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Moldicidal properties of seven essential oils

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ABSTRACT

When wood and wood products are exposed to moisture during storage, construction or while in-service, mold growth can occur in 24 to 48 hours. Mold growth could be suppressed or prevented if wood was treated with an effective mold inhibitor. The objective of this study was to evaluate the mold inhibiting properties of natural plant extracts such as essential oils. Seven essential oils were evaluated for their ability to inhibit growth of *Aspergillus niger*, *Trichoderma viride*, and *Penicillium chysogenum* on Southern yellow pine (SYP) specimens that were either dip-treated or exposed to volatiles of the test oils. Dip treatment with thyme or geranium (Egyptian) oil inhibited growth of test fungi for 20 weeks. Vapors from dill weed oil also inhibited all test fungi for at least 20 weeks when the vapor source remained in the test apparatus. Essential oils may be useful as moldicidal surface-treatments or fumigants for wood and wood products.

Key words: Mold fungi, essential oil, plant extract, moldicide

[3]The use of trade or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

1. INTRODUCTION

Moisture management remains the most important factor for controlling mold growth on wood and wood products during distribution, storage and construction, and while in service. Treatment of wood with mold inhibitors either by surface or vapor treatment would afford additional protection during inadvertent moisture exposure. Broader applications for such treatments could be realized if they were appropriate for interior applications, and if they would remain efficacious for some time after being placed in service. Chemical fungicides that are commonly used to control the growth of mold on wood may not be appropriate for interior applications. Plant extracts that demonstrate low toxicity to humans could provide protection for interior applications as well as short-term protection during storage and construction. Essential oils are known for their naturally occurring anti-microbial properties conferred by monoterpenes, diterpenes and hydrocarbons with various functional groups.

For many centuries, essential oils have been used for medicinal purposes (Inouye et al. 1998). In the middle of the twentieth century, it was reported that several essential oils demonstrated *in vitro* anti-fungal activity and vaporous antibacterial activities (Maruzzella and Ligrouri 1958; Maruzzella and Sicurella 1960). In the 1990's, Muanza *et al.* searched for potential bioactive plant extracts against bacteria and fungi (Muanza *et al.* 1994; 1995). Many other researchers have since reported on anti-microbial (Cowan 1999; Hammer *et al.* 1999; Hoffman *et al.* 2004; Mau 2001; Sivropoulou *et* al. 1995; 1997) or, more specifically, anti-fungal activities (Adam *et* al. 1998; Deferera *et* al. 2000; Moretti *et* al. 1998; Muller-Riebau *et* al. 1995; Sridhar *et* al. 2003) of essential oils which are widely used today in food applications and pharmaceuticals. Published research on fungal inhibition by essential oils on cellulose-based products has been limited to packaging and preservation of periodicals (Rakotonirainy and Lavedrine 2005; Scheffer and Duncan 1946) or inhibition of wood decay fungi (Wang *et al.* 2005).

The objective of this study was to evaluate the ability of seven essential oils to inhibit mold growth on southern yellow pine by dip treatment or vapor exposure.

2. Experimental Methods

2.1 Essential oils

Seven essential oils, ajowan, dill weed, geranium (Egyptian), lemongrass, rosemary, tea tree and thyme, were obtained from New Directions Aromatics Inc. (San Francisco, California), and were derived from steam distillation. All oils were tested neat. Major 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, *Penicillium chrysogenum* PH02, and *Trichoderma viride* ATCC 20476 were grown on 2% malt agar for 2 weeks. Spore suspensions 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 (ASTM 1998). Spores of individual mold strains were diluted to 100 mL with DI water to yield 3 x 10^7 spores/ml. The spray bottle was adjusted to deliver 1 mL inoculum per spray.

2.3 Test specimens

SYP specimens (7x20 mm cross section by 7 cm long) were cut from southern pine mill ends obtained from a Mississippi sawmill and stored at 0°C.

2.4 Dip treatment

Five random replicate specimens were dip-treated for 15 seconds in individual essential oils. Vegetable oil served as a control. Specimens were held in a Petri dish test apparatus overnight according to ASTM test methods D4445-91 (ASTM 1998) prior to inoculation with the test fungi. Three random replicates were weighed before and after dipping to estimate essential oil retention levels.

2.5 Vapor treatment

2.51 Constant vapor source

Five untreated specimens were held at room temperature overnight in a glass Petri dish test apparatus with a small glass dish (4 cm diameter) containing 3 ml of an individual test oil placed beside the specimens. Spore suspensions were sprayed onto specimens 24 hr post-vapor exposure. The dish of essential oil remained in the test apparatus throughout the course of the 20-wk incubation. Vegetable oil served as the control.

2.5.2 Twentyfour hour vapor exposure

Ten specimens were exposed to dill weed oil vapors for 24 hours as described above. The dish containing the oil was weighed before and after the exposure period. Specimens were inoculated with spores and incubated to determine if residual vapor was inhibitory.

2.5.3 Minimum inhibitory vapor concentration

Three specimens challenged with a mixture of fungal spores were continuously exposed to filter paper disks saturated with 0.1, 1.0, 10, 100, or 1000 µl of dill weed oil in 452 cm^3 jars. Vegetable oil served as a control. Specimens were rated for mold growth following 4-wk incubation.

2.6 Test apparatus

Each Petri dish test apparatus (150x25 mm) (B-D Falcon, Los Angeles, Calif.) or lidded glass jar (8 cm x 9 cm) 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 hr post-treatment. Petri dishes were sealed in a polyethylene bag and lids were tightened on the glass jars to prevent drying. All tests apparatuses were incubated at 27°C, 70% relative humidity (RