilled water) followed by heating in a drying oven for 8 h at 103 ± 2 °C (LT, leaching + thermo); and artificial ageing in Xenotest for 1 week based on a modified EN 927-6 (2006) (modified parameter of irradiance 0.55 W/m^2 and temperature on black panel 50 °C) followed by heating in a drying oven for 8 h at 103 ± 2 °C (XT, Xenotest + thermo). All non-aged (N type) and all aged (T, LT, and XT types) samples were conditioned to a 12% equilibrium moisture state (EMC) in a conditioner room and then weighed to an accuracy of 0.001 g (m_0) before exposure to the mycological tests with decay fungi.

Mycological Tests

Mycological tests of oils present in filter papers against wood-destroying fungi

An inoculum of the testing fungus was placed on malt-dextrose agar in the centre of a petri dish with a diameter of 100 mm. Immediately afterwards, 4 pieces of filter papers were placed in the petri dish at a distance of 20 mm from the inoculum (1

reference = filter paper dipped only in 96% ethanol; 3 treated = filter papers treated with one concentration of one essential oil). These tests were performed under sterile conditions at 25 °C in 240 petri dishes. Evaluation of the mycelia growth tests was performed on the basis of the growth inhibition index of fungal mycelia on filter paper impregnated with the essential oil (I_{Paper}) between 7 and 14 days, coming out from inhibited growth (slowed growth, where $I_{\text{Paper}} > 0\%$; or a total of stopped growth, where $I_{\text{Paper}} = 100\%$) by Eq. 1,

$$I_{\text{Paper}} = [(L_C - L_{\text{Oil}}) / L_C] \times 100 \quad (\%) \quad (1)$$

where L_C is the growth of mycelia during the time interval on the reference-control filter paper (mm), and L_{Oil} is the growth of mycelia during the time interval on the tested filter paper treated with essential oil of a defined concentration (mm).

Mycological tests of oils present in filter papers against moulds

Four filter papers were placed on Czapek-Dox agar in a 100-mm petri dish (1 reference = paper dipped only in 96% ethanol; 3 treated = filter papers dipped in one concentration of one essential oil). Then, a suspension of spores of the testing mould was sprayed on all surfaces of the petri dish, *i.e.*, on the papers and agar. These tests were performed under sterile conditions at 28 °C in 240 petri dishes. Evaluation of the mould growth was performed on the 5th, 7th, 10th, 14th, 21st, and 28th days. The growth of moulds from the spores on the top side of filter papers was visually observed on daily light in the laboratory at 10x magnification.

Mycological tests of oils present in beech samples against wood-destroying fungi

These tests were performed according to EN 113 (1997), with some modifications in the methods as follows (Reinprecht and Grznárik 2014): use of specimens with smaller dimensions of 25 × 25 × 3 mm (L × R × T) instead of 50 × 25 × 15 mm (L × R × T); treatment of specimens by dipping instead of vacuum impregnation; shorter time of fungal test (8 weeks instead of 16 weeks), similar also to Chittenden and Singh (2011); and fungal tests performed in petri dishes with diameters of 100 mm instead of in 1-L Kolle's flasks.

Four beech wood samples (1 reference = sample dipped only in 96% ethanol; 3 treated = samples dipped in one concentration of one essential oil), all with known initial mass (m_o), were placed in one petri dish with growth mycelium of testing fungus on four small polyethylene perforated mats. These tests were performed under sterile conditions at 25 °C in 160 petri dishes.

After mycological tests, the mycelia were carefully brushed from the beech samples, the samples conditioned to an EMC similar to their state before the fungal attack (12%), and weighed again to an accuracy of 0.001 g (m_F). The decay resistance of the beech samples was calculated on the basis of their mass losses caused by the fungi (Δm) by Eq. 2:

$$\Delta m = [(m_o - m_F) / m_o] \times 100 \quad (\%) \quad (2)$$

Colour Tests

Colour changes of beech samples treated with essential oils were evaluated after their treatment with essential oils and conditioning to 12% EMC and also after artificial ageing in Xenotest for 1 week (EN 927-6 2006) and conditioning to 12% EMC.

