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2 **PLANT ESSENTIAL OILS FOR ENVIRONMENT-FRIENDLY**
3 **PROTECTION OF WOOD OBJECTS AGAINST FUNGI**
4

5 **Mohsen Bahmani^{1*}, Olaf Schmidt²**

6 ***Corresponding author:** mohsen.bahmani@nres.sku.ac.ir

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11 **ABSTRACT**
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13 The study was aimed at testing environmental-friendly plant essential oils for their
14 protecting efficiency of valuable woods against colonization by mould and wood-decay fungi.
15 Sixteen essential oils were applied to *Fagus orientalis* and *Pinus taeda* wood samples by vacuum
16 impregnation and infected with *Aspergillus niger*, *Penicillium commune* (moulds), *Coniophora*
17 *puteana* (brown rot), *Trametes versicolor* (white rot), *Chaetomium globosum* (soft rot) and
18 natural infection by airborne spores. Lavender oil, lemon grass oil and thyme oil were the most
19 effective oils against all fungi tested.
20

21 **Keywords:** Beech, moulds, pine, wood decay fungi, wood protection.
22

23 **INTRODUCTION**
24

25 Old woods such as archaeological woods, woods in historical buildings, works of art,
26 antique furniture are important parts of the cultural heritage that gives us valuable information
27 about the past. Due to the high historic potential they must be protected against damages. A large
28 part of historic wood is found inside such as carvings, altarpieces in churches, mosques and
29 museums (e.g. Blanchette 2000, Cojocariu and Tanase 2010, Ortiz *et al.* 2014). Historic wooden

¹ Department of Natural Resources and Earth Science, Shahrekord University, P.O. Box 115, Shahrekord, Iran

²Wood Biology, University of Hamburg, Leuschnerstr. 91, 21031 Hamburg, Germany

30 structures may be attacked by various degradation agents (Blanchette 2000). Among them, the
31 infection and subsequent growth of moulds and other fungi may cause considerable damages
32 (Sterflinger 2010). Fungal damages are often caused by high level of ascension humidity, lack of
33 ventilation and intermittent warming and condensation. A method to protect valuable woods may
34 be the use of plant essential oils. Essential oils have benefits such as they can provide safe and
35 environmentally friendly preservatives. They are effective against bacteria (Burt 2004, Wong *et al.*
36 *al.* 2008), fungi (Mahmoud 1994, Voda *et al.* 2003, Wang *et al.* 2005, Singh and Singh 2012,
37 Boulogne *et al.* 2012, Fidah 2016) and insects (Isman *et al.* 2011, Fatimah and Morrell 2015).
38 Some studies were done on the efficacy of essential oils against moulds (Voda *et al.* 2003, Yang
39 and Clausen 2006, Pánek *et al.* 2014). Caccioni *et al.* (1998) tested mould (*Penicillium* species)
40 inhibition by citrus essential oils measuring the growth in oil-containing nutrient broth. Su *et al.*
41 (2006) obtained growth inhibition of moulds and wood-decay fungi by *Eucalyptus citriodora*
42 essential oil dispersed in agar. Viuda-Martos *et al.* (2008) showed antimould activity of lemon,
43 mandarin, grapefruit and orange essential oils in agar. Yang and Clausen (2007) obtained mould
44 inhibition on pine stakes dipped in geranium and thyme oil. Zani *et al.* (2011) found mould
45 inhibition by oils of *Origanum compactum*, *Eugenia caryophyllata* and *Ocimum basilicaum* using
46 the disc diffusion method and minimum inhibitory concentration in agar dilution test. Pánek *et al.*
47 (2014) evaluated ten essential oils against wood-destroying fungi and moulds. They stated that
48 essential oils containing phenol components such as carvacrol, eugenol, thymol and cisisoasarol
49 trimethyleter have the most effect for beech wood protection against fungi in interior conditions.
50 Experiments with woods impregnated with plant extracts are rare. Goktas *et al.* (2010) showed
51 some protection of beech and pine wood against *Oligoporus placenta* (brown rot) and *Trametes*
52 *versicolor* (white rot) when the samples were treated with extracts of *Muscari neglectum* and
53 *Gynandrinum sisyryinchium*. Chittenden and Singh (2013) monitored antifungal activity of
54 essential oils against wood degrading fungi and concluded that eugenol and cinnamaldehyde are
55 applicable for protection of timber when not exposed to wet conditions. Sailer (2001) obtained
56 lower mass loss in cultures with *T. versicolor* and *Coniophora puteana* (brown rot) when the
57 wood samples of pine, larch, beech and oak were impregnated with linseed and hempseed oil.
58 Our study evaluates the efficacy of 16 plant essential oils against moulds and wood decay fungi
59 using impregnated beech and pine wood samples to test their possible application for protecting
60 valuable wood objects.

