ELSEVIER

International Biodeterioration & Biodegradation 59 (2007) 302–306

INTERNATIONAL BIODETERIORATION & BIODEGRADATION

www.elsevier.com/locate/ibiod

Antifungal effect of essential oils on southern yellow pine

Vina W. Yang*, Carol A. Clausen

US Department of Agriculture, Forest Service, Forest Products Laboratory, One Gifford Pinchot Drive, Madison, WI 53705-2398, USA

Received 24 May 2006; received in revised form 3 August 2006; accepted 10 September 2006
Available online 27 October 2006

Abstract

Moisture management remains the most critical factor for controlling mold growth on wood and wood products during storage, construction, and while in service. When moisture management practices fail to adequately control moisture, plant extracts demonstrating antifungal properties may provide protection for these applications. The objective of this study was to evaluate the antifungal properties of natural plant extracts, such as essential oils, for use on wood. Seven essential oils were evaluated for their ability to inhibit growth of *Aspergillus niger*, *Trichoderma viride*, and *Penicillium chrysogenum* on southern yellow pine (SYP) stakes that were either dip treated or exposed to vapors of the test oils. Thyme and Egyptian geranium oil inhibited growth of all test fungi for 20 weeks. Likewise, dill weed oil vapors inhibited all test fungi for at least 20 weeks. Comparison of two mold test apparatuses—Petri dish test and tank test chambers—gave similar results for thyme oil. These findings support the application of essential oils for surface treatment or vapor exposure of wood to prevent mold infestation.

Keywords: Essential oil; Mold; Antifungal; Moldicide

1. Introduction

Potential health risks caused by mold growth in residential and non-residential wooden structures have been a major concern for homeowners, building contractors, and insurance companies alike. Lawsuits claiming health problems caused by indoor mold exposure exceeded 2.8 billion US dollars in 2002 (Hartwig and Wilkinson, 2003). Chemical fungicides commonly used to control the growth of mold on wood may not be appropriate for many indoor applications. Natural alternatives that are user friendly and demonstrate low toxicity to humans are desirable for this application. Essential oils are known for their natural components, such as monoterpenes, diterpenes, and hydrocarbons with various functional groups. In the 1990s, Muanza and others searched for potential bioactive plant extracts against bacteria and fungi (Muanza et al., 1994, 1995). Many other researchers have since reported on antimicrobial (Cowan, 1999; Hammer et al., 1999; Hoffman et al., 2004; Mau et al., 2001; Sivropoulou et al., 1995, 1997) and antifungal activities (Adam et al., 1998; Deferera et al., 2000; Moretti et al., 1998; Muller-Riebau et al., 1995; Rakotonirainy and Lavedrine, 2005; Scheffer and Duncan, 1946; Sridhar et al., 2003; Wang et al., 2005) of essential oils in food applications, pharmaceutical research and other areas. However, little has been published previously on the use of essential oils as antimold agents on wood and wood products.

The objective of this study was to evaluate the ability of seven commercially available essential oils for mold inhibition on wood. Essential oils were evaluated by two methods, dip treatment and vapor exposure, and were compared in both Petri dish test chamber and tank test chamber.

2. Materials and methods

2.1. Essential oils

Seven essential oils derived from steam distillation—ajowan, dill weed, Egyptian geranium, lemongrass, rosemary, tea tree, and thyme—were obtained from New Directions Aromatics, Inc., San Francisco, California. All oils were used at full strength unless specified otherwise. Major

*Corresponding author. Tel.: +1 608 231 9419; fax: +1 608 231 9508.
E-mail address: vyang@fs.fed.us (V.W. Yang).