Total colour changes of beech samples were evaluated in the CIELab system using Colour Reader CR-10 (Konika Minolta, Japan) by the well-known Eq. 3 (ISO 7724 1984):

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (3)$$

Statistical Analysis

Results obtained by the mycological and colour tests were evaluated by mean values, standard deviations, and statistically by Duncan's test (Statistica 12, StatSoft CR s.r.o., Czech Republic). The results from some tests were also evaluated by histograms (generated in Microsoft® Excel 2013; Microsoft Corporation, USA).

RESULTS AND DISCUSSION

Mycological Tests of Filter Papers Treated with Oils

The results of mycological tests using essential oils in filter papers are shown in Table 2 and in Figs. 1 and 2.

Table 2. Effective Concentration (%) of Essential Oils against Growth of Decay Fungi *C. puteana* and *T. versicolor* (during 14-day Test) and Moulds *A. niger* and *P. brevicompactum* (during 28-day Test) on Treated Filter Papers

Decay fungus or mould	Essential oil									
	Birch	Clove	Lavender	Oregano	Sweet Flag	Savory	Sage	Tea tree	Thyme	Oil mixture
<i>C. puteana</i>	3.5%	3.5%	10%	1%	3.5%	3.5%	100%	100%	3.5%	10%
<i>T. versicolor</i>	10%	10%	100%	10%	10%	10%	100%	100%	10%	100%
<i>A. niger</i>	10%	3.5%	100%	10%	10%	10%	*	100%	10%	100%
<i>P. brevicompactum</i>	10%	3.5%	*	10%	3.5%	10%	*	100%	3.5%	100%

Notes: Reference-control papers were fully covered by mould after 5 days
* non-effective also at c = 100% (treated papers fully covered by mould after 28 days)

The preliminary mycological tests showed similar efficacy of individual essential oils against the growth of wood-destroying fungi and moulds. The highest anti-fungal efficacy occurred in oils containing carvacrol and thymol, e.g., oregano (effective against *C. puteana* even at 1%), thyme, and savory oils. Good efficacy of thyme oil against moulds was also determined by Yang and Clausen (2006). Oils containing methyl salicylate and eugenol, e.g., birch and clove oils, also had high anti-fungal efficiency. At a 3.5% concentration, clove oil was effective against the two moulds *A. niger* and *P. brevicompactum*, and also against brown-rot fungus *C. puteana*. The efficacy of sweet flag oil with active compound cis-isoasarol trimethyleter was also interesting, because it

showed the best result against white-rot fungus *T. versicolor* among all tested essential oils (Fig. 2).

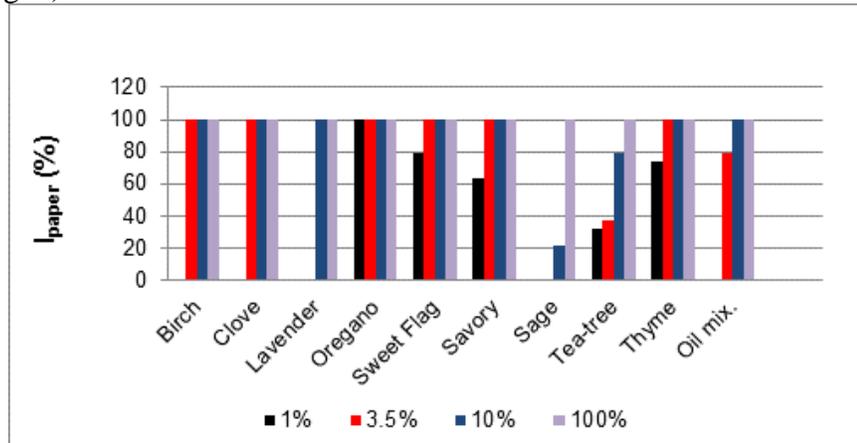


Fig. 1. Inhibition efficacy (based on mean values of I_{Paper} between 7 days and 14 days) of essential oils used in 1, 3.5, 10, and 100% concentrations against the growth of the brown-rot fungus *Coniophora puteana* on treated filter papers