MATERIAL AND METHODS

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62
63 The wood samples derived from trees of Oriental beech (*Fagus orientalis* Lipsky) and
64 Loblolly pine (*Pinus taeda* L.) felled in September 2016 in the Kheyroud-Kenar forests, Iran. The
65 sample size of 30 x 30 x 5₁ mm was used to obtain great radial surfaces for intense impregnation
66 with oils and for subsequent sample placement in Petri dishes. The samples were numbered, dried
67 at 103 °C, weighed for dry matter determination, grouped in large glass dishes according to the
68 planned oil treatments and autoclaved for 15 min at 121 °C. This basic sterilization was thought
69 to be necessary to reduce subsequent mould infections in cultures with the slowly growing
70 basidiomycetes.

71 As essential oils, 16 oils (see Tab. 2) were purchased from several German manufacturers
72 specialized in those products. Oils were obtained as 100% concentrations to avoid unknown and
73 diverse dilution chemicals. For wood impregnation, 10 % concentrations were made by diluting
74 with native olive oil. Impregnation was done in a desiccator with 15 min initial vacuum by a
75 pump, oil addition via a funnel in the desiccator lid, followed by 15 min duration for oil uptake.
76 There was no sample sterilization after impregnation to avoid oil removal from the treated
77 samples due to pressure variations in the autoclave. However, the desiccator and all other glasses
78 for impregnation had been disinfected in advance to minimize wood infections from the
79 laboratory. Oil uptake was determined by weighing some samples after impregnation. The
80 impregnated wood samples were lightly touched on clean filter papers to remove oil from the
81 surfaces and immediately placed on fungal mycelia grown on 2% malt-1.5% agar (Oxoid) in Petri
82 dishes.

83 **Table 1.** Fungi used.

Species	Wood damage	Coding	Origin
<i>Aspergillus niger</i>	Moulding	15	Soil of pot flower
<i>Penicillium commune</i>	“	311	Chinese bamboo
Natural infection	“		Airborne spores
<i>Coniophora puteana</i>	Brown rot	167	Fruiting body, Hamburg
<i>Trametes versicolor</i>	White rot	CTBA 863	France
<i>Chaetomium globosum</i>	Soft rot	10, ATCC 44753	

85 Table 1 lists the used fungi with their coding/origin kept in the laboratory strain collection
86 of the second author. Each 2 (for moulds) or 3 (for decay fungi) samples per oil treatment were
87 placed on the fungal mycelia grown in Petri dishes. Natural infection by the surrounding
88 laboratory air was performed by storing the open dishes with the samples for 1 hour. Thereafter,
89 all dishes were sealed with Parafilm against drying. Untreated samples as well as samples
90 impregnated with 100% olive oil were used as controls. Incubation was done at room temperature
91 for 8 to 10 weeks and growth was weekly controlled. The closed mould dishes were several times
92 roughly shaken during incubation to induce further sample infection by spores from the
93 surrounding basal mycelial colony, because moulds spread mainly by their spores and lesser by
94 mycelial growth (Schmidt 2006). After incubation, samples were cleaned from superficial
95 mycelium, weighed for wet wood determination, again dried at 103 °C and finally weighed for
96 mass loss determination. The used method investigates the colonization/infection of wood by
97 fungi and not a subsequent wood decay inhibited by the low wood moisture content. European or
98 American test standards were not applied as the above technique is successfully used for several
99 years in the laboratory of the second author.