0964-8305/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ibiod.2006.09.004

Table 1
List of essential oils tested

Common name	Botanical name	Major component(s)	Functional group
Ajowan	*Carum opticum*	Thymol	Monoterpene phenol
Dill	*Anethum graveolens*	Carvone	Ketone
Egyptian geranium	*Pelargonium graveolens*	Citronellol	Monoterpene alcohol
Lemongrass	*Cymbopogon flexuosus*	Citral	Monoterpene aldehyde
Rosemary	*Rosmarinus officinalis*	Verbenone, Camphor, Cineole	Ketone, terpene oxide/hydrocarbons
Tea tree	*Melaleuca alternifolio*	Terpineol-4	Monoterpene alcohol
Thyme	*Thymus zygis*	Geraniol, Linalol, Thujanol, Carvacrol, Thymol	Monoterpene phenol, alcohol, esters

components and functional groups of test oils are shown in Table 1 (Edwards, 1999; Schnaubelt, 1998).

2.2. Fungal strains

Three mold fungi, *Aspergillus niger* 2.242 provided by University of Virginia, *Penicillium chrysogenum* PH02 from Forest Products Laboratory, Madison, Wisconsin, and *Trichoderma viride* ATCC 20476 were grown on 2% malt agar (Difco, Becton, Dickinson & Company, Sparks, Maryland) for 2 weeks. *Aureobasidium pullulans* provided by Forest Products Laboratory was grown on 2% potato dextrose agar (Difco, Becton, Dickinson & Company, Sparks, Maryland) for 2 weeks explicitly for inoculation of the soil in the tank test chamber. Spore suspensions of remaining test fungi were prepared by washing the surface of each malt agar plate with 10–15 mL of sterile deionized water (DI) according to ASTM standard D4445-91 (American Society for Testing and Material, 1998). In one set of tests, a mixture of three mold spore suspensions was transferred to a spray bottle and diluted to 100 mL with DI water to yield 3×10^7 spores/mL. Spores of individual mold strains were prepared as described above for subsequent tests with individual test fungi. The spray bottle was adjusted to deliver 1 mL inoculum per spray.

2.3. Test specimens

Southern yellow pine (SYP) specimens (7×20 mm cross-section by 7 cm long), cut from southern pine mill ends obtained from a Mississippi sawmill and stored at 0 °C, were used in the Petri dish chamber method. Test specimens of kiln-dried SYP, cut into a series of 75×100 mm samples, 12.5 mm thick were used in the tank test chamber method.

2.4. Dip stake treatment

Five random replicate specimens were dip treated for 15 s in individual essential oils. Vegetable oil served as a control. Specimens were held in a closed container overnight at room temperature according to ASTM test methods D4445-91 and D3273-00 (American Society for Testing and Material, 1986, 1998) before inoculation with spores of the test fungi. Additionally, thyme and tea tree oil dilutions of 1:2, 1:4 and 1:8 were tested individually and in combination for mold inhibition for 22 weeks. Three random replicates were weighed before and after dipping to estimate essential oil retention levels.

2.5. Vapor exposure treatment

Five untreated specimens were held overnight at room temperature in a closed glass Petri dish (150×250 mm). A 4-cm diameter glass dish containing 3 ml of a test oil was placed next to the specimens 24 h before inoculation with spores of the test fungi. The test oil remained in the Petri dish chamber for the duration of the 20 weeks test. Vegetable oil served as a control.

2.6. Petri dish test chamber

Each Petri dish test chamber (150 by 25 mm) (B-D Falcon, Los Angeles, California) contained four layers of blotting paper that was saturated with 30 mL DI water and covered with a polyethylene mesh spacer to elevate specimens. Specimens were sprayed with 1 mL of mixed or individual mold spore inoculum 24 h post-treatment. Petri dish test chambers were sealed in polyethylene bags to ensure 100% relative humidity (RH) exposure and prevent drying by incubating at 27 °C, 70% RH for the duration of the test. Specimens were evaluated for mold growth at 4, 6, 10, 12, 16, 20 and 22 weeks and rated on a scale of zero to five, with zero indicating no growth and five indicating heavy mold growth. Specimen rating ceased when test oils failed to substantially inhibit growth of test fungi.

2.7. Tank test chamber

A self-contained stainless steel environmental chamber ($28 \times 20 \times 26$ cm) containing water, soil, and hangers for suspending test samples was covered with a pitched roof to prevent condensation from dripping onto specimens. Test chambers were set up in a conditioning room at 27 °C and 70% RH. This test set-up, a modification of ASTM D3273-00 (American Society for Testing and Material, 1986) for resistance to mold growth on the surface of interior coatings in an environmental chamber, did not include an internal heater, electrical fan, or water circulator. The tank lid is sealed with rubber tubing so that the humidity remains at 100% for the duration of the test and the temperature in the chamber is the same as the conditioning room, 27 °C.

Non-sterile potting soil was placed in a tray to a depth of 1 in. above the water level. Soil was inoculated with mold spores from three test fungi, *Aureobasidium pullulans, Aspergillus niger,* and *Penicillium chrysogenum,* 2 weeks before placing the test specimens in the chamber. Test specimens were vertically suspended across the width of the chamber over inoculated soil.

In one tank test chamber, specimens dip treated with thyme and Egyptian geranium oils were inoculated with a mixed spore suspension 24 h post-treatment. In a second tank test chamber for vapor exposure, a glass Petri dish containing 5 mL dill weed oil was placed on the soil surface for 24 h before specimens were inoculated with the mixed spore suspension. The dish containing the test oil remained in the tank test chamber for the duration of the test.

3. Results and discussion

Essential oils evaluated in this study were selected for their previously reported antimicrobial properties in pharmaceutical, food, and packaging applications. Antifungal effects of essential oils on wood against three common airborne mold fungi were treated by two different methods, dip treatment and vapor exposure, and the results