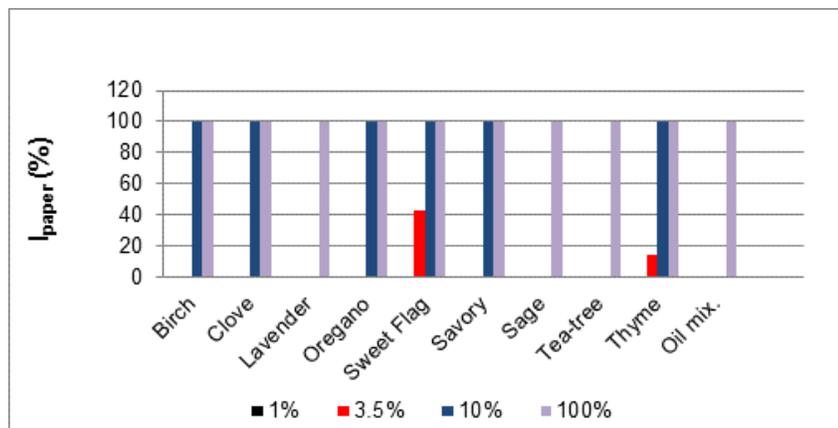


Fig. 2. Inhibition efficacy (based on mean values of I_{Paper} between 7 days and 14 days) of essential oils used in 1, 3.5, 10, and 100% concentrations against the growth of the white-rot fungus *Trametes versicolor* on treated filter papers

Sage, lavender, oil mixture, and tea tree oils had lower or no efficacy against decay fungi and moulds. These oils do not contain phenolic compounds such as carvacrol, thymol, eugenol, *etc.*; therefore, these results confirmed the higher anti-fungal effect of oils that contain phenol compounds, in accordance with the works of Voda *et al.* (2003), Yang and Clausen (2006), Maoz *et al.* (2007), and Chittenden and Singh (2011).

Generally, lower anti-fungal effects of tested essential oils against growth of white-rot fungus *Trametes versicolor* were observed. This result corresponds with the work of Voda *et al.* (2003). It could be explained by the enzymatic nature of this fungus (production of ligninolytic enzymes), as opposed to brown-rot fungus *C. puteana*, because *T. versicolor* can deactivate not only lignin, but also phenolic or some other anti-fungal compounds in essential oils (Bayramoglu and Arica 2009).

Mycological Tests of Beech Samples Treated with Oils

Table 3 shows retention, R , of pure essential oils in beech wood samples (kg/m^3).

Table 3. Retention (R) of Pure Essential Oils in Treated Beech Samples after 24 Hour Impregnation Carried out by Dipping in 10% Ethanol Solutions of Oils

Kind of oil	Birch	Clove	Lavender	Oregano	Sweet flag	Savory	Sage	Tea tree	Thyme	Oil mixture
R (kg/m ³)	37.70 (5.70)	37.77 (4.94)	34.47 (3.23)	35.97 (4.37)	36.41 (4.57)	35.76 (4.92)	35.70 (4.14)	37.80 (4.53)	36.63 (4.87)	38.12 (5.65)
Arithmetic means in each series are from 12 samples (n = 12) Numbers in the parentheses are the standard deviations										

Table 4. Mass Losses (Δm) of Beech Samples Treated with Essential Oils caused by Fungus *Coniophora puteana* during 8 weeks (by Modified EN 113 = m-EN 113)

