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Antifungal activities of two essential oils used in the treatment of three commercial woods deteriorated by five common mold fungi

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ABSTRACT

In the past ten years natural extracts have been used as important potential applications to prevent mold growth on in-service wood. The growth of fungal hyphae of five common mold fungi (*Alternaria alternata*, *Fusarium subglutinans*, *Chaetomium globosum*, *Aspergillus niger*, and *Trichoderma viride*) on wood surface of *Pinus sylvestris*, *Pinus rigida* and *Fagus sylvatica* treated with the essential oil (EO) of *P. rigida* (wood) and *Eucalyptus camaldulensis* (leaves) was visually estimated. EOs were applied by vapor method and the mold growth inhibition was measured. The chemical constituents of the EOs was analyzed by GC/MS, which referred to the presence of α-terpineol (34.49%), borneol (17.57%), and fenchyl alcohol (14.20%) as the major components in *P. rigida* wood oil, and eucalyptol (60.32%), α-pinene (13.65%), and γ-terpinene (8.77%) in *E. camaldulensis* leaves. Complete inhibition against the growth of *A. alternata*, *F. subglutinans*, *C. globosum*, and *A. niger* except of *T. viride* by applying *P. rigida* wood EO at 5000 ppm and complete growth with all the studied fungi except of *C. globosum* at 156.25 ppm was found. Good inhibitions against *C. globosum* at 5000 ppm and 156.25 ppm and no inhibition against *A. niger* and *T. viride* and little inhibition against *F. subglutinans* at high concentration was found by the application of EO from *E. camaldulensis* leaves. These findings support the potential use of the EOs for wood protection against mold infestation for surface-treatment or fumigation of wood products.

1. Introduction

For the building industry, the protection against mold growth on wood is a critical economic concern as well as choice of nontoxic compounds which are applicable in interior wood protection. Wood deterioration is a complex biological process that can involve a wide variety of microorganisms, such as fungi and bacteria, and is influenced by the changing environmental conditions in which the wood is placed (Eaton and Hale, 1993; Zabel and Morrell, 1992). Fungi are important biodegrading organisms and are considered as serious degrading agents where the presence of vegetative cells or spores on the surface of wood or other materials like paper may indicate a possible degradation in the future (Fabbri et al., 1997; Mesquita et al., 2009).

Mold fungi growing on wood surfaces cause sapstain and simple sugars and starch present in ray cells and axial cell lumens are consumed by molds (Kerner-Gang and Schneider, 1969; Mansour and Salem, 2015). Sapstain is a major problem for timber producers as well as pulp and paper manufacturers since fungal colonization and disfigurement of freshly felled material prior to drying can result in significant economic losses.

Although mold fungi cause little or no significant damage to the structural elements of the timber (Blanchette et al., 1992), they have a detrimental effect on the aesthetic value of the wood due to the colonization by their pigmented mycelium. This is due to the production and deposition of granules of melanin in and around the fungal hyphae (Brisson et al., 1996). Ray parenchyma and cell lumens are colonized by mold fungi as well as scavenge of proteins and triglycerides deep throughout the sapwood of the lumber (Breuil, 1998).

Molds like *Alternaria* and *Trichoderma* species, are very destructive in museums, have a well-developed lignocellulolytic

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enzyme system (Garg et al., 1995).

Alternaria fungi have black pigment (melanin) that causes distinctly seen dark grey discoloration on wood joints (Domsch et al., 2007). *Alternaria alternata* and other mold fungi isolated from treated wood are recognized as soft-rot fungi and they have been found on painted or preservative-treated wood (Kim et al., 2007; Pournou and Bogomolova, 2009; Råberg et al., 2009). Fungal enzymatic activity on wood showed that *A. alternata* was the most active tyrosinase producer and it was the hardiest to wood preservatives fungal strain (Bridžiuvienė and Raudonienė, 2013). *A. alternata* produces mycotoxins (tenuazonic acid) which cause toxic leucopenia in humans and is considered an important source of allergens as well (Breitenbach and Simon-Nobbe, 2002). *A. alternata* showed phenoloxidase activity that degrades lignin (Bridžiuvienė and Raudonienė, 2013).

Trichoderma rarely have been reported to cause soft rot (Kim et al., 2007; Mansour and Salem, 2015). *Trichoderma* species are not capable of invading living wood and are not an effective colonizer of living tissue (Lundborg and Unestam, 1980; Kubicek et al., 2008). They are associated with other fungi that are pathogenic to the host tree (Jin et al., 1992), and were isolated with greater frequency from the roots of Douglas fir decayed by *Phellinus weirii* than from sound, un-decayed regions (Goldfarb et al., 1989). Confocal micrograph showed *Trichoderma viride* hyphae in ray parenchyma cells of common radiata pine (Xiao et al., 1999). Greater persistence of green mold contamination in wood, wooden pallets and concrete were observed (Abosriwil and Clancy, 2002) and produce cellulase and hemicellulases (Gautam, 2010).