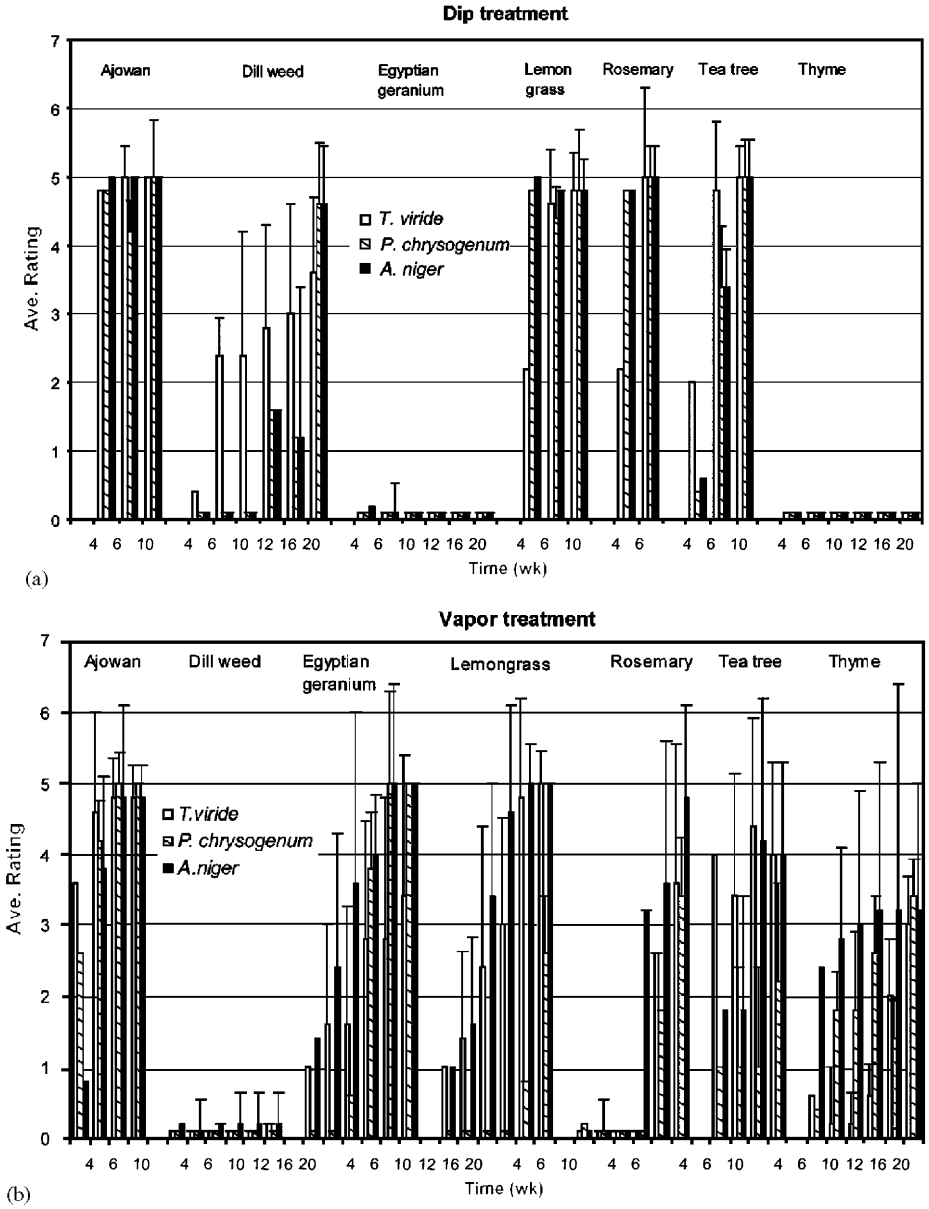

Fig. 1. (a) Mold resistance of SYP specimens dip treated with seven individual essential oils and challenged with three mold fungi in a Petri dish test chamber. (b) Mold resistance of SYP specimens exposed to vapors of seven individual essential oils and challenged with three mold fungi in a Petri dish test chamber.

are presented as the average ratings of five specimens in Figs. 1a and b.

3.1. Dip stake results

Specimens were initially rated after being incubated for 4 weeks. Ratings continued periodically through 20 weeks or until test oils failed to substantially inhibit test fungi. Results of the dip stake method showed that ajowan, lemongrass, rosemary, and tea tree were about 80% covered with mold growth at week 6 and 100% covered at week 10 (Fig. 1a). The inhibitory effect on the surface of wood specimens was low for these four essential oils using the dip stake method. Dill weed oil inhibited *P. chrysogenum* PH02 and *A. niger*, but not *T. viride*, for up to 10 weeks. Egyptian geranium and thyme completely inhibited all test fungi for at least 20 weeks (rated zero). Oil treatment may have some effects on moisture exclusion, but control stakes dipped with vegetable oil showed 100% mold coverage at week 4. Diluted thyme oil (1:8) showed no mold growth up to 22 weeks, whereas a 1:2 dilution of tea tree oil only demonstrated mold inhibition for 6 weeks. The combination of thyme and tea tree oils was less inhibitory than thyme oil alone, therefore no synergy was observed in this combination. Rather, it appears that thyme oil's antimold properties were diluted by tea tree oil

(data not shown). Retention levels of undiluted oils, as well as controls, averaged approximately 0.04 g/cm^3 on southern pine.

3.2. Vapor exposure results

Test fungi showed a completely different response to vapor exposure of essential oils. The most effective mold inhibitor was dill weed oil vapor. It retarded growth of all three molds for at least 20 weeks. Rosemary vapor inhibited *T. viride* and *P. chrysogenum* for 12 weeks and *A. niger* for 10 weeks (Fig. 1b). These findings suggest that ketone volatilization may play a role in preventing spore germination for dill weed and rosemary oils. Since other essential oils that contain ketones in varying amounts may not demonstrate inhibition of mold fungi, it is more likely that specific ketones or the combination of components in an essential oil could be responsible for anti-fungal properties noted in this study. Further investigation is needed to elucidate the role of specific ketones on mold retardation. Lemongrass vapor retarded *P.chrysogenum* growth for 12 weeks, but was ineffective against the other two test fungi. Ajowan and tea tree vapors did not inhibit mold fungi. Contrary to dip treatment results, Egyptian geranium and thyme oil vapors did not inhibit mold fungi under the conditions of this study, suggesting that the monoterpene components of these two oils either inhibit spore germination or vegetative growth upon contact.