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RESULTS AND DISCUSSION

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Efficacy of essential oils against mould fungi

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105 The tested oils differed considerably in their efficacy against moulds. Figure 1 shows as an
106 example beech wood sample incubated with *Aspergillus niger*. The sample with olive oil (left)
107 was fully overgrown, fennel oil yielded medium growth (centre) and lemon grass oil (right)
108 inhibited the mould totally.

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110



111 **Figure 1.** Beech wood samples impregnated with olive oil (left), fennel oil (centre) and lemon
112 grass oil (right) incubated with *Aspergillus niger*.

113 **Table 2.** Growth of mould fungi on oil-impregnated *Fagus orientalis* wood samples

Oil (Plant origin)	<i>Aspergillus niger</i>			<i>Penicillium commune</i>			Natural infection		
	Growth after weeks								
	2	4	10	2	4	10	2	4	10
Control	0	2	3	0	2	3	2	3	3
Control Olive	0	2	3	0	1	3 (thin)	1	3	3
Ajowan <i>Trachyspermum copticum</i>)	0	0	1	0	1	2-3*	0	0	margin
Bergamot (<i>Citrus x limon</i>)	0	3	3	0	0	0	0	0	0
Cedar wood (<i>Juniperus mexicana</i>)	0	3	3	0	1- 2	1-3	0	0	margin
Chamomile (<i>Matricaria chamomilla</i>)	0	3	3	0	0	2	0	0	0
Eucalyptus (<i>Eucalyptus globolus</i>)	0	3	3	0	0	0	0	0	0
Fennel (<i>Foeniculum vulgare</i>)	0	2-3	2-3	0	0	1 (thin)	0	0	margin
Geranium (<i>Pelargonium graveolens</i>)	0	1	2	0	0	traces	0	0	0
Lavender (<i>Lavandula angustifolia</i>)	0	0	traces	0	0	0	0	0	margin
Lemongrass (Citronella) (<i>Cymbopogon winterianus</i>)	0	0	0	0	0	margin	0	0	0
Mint (<i>Mentha arvensis</i>)	0	2	2 (thin)	0	0	0	0	0	margin
Neem (<i>Melea arachdirachta</i>)	0	0	3	0	0	traces	0	0	3(thin
Oenothera (<i>Oenothera biennis</i>)	1-2	2	3 (thin)	0	0	traces	0	0	traces
Peppermint (<i>Mentha piperita</i>)	0	1	3 (thin)	0	0	traces	0	0	margin
Star anise (<i>Ilium verum</i>)	0	1	3 (thin)	0	0	0	0	0	0
Tea tree (<i>Melaleuca alternifolia</i>)	0	1	2 (thin)	0	0	traces	0	0	0
Thyme (<i>Thymus vulgaris</i>)	0	0	0	0	0	traces	0	0	0

114 0 - no growth on sample surface; 1 - about 1/3 of sample surface overgrown; 2 - about half surface overgrown; 3 -
 115 whole surface overgrown; margin - sample sides overgrown; *- growth differs among samples.

116
 117 Table 2 summarizes the results for the beech wood samples. The control wood samples
 118 were fully overgrown by moulds within 4 to 10 weeks of incubation. Growth became obvious by
 119 thin hyphae on the upper sample area followed by the development of new conidial structures
 120 (conidiophores with conidia). If this growth had happened by mycelium reaching the upper
 121 sample area from the fungus colony on the agar and/or by flying spores within the Petri dish
 122 could not be distinguished. Remarkable is the slight decrease of infection intensity by the olive
 123 oil.

124 Considerable differences were found among the 16 oils tested. Most oils yielded only
 125 some mould inhibition. However, the oils from lavender, lemon grass and thyme (fat) showed
 126 sufficient inhibition on both moulds and the natural infection by airborne spores, allowing only
 127 some growth on the sample side area within 10 weeks of incubation. Thyme oil was already
 128 shown by Yang and Clausen (2007) to inhibit the growth of *A. niger* and *Penicillium*
 129 *chrysogenum*).