Essential oil	Mass loss by <i>C. puteana</i> (%)			
	m-EN 113	thermo + m-EN 113	leaching + thermo + m-EN 113	Xenotest + thermo + m-EN 113
Birch	0.08 (0.047) ^a	0.46 (0.306) ^a	4.72 (3.237) ^a	0.41 (0.329) ^a
Clove	0.04 (0.029) ^a	0.23 (0.223) ^a	0.90 (0.318) ^a	0.56 (0.336) ^a
Lavender	8.02 (1.473) ^a	19.26 (1.819) ^a	22.70 (4.243) ^d	19.72 (3.045) ^b
Oregano	0.02 (0.022) ^a	0.17 (0.102) ^a	0.66 (0.346) ^a	0.20 (0.083) ^a
Sweet flag	0.04 (0.031) ^a	0.26 (0.152) ^a	0.57 (0.323) ^a	0.29 (0.156) ^a
Savory	0.07 (0.021) ^a	0.25 (0.124) ^a	2.24 (0.956) ^a	0.71 (0.438) ^a
Sage	17.80 (2.074) ^a	21.74 (3.043) ^c	24.58 (3.320) ^d	18.45 (2.951) ^a
Tea tree	8.52 (1.596) ^a	20.03 (1.233) ^a	21.08 (1.596) ^b	20.04 (0.906) ^b
Thyme	0.06 (0.025) ^a	0.11 (0.077) ^a	0.87 (0.150) ^a	0.81 (0.422) ^a
Oil mixture	5.34 (1.200) ^a	21.15 (2.612) ^b	21.65 (3.030) ^c	19.26 (2.242) ^b
Reference	27.01 (2.025)	23.92 (1.824)	25.22 (1.673)	23.57 (0.791)
Before the mycological tests with fungus <i>C. puteana</i> (by m-EN 113) the beech samples were exposed to accelerated ageing (thermo; leaching by EN 84; Xenotest by EN 927-6) Statistical evaluations of the mass losses (Δm) within each treatment are shown by: (1) mean values from 6 replicates; (2) standard deviations in parentheses; (3) Duncan's test in relation to the references (^a 99.9% significance level; ^b 99% significance level; ^c 95% significance level; and ^d no statistical significance at $p \geq 0.05$)				

Results from the decay attack of treated beech samples with brown-rot fungus *C. puteana* (Table 4) correspond to the results obtained during the testing of filter papers treated with the same essential oils against growth activity of this fungus (Fig. 1). Essential oils with high effect against the degradation activity of *C. puteana* (oregano, thyme, sweet flag, clove, savory, and birch oils) also had a high anti-decay efficacy after thermo, leaching and thermo, or Xenotest and thermo ageing processes. The lowest mass losses caused by *C. puteana* (Δm values below 1%) after the most aggressive ageing process (leaching + thermo) were in beech samples treated with sweet flag, oregano, thyme, and clove oils (Table 4).

Sage, tea tree, lavender, and oil mixture had only a minimal effect against the decay activity of *C. puteana* in treated and aged beech samples compared to untreated ones (reference), at which mass losses of these samples ranged from 5% to approximately 25% (Table 4). These results confirmed insufficient efficacy of these oils from previous tests with treated filter papers (Fig. 1 and Table 2).

The results of experimentation with *C. puteana* have shown some differences to the work of Chittenden and Singh (2011). During the decay process, they observed higher mass losses in wood treated with essential oils after leaching. This fact could be explained by harsher leaching conditions in their experiment, because they used vacuum and more leaching cycles.

Mycological tests with the white-rot fungus *Trametes versicolor* after all combinations of ageing on beech samples treated with essential oils were also done. However, the achieved results were not clear (high standard deviations of mass losses), and most oils had no preservative effect against this fungus. After leaching (EN 84 1997) and artificial ageing in Xenotest (EN 927-6 2006), both in combination with heating (thermo), the mass losses of beech samples treated with all tested essential oils were over 15%. Only beech wood treated with sweet flag oil and aged only by heating (thermo) had a smaller mass loss ($\Delta m = 1.70$; $SD = 1.80$) during the mycological tests. *T. versicolor*'s lower sensitivity to essential oils confirmed the previous results of Voda *et al.* (2003). The results of Bayramoglu and Arica (2009) showed a very high production of the extracellular ligninolytic enzyme laccase by *T. versicolor*. This enzyme destroys lignin and continuously deactivates phenolic compounds with the highest anti-fungal effects in essential oils, such as thymol, carvacrol, and eugenol.