3.3. Petri dish test chamber versus tank test chamber

Both dip treatment and vapor exposure in separate tank test chambers showed positive inhibition for all test fungi on treated specimens for at least 20 weeks for thyme oil and up to 8 weeks for dill weed oil (Table 2). In a comparison of thyme oil in two different test apparatus configurations, Petri dish and tank chambers, mold inhibition results were comparable. Dill weed oil vapor demonstrated lower efficacy in the tank test than in the Petri dish chamber, which could be due to the proportionally larger volume of the tank compared to the Petri dish. Minimum effective concentrations of dill weed vapor have not yet been established.

RH can play an important role for mold infestation. *Penicillium*, *Aspergillus*, and *Cladosporium* sp. are commonly associated with wood and wood products exposed to humidity conditions lower than saturation. Minimum water activity levels (aw) are reported as 0.8 for *Aspergillus* and *Penicillium* or lower for some mold fungi (Flannigan and Miller, 1993; Quarles, in press). The test apparatuses in this study are designed to provide continual conditions of 100% RH.

Antifungal properties of thyme and Egyptian geranium oils may play an important role in wood protection from molds. Three active components of thyme oil, namely geraniol, thymol, and carvone, provide significant inhibition of mold growth and can serve as a broad spectrum biocide against commonly occurring molds (Scheffer and Duncan, 1946). Ajowan was not an effective mold inhibitor under the conditions of this study, which is contrary to the results observed by Sridhar et al. (2003).

Table 2
Inhibition of mold growth[a] on SYP in a tank test chamber

Treatment	Essential oil	Time (weeks)	Rating[b]
Dip treated	Thyme	4	0
		8	0.2
		10	0.4
		16	0.6
		20	0.4
	Egyptian geranium	4	0
		8	1.2
		10	3.2
		16	3.8
		20	4
Vapor exposure	Dill weed	4	0
		8	3.6
		10	4
		16	4
		20	3.8

[a]Inoculum consists of a mixture of spores from *A. niger*, *T. viride* and *P. chrysogenum* with a final concentration of 3×10^7/mL.
[b]Value is the average rating of five specimens per treatment. Rating system: 0 = no growth, 1 = 20%, 2 = 40%, 3 = 60%, 4 = 80% and 5 = 100% mold coverage.

Utilizing fungistatic vapor to control mold growth was explored as early as 1946 by Scheffer and Duncan. Volatilization of a biocide can be an undesirable property that affects efficacy, but in this circumstance, the tendency for high volatilization could prove advantageous to broaden the range of useful applications for essential oils. Vapor inhibition of molds could provide protection for large volumes of wood products in a closed environment. Dill weed and rosemary oil vapors appear to be fungicidal under the vapor exposure conditions employed in this study. However, minimum effective concentrations need to be established before the economic feasibility of this application can be determined. Combinations of different chemicals are frequently more effective than single compounds when a variety of fungi are to be controlled. Antifungal synergy among essential oils needs to be further explored, particularly by the tank test chamber method.

4. Conclusions

Thyme and Egyptian geranium oils were efficacious against *T.viride*, *P.chrysogenum,* and *A. niger* on southern pine dip treated with the oils, while dill weed oil inhibited all test fungi by vapor exposure. Thyme oil provided protection from mold growth on SYP for at least 20 weeks, even when diluted in 1:8 ratio. These antimycotic natural compounds may be useful for inhibition of mold fungi on wood in service or during storage of building materials, such as framing lumber, millwork, or truss systems.

References

Adam, K., Sivropoulou, A., Kokkini, S., Lanaras, T., Arsenakis, M., 1998. Antifungal activities of *Origanum vulgare* subsp. *Hirtum, Mentha spicata*, *Lavandula angustifolia* and *Salvia fruiticosa* essential oil against human pathogenic fungi. Journal of Agricultural and Food Chemistry 46, 1739–1745.

American Society for Testing and Material, 1986. Standard test method for resistance to growth of mold on the surface of interior coatings in an environmental chamber. ASTM D3273-00, West Conshohocken, PA, vol. 06.01, pp. 411–413.