130

131 **Table 3.** Growth of mould fungi on oil-impregnated *Pinus taeda* wood samples.

Oil	<i>Aspergillus niger</i>			<i>Penicillium commune</i>			Natural infection		
	Growth after weeks								
	2	4	8	2	4	8	2	4	8
Control	0	2	3	0	2	3	2	3	3
Control Olive	0	2	3	0	1	3 (thin)	1	3	3
Ajowan	0	0	1	0	1	2-3	0	0	margin
Bergamot	0	3	3	0	0	0	0	0	0
Cedar wood	0	3	3	0	1-2	1-3	0	0	margin
Chamomile	0	3	3	0	0	2	0	0	0
Eucalyptus	0	3	3	0	0	0	0	0	0
Fennel	0	2+3	2-3	0	0	1	0	0	margin
Geranium	0	1	2	0	0	traces	0	0	0*
Lavender	0	0	traces	0	0	0	0	0	margin
Lemongrass	0	0	0	0	0	margin	0	0	0
Mint	0	2	2 (thin)	0	0	0	0	0	margin
Neem	0	0	3	0	0	traces	0	0	3 (thin)
Oenothera	1-2	2	3 (thin)	0	0	traces	0	0	Traces
Peppermint	0	1	3 (thin)	0	0	traces	0	margin	margin
Star anise	0	1	3 (thin)	0	0	0	0	0	0
Tea tree	0	1	1-2 (thin)	0	0	traces	0	0	0
Thyme	0	0	0	0	0	traces	0	0	0

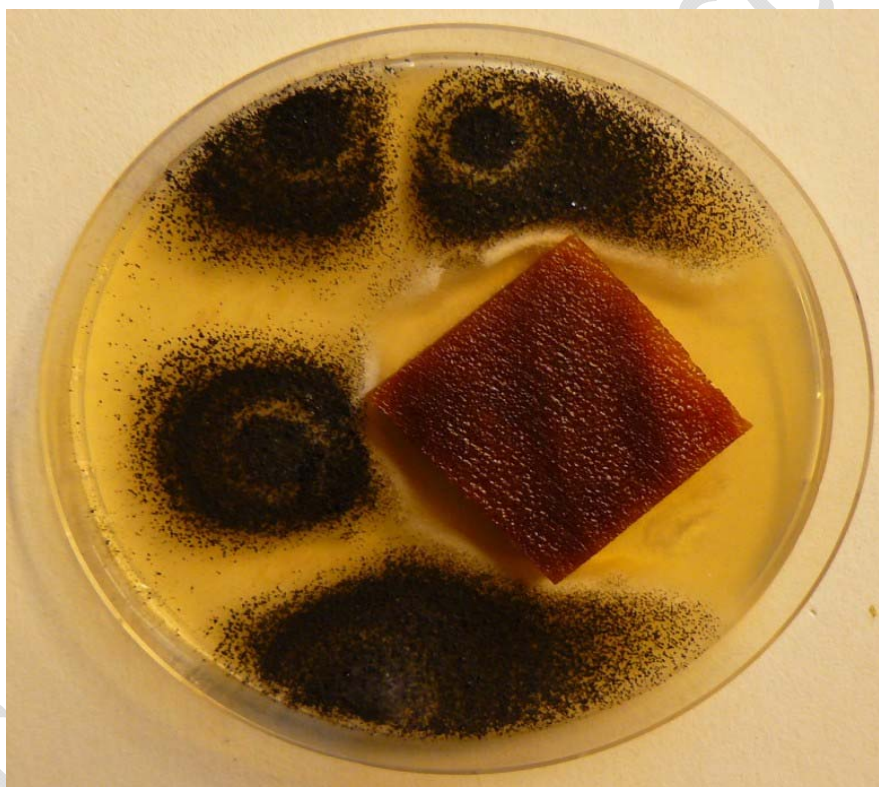
132 * = inhibition zone occurred against neighbouring mould colonies (see Figure 2).