Colour Changes

The effect of essential oils on colour changes of beech wood is important for practice. However, these changes should be stable after the action of UV-light and water. The colour parameters of natural beech wood in CIELab were as follows: $L = 68.7$, $a = 7.6$, and $b = 15.3$. The colour changes of beech wood treated with essential oils are shown in Table 5. The stability of the new colours of treated beech wood after accelerated ageing in Xenotest during 1 week (by modified EN 927-6 2006) is shown in Table 6.

Colour tests showed that the colour of treated beech samples changed individually in accordance with the natural colour of these oils. When using the birch, oregano, sweet flag, savory, and tea tree oils, which have a yellow tone, the beech wood also obtained more yellow tones, and its Δb value significantly changed – increased about 17.5 ± 1 . The total colour change of beech wood during the application of these oils was very high, with a ΔE value above 17.0 (Table 5).

Table 5. Colour Changes of Beech Wood after Treatment with Essential Oils

Essential oil	ΔL	Δa	Δb	ΔE
Birch	-4.4 (2.54) ^a	0.3 (1.24) ^d	17.4 (1.42) ^a	18.1 (1.69) ^a
Clove	-3.2 (2.23) ^b	1.8 (0.98) ^a	-0.2 (3.51) ^d	5.3 (1.63) ^a
Lavender	-3.8 (2.77) ^a	1.4 (1.16) ^b	-0.2 (1.58) ^d	4.7 (2.25) ^a
Oregano	-3.9 (2.21) ^a	1.8 (0.77) ^a	18.3 (2.67) ^a	18.2 (3.43) ^a
Sweet flag	-2.8 (2.88) ^b	0.3 (0.88) ^d	16.7 (3.36) ^a	17.2 (3.45) ^a
Savory	-3.8 (2.14) ^a	1.1 (0.91) ^c	16.9 (1.49) ^a	17.5 (1.73) ^a
Sage	-3.1 (2.90) ^b	0.8 (1.37) ^d	-0.5 (3.72) ^d	4.9 (2.94) ^a
Tea tree	-3.2 (2.69) ^b	1.8 (1.01) ^a	17.5 (1.72) ^a	18.0 (1.88) ^a
Thyme	-2.9 (1.99) ^b	0.7 (0.86) ^d	-1.1 (0.90) ^d	3.5 (1.69) ^a
Oil mixture	-0.4 (3.05) ^d	-0.1 (1.44) ^d	-0.5 (3.53) ^d	4.3 (1.95) ^a
Reference	-	-	-	-

Arithmetic means in each series are from twelve samples (n = 12)
 Numbers in the parentheses are the standard deviations
 Results are evaluated within each treatment by Duncan's test in relation to the references
 (^a99.9% significance level; ^b99% significance level; ^c95% significance level; and ^dno statistical significance at p \geq 0.05)

Table 6. Colour Changes of Treated and Reference (Control) Beech Wood after Artificial Ageing in Xenotest during 1 Week

Essential oil	ΔL	Δa	Δb	ΔE
Birch	-1.8 (3.25) ^d	0.3 (1.21) ^c	4.9 (1.44) ^c	6.1 (1.77) ^d
Clove	-4.9 (2.11) ^a	0.5 (1.52) ^b	6.7 (1.50) ^d	8.5 (2.36) ^d
Lavender	0.9 (2.50) ^d	-1.7 (0.95) ^d	5.3 (1.26) ^d	6.2 (1.20) ^d
Oregano	-0.4 (1.98) ^d	-2.1 (1.09) ^c	4.6 (1.92) ^c	5.7 (1.47) ^c
Sweet flag	-1.6 (2.75) ^d	-0.2 (1.09) ^d	5.1 (1.50) ^d	6.0 (1.82) ^d
Savory	-0.6 (2.06) ^d	-1.3 (1.13) ^d	5.6 (1.12) ^d	6.2 (1.00) ^d
Sage	-0.2 (2.21) ^d	-0.8 (1.03) ^d	6.1 (2.33) ^d	6.7 (2.19) ^d
Tea tree	0.0 (2.79) ^d	-1.2 (1.12) ^d	5.4 (1.11) ^d	6.3 (1.10) ^d
Thyme	-0.5 (2.53) ^d	-1.0 (0.96) ^d	5.6 (2.18) ^d	6.3 (2.17) ^d
Oil mixture	-0.6 (1.65) ^d	-0.6 (0.83) ^d	5.6 (1.80) ^d	6.1 (1.47) ^d
Reference	-0.9 (3.68)	-1.0 (1.65)	6.7 (2.87)	7.6 (3.36)