T. viride, *Chaetomium globosum* and *Alternaria* sp. are the fungi with proven cellulolytic activity detected in the deteriorated wooden sculptures and art photographs temporarily stored in the quarantine room of the Cultural Center of Belgrade (Ljaljević-Grbić et al., 2013). *Aspergillus niger* is a producer of many pectinases and hemicellulose degrading enzymes, like xylanases and arabinases (Delgado et al., 1992; van Peij et al., 1997; Parenicová et al., 2000). Also, xylan (major structural heteropolysaccharides of hardwood) degradation occurs in certain strains including *A. niger* and *T. viride* (Filho et al., 1996). *A. niger* and *Fusarium oxysporum* produces cellulase and xylanase enzymes which are capable to biodegrade the forest waste from *Pinus roxburghii, Cedrus deodara, Toona ciliata* and *Celtris australis* (Kaushal et al., 2012) and hardwood has shown better degradation as compared to the softwood.

C. globosum is the most common species of *Chaetomium* found in buildings (Andersen and Nissen, 2000) and produces the highly cytotoxic chaetomins and chaetoglobosins that inhibit cell division and glucose transport (Ueno, 1985). *C. globosum* was stated to be a very active organism in the decay of leather from book bindings (Strzelczyk et al., 1989) and was found in both wood-pulp paper and laid-paper (Mesquita et al., 2009).

Mycelial growth by most molds is hyaline and relatively insignificant in causing spoilage to *Pinus sylvestris* timber (Payne and Bruce, 2001). Rapid air or kiln drying of wood or through the use of diffusible chemical preservatives can be used to control of sapstain (Byrne, 1998). Mold fungi that grow on wood surface are able to degrade lignin, but the phenoloxidases (peroxidase, tyrosinase and laccase) that take part in lignin decomposition are not characteristic of every fungus (Bridžiuvienė and Raudonienė, 2013).

Synthetic fungicides are commonly used to control and prevent the growth of mold and decay fungi on wood, but are not environmentally suitable for many indoor applications and the search for naturally, environmentally-friendly alternatives, which exhibit negligible toxicity to human has become a necessity (Verma and Dubey, 1999; Qi and Jellison, 2004; Li et al., 2013; Wang et al., 2005; Kiran and Raveesha, 2006). Natural extracts have therefore become potentially useful for protecting woods from mold fungi (Philp et al., 1995; Jeloková and Šindler, 1997; Qi and Jellison, 2004; Wang et al., 2005; Li et al., 2013; Mansour and Salem, 2015; Salem et al., 2015). Essential oils (EOs), a complex mixture of odorous and volatile compounds, are known for their natural components, such as monoterpenes, diterpenes and hydrocarbons with various functional groups and, have been studied for their antibacterial and antifungal activities (Mazzanti et al., 1998; Hammer et al., 2002; Wang et al., 2005; Pawar and Thaker, 2006; Salem et al., 2014).

As part of the continuous research for using natural products as a bio-agents for protecting woods against mold fungi, our ongoing work was to evaluate the antifungal effect of essential oils from *Pinus rigida* (wood) and *Eucalyptus camaldulensis* Dehnh. (leaves) against the growth of five common mold fungi namely, *A. alternata*, *Fusarium subglutinans*, *C. globosum*, *A. niger*, *and Trichoderma viride*. The evaluation was done by application of these essential oils as biocides for preservation of three commercial woods (*P. sylvestris*, *P. rigida* and *Fagus sylvatica*) against the biodeterioration caused by the studied mold fungi.

2. Materials and methods

2.1. Chemicals

All of the chemicals used in the present study were of high analytical grade from Fluka and Sigma–Aldrich Co. (USA).

2.2. Preparation of wood samples

Sapwood samples of Scots pine (*P. sylvestris* L., Pinaceae), Pitch pine (*Pinus rigida* Mill., Pinaceae), and European beech (*F. sylvatica* L.) (Fagaceae), were provided from a woodworking shop in Alexandria City, Egypt, August 2014. The wood samples (10 × 10 × 5 mm) were oven-dried at 105 °C for 24 h, then autoclaved at 121 °C for 20 min.

2.3. Essential oil extraction

Fresh leaves of *E. camaldulensis* were cut to small pieces and the air-dried *P. rigida* ground wood was hydro-distillated for 3 h, in a Clevenger apparatus (Salem et al., 2013). The essential oils (EOs) were dried over anhydrous Na_2SO_4, and measured with respect to the mass weight (4.5 and 2.12 ml 100 g^{-1} sample weight for *E. camaldulensis* and *P. rigida*, respectively). The EOs were kept dry in sealed Eppendorf tubes and stored at 4 °C prior chemical analysis.

2.4. Preparation of essential oils

The extracted EOs were prepared at the concentrations of 5000, 2500, 1250, 625, 312.5, and 156.25 ppm for antifungal activity bioassay. The respective amount of oil was diluted in 10% dimethyl sulfoxide (DMSO): sterilized distilled water (SDW) (1:1 *v/v*) and 0.5 ml of Tween 80 was added to emulsify carrier oils in water.

2.5. Vapor treatment of essential oils for mold inhibition

Wood samples were vapor treated with the EOs prepared at the concentrations of 5000 ppm, 2500 ppm, 1250 ppm, 625 ppm, 312.5 ppm, and 156.25 ppm using the evaporation method (Lopez et al., 2005; Nedorostova et al., 2009). Wood samples were put in petri dishes contains 8 layers of filter papers (Wattman No. 1) overlaid by a mesh (polyethylene spacer). The dishes were autoclaved at 121 °C for 20 min and left to cool, then the oils with the respective concentration were impregnated over the filter papers and kept for 48 h to allow the EO evaporation which the fumigants