133

134 The results for pine wood are summarized in Table 3. As with beech wood, the oils from
135 lavender, lemon grass and thyme were the most effective oils for pine wood protection from
136 moulds. This result is not astonishing because the characteristic feature of mould fungi on wood
137 is their growth restricted to the surfaces and outermost wood regions (Schmidt 2006). The
138 substrate underneath, in this case beech or pine wood, has no influence because these fungi do
139 not feed from the woody cell wall.

140 Remarkable is the formation of an inhibition zone in the agar by geranium oil. Figure 2
141 shows such zone spreading from the treated wood sample towards the neighbouring mould
142 colonies. If this was due to oil diffusion through the agar or by vapour remains unclear. Zyani et
143 al. (2011) showed that some oil constituents are active as vapour.

144



145

146

147 **Figure 2.** Inhibition zone by geranium oil against neighbouring mould growth.

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149 **Efficacy of essential oils against wood decay fungi**

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151 Table 4 shows the influence of the essential oils in impregnated beech wood samples on
152 wood-decay fungi. The controls without any oil were totally overgrown by mycelium already

153 after 2 weeks of incubation. However, already the olive oil controls showed a slight to strong
 154 growth inhibition particularly with the brown-rot species *Coniophora puteana*. Among the 16
 155 oils tested, like against the deuteromycetes (Tab. 2 and 3), the oils from lavender, lemon grass
 156 and thyme were effective in view of growth inhibition; additionally ajowan oil inhibited the three
 157 rot fungi.

158

159 **Table 4.** Growth of wood-decay fungi on oil-impregnated *Fagus orientalis* wood samples.

Oil	<i>Coniophora puteana</i>			<i>Trametes versicolor</i>			<i>Chaetomium globosum</i>		
	Growth after weeks								
	2	4	10	2	4	10	2	4	10
Control	3	3	3	3	3	3	3	3	3
Olive	1	1	1-2	2 (thin)	2	3	2	3	3
Ajowan	0	0	0	0	0	margin	0	0	0
Bergamot	2-3	2-3	3	2	2	2-3	0	0	margin
Cedar wood	2	2	3	0	1	3	1	1	3 (thin)
Chamomile	3	3	3	1	2	3 (thin)	0	3	3
Eucalyptus	3 (thin)	3 (thin)	3	0	1	2-3 (thin)	0	0	0
Fennel	0	0	0	1	1	1-2 (thin)	0	0	traces
Geranium	1	2	2-3	0	0	1 (thin)	0	0	0
Lavender	0	0	margin	0	0	traces	0	0	0
Lemongrass	0	0	0	0	0	0	0	0	0
Mint	1	2-3	3	0	1	1 (thin)	0	0	0
Neem	3	3	3	2 (thin)	2	2	0	3	3
Oenothera	3	3	3	2 (thin)	2 (thin)	2 (thin)	1	3	3
Peppermint	0	0	1-2	2	2	2-3	0	0	margin
Star anise	0	1	1	0	0	traces	0	0	margin
Tea tree	0	traces	1-3	0	1	2 (thin)	0	0	0-1
Thyme	0	0	traces	0	0	0	0	0	0

160

161 Table 5 shows the results obtained for wood-decay fungi and pine wood. Effective oils
 162 to protect the softwood derived from ajowan, geranium, lavender, lemon grass and thyme.

163 Most plant essential oils contain many constituents. Lavender oil and tea tree oil, for
 164 example, consist of over 100 active compounds. Su *et al.* (2006) identified 67 compounds from
 165 the essential oil of *Eucalyptus grandis*. Because quite different oils inhibited the fungi tested it
 166 can be assumed that several constituents were effective. Thymol is known as a strong
 167 antimicrobial agent. However, to correlate the antifungal activity to a single constituent or to
 168 classes of compounds is difficult. The various components of any oil may act synergistically

169 (Caccioni et al. 1998) while several compounds may have even a stimulating action on fungi, as
 170 shown by French (1985) for spore germination. Various possible action mechanisms by which
 171 fungal growth may be reduced or inhibited have been proposed (Viuda-Vartos et al. 2008) such
 172 as effects on the functionality and structure of cell membranes, changes in cell structure,
 173 inhibition of respiration, and interactions with enzymes.