Arithmetic means in each series are from twelve samples (n = 12)
 Numbers in the parentheses are the standard deviations
 Results are evaluated within each treatment by Duncan's test in relation to the references
 (^a99.9% significance level; ^b99% significance level; ^c95% significance level; and ^dno statistical significance at p \geq 0.05)

However, further colour changes of treated and reference (control) beech samples after accelerated ageing in Xenotest were very similar, and no significant statistical difference was observed between them (Table 6). After ageing, samples treated with clove oil had slightly darker tones ($\Delta L = -4.9$). All treated and reference samples obtained a more yellow tone, and the Δb values were from 4.9 to 6.7. In summary, essential oils had no negative effect on the colour stability of beech wood after being exposed to daylight.

Final Discussion

The results achieved in this work have shown the ability of essential oils to stop or slow the growth of moulds and wood-destroying fungi on the surface of cellulose (filter papers), as well as the ability to significantly decrease activity of decay fungi in wood (beech). However, the partial decline of the anti-decay efficacy of essential oils against the brown-rot fungi in beech wood was monitored after its previous ageing in the presence of water-leaching or in the presence of UV light and water-leaching in Xenotest. The protective effect of essential oils against *C. puteana* is important for wood products in interiors, where this fungus can attack wet wood together with other domestic brown-rot fungi, e.g., dry-rot fungus *Serpula lacrymans*. The tested *C. puteana* is less sensitive to biocides than the very dangerous *S. lacrymans* (Bech-Andersen 1995; Kizlink *et al.* 1996; Milata *et al.* 2012), so it is hoped that the best essential oils can potentially be used as health-friendly wood biocides in interior conditions.

The tasks for exterior wood protection will be to explore the interaction of essential oils with paints protecting wooden surfaces against sunlight and moisture, to search for possibilities for eco-friendly fixation of oils in wood, and to find active compounds in oils or in other natural products that are effective against white-rot fungi. Of note, the present work has shown the potential of using of the cis-isoasarol trimethyleter from sweet flag for this purpose. Another question will be to research the interactions of essential oils with other natural substances added to wood, such as tannins, flavonoids, or resins, with the aim to increase their efficacy against biodegradation of wood products.

CONCLUSIONS

1. Essential oils containing phenol compounds like carvacrol, eugenol, thymol, and cis-isoasarol trimethyleter (e.g., thyme, oregano, sweet flag, and clove oils, respectively) were the most effective for beech wood protection against fungi.
2. The minimum effective concentration of essential oils for protection of filter papers against brown-rot fungus *C. puteana* and moulds was 3.5%.
3. White-rot fungus *T. versicolor* was less sensitive to effective compounds contained in essential oils than *C. puteana*. This is caused by its enzymatic apparatus by which it degrades not only lignin but also the most bioactive phenol compounds in these oils.
4. Only sweet flag oil was more effective against white-rot fungus *T. versicolor*, but only without accelerated ageing of treated beech samples in water (without leaching or ageing in Xenotest).
5. Generally, essential oils are suitable for wood protection against wood-destroying fungi and moulds in interior conditions.
6. After accelerated ageing in Xenotest, the colour changes of beech wood treated with essential oils were the same as untreated wood, so used treatments does not worsen its colour stability.

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