American Society for Testing and Material, 1998. Standard test method for fungicides for controlling sapstain and mold on unseasoned lumber (laboratory method). ASTM Standard D4445-91, West Conshohocken, PA, vol. 11.01, pp. 497–500.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews 12, 564–582.

Deferera, D.J., Ziogas, B.N., Polissiou, M.G., 2000. GC-MS Analysis of essential oil from some Greek aromatic plants and their fungitoxicity on *Pennicillium digitatum*. Journal of Agricultural and Food Chemistry 48, 2576–2581.

Edwards, V., 1999. The Aromatherapy Companion. Published by Storey Books, Pownal, Vermont, pp. 55–62.

Flannigan, B., Miller, J.D., 1993. Indoor humidity and the building envelope. In: Ross, W.B., TenWolde, A. (Eds.), Bugs, Mold and Rot II. National Institute of Building Sciences, Washington, DC, pp. 43–50.

Hammer, K.A., Carson, C.F., Riley, T.V., 1999. Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 86, 985–990.

Hartwig, R.P., Wilkinson, C., 2003. Mold and Insurance from Insurance Information Institute. 〈www.iii.org〉

Hoffman, B.R., DelasAlas, H., Wiederhold, R.E., William, L., 2004. Screening of antibacterial and antifungal activities of ten medicinal plants from Ghana. Pharmaceutical Biology 42 (1), 13–17.

Mau, J.L., Chen, C.P., Hsieh, P.C., 2001. Antimicrobial effect of extracts from Chinese chive, cinnamon and *Corni fructus*. Journal of Agricultural and Food Chemistry 49, 183–188.

Moretti, M.D., Peana, A.T., Franceschini, A., Carta, C., 1998. In vivo activity of Salvia officinalis oil against Botrytis cinerea. Journal of Essential Oil Research 10, 157–160.

Muanza, K., Kim, B.W., Euler, K.L., William, L., 1994. Antibacterial and antifungal activities of nine medicinal plants from Zaire. International Journal of Pharmacology 32, 337–345.

Muanza, D.N., Euler, K.L., William, L., 1995. Screening for antitumor and anti-HIV activities of nine medicinal plants from Zaire. International Journal of Pharmacology 33, 98–106.

Muller-Riebau, F., Berger, B., Yegen, O., 1995. Chemical composition and fungitoxic properties to phytopathogenic fungi of essential oil of selected aromatic plants growing wild in Turkey. Journal of Agricultural and Food Chemistry 43, 2262–2266.

Quarles, S.L., Mold growth in structures: an Overview. American Chemical Society Symposium Series, in press.

Rakotonirainy, M.S., Lavedrine, B., 2005. Screening for antifungal activity of essential oils and related compounds to control the biocontamination in libraries and archives storage areas. International Biodeterioration and Biodegradation 55, 141–147.

Schnaubelt, K., 1998. Advanced aromatherapy: the science of essential oil therapy. Published by Healing Arts Press, Rochester, Vermont (pp. 9–41).

Scheffer, T.C., Duncan, C.G., 1946. Fungistatic vapors for control of mold in packages and equipment. Industrial and Engineering Chemistry 38, 619–621.

Sivropoulou, A., Kokkini, S., Lanaras, T., 1995. Antimicrobial activity of mint essential oil. Journal of Agricultural and Food Chemistry 43, 2384–2388.

Sivropoulou, A., Nicolaou, C., Papanikolaou, E., Dokkini, S., Lanaras, T., Arsenakis, M., 1997. Antimicrobial, cytotoxic and antiviral activities of *Salvia fruticosa* essential oil. Journal of Agricultural and Food Chemistry 45, 3197–3201.

Sridhar, S.R., Rajagopal, R.V., Rajavel, R., Masiilamani, S., Narasimhan, S., 2003. Antifungal activity of some essential oils. Journal of Agricultural and Food Chemistry 512, 7596–7599.

Wang, S-Y., Chen, P-F., Chang, S-T., 2005. Antifungal activities of essential oils and their constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) leaves against wood decay fungi. Bioresource Technology 96, 813–818.