174

175 **Table 5.** Growth of wood-decay fungi on oil-impregnated *Pinus taeda* wood samples.

Oil	<i>Coniophora puteana</i>			<i>Trametes versicolor</i>			<i>Chaetomium globosum</i>		
	Growth after weeks								
	2	4	8	2	4	8	2	4	8
Control	3	3	3	3	3	3	3	3	3
Control Olive	3	3	3	3 (thin)	3	3	3 (thin)	3	3
Ajowan	0	0	margin	0	0	0	0	0	0
Bergamot	1	1-2	1-2	0-1	0-1	1	0	1	1
Cedar wood	2	1	3	2 (thin)	3	3	0	margin	1-3
Chamomile	2	2	3 (thin)	2	2	3 (thin)	0	1-2	2 (thin)
Eucalyptus	0	0	0	0	0	2 (thin)	0	0	0-3 (thin)
Fennel	0	1-2	1-3	0	2	2	0	0	0
Geranium	0	0	margin	0	0	0	0	0	0
Lavender	0	0	0	0	0	0	0	0	margin
Lemongrass	0	0	0	0	0	0	0	0	0
Mint	1	2	2-3	0-1	0-1	0-2 (thin)	0	0	margin
Neem	3 (thin)	3	3	2 (thin)	2	2	0	1-2	2-3 (thin)
Oenothera	2-3	3	3	2	2-3	3	0	1-3	3
Peppermint	1	1	1	0	0	margin	0	0	0
Star anise	0	0	margin	1	1-2	2 (thin)	0	0	0
Tea tree	0	0	0	0	0	0	0	0	0
Thyme	0	0	traces	0	0	traces	0	0	0

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177

178 The weight measurements after incubation (wet weight) indicated that most or even all oil
 179 introduced into the wood sample by the impregnation procedure was still present in the samples.
 180 Subsequent drying at 103 °C and weighing for mass loss determination did not yield any usable
 181 result because the oils resist this temperature. Reasons for the missing mass loss may be: The
 182 wood samples with open cells (fibres or tracheids) at both sample surfaces could be intensively
 183 impregnated with oils due to their great radial areas and small height. Oil uptake was about 560
 184 kg oil (= 56 kg essential oil) per m³ beech wood and about 620 (62) kg/m³ pine wood. Typical of
 185 basidiomycetous brown- and white-rot fungi is their growth within the lumina of wood cells and

186 excreting from there the enzymes and free radicals for cell wall degradation into the walls; soft-
187 rot fungi (type I) such as *Chaetomium globosum* initially also invade the cell lumina and grow
188 from there into the cell wall (Liese 1955). Wood decay fungi need water and oxygen for their
189 metabolism. Water is also needed for the wood-degrading agents to reach the cell wall
190 components. Due to the applied methods, there was not enough water for wood decay. From
191 these aspects it becomes probable that the wood-decay fungi could not penetrate into the samples
192 with oil-filled lumina and low moisture content and therefore could not decay the samples.
193 However, the aim of our study was to test if the fungi can colonize/infect oil-treated wood.
194 Practical use of essential oils is restricted to indoor use due to their high volatility and insufficient
195 fixation. As application method the oils may be added to woods by brushing.

197 CONCLUSIONS

198 Our experiments showed that several plant essential oils are able to protect wood samples
199 of a hardwood (*Fagus orientalis*) and softwood (*Pinus taeda*) against colonization by fungi.
200 The oils inhibited the growth of common moulds (*Aspergillus niger*, *Penicillium commune*,
201 airborne spores) and important wood-decay fungi (*Coniophora puteana*, *Trametes versicolor*,
202 *Chaetomium globosum*). Results indicated that lavender oil, lemon grass oil and thyme oil were
203 the most effective oils against fungi. In view of wood decay it became obvious that at least the
204 outer wood region is protected against deterioration if water and air are missing as a result of
205 filling the wood cells with oil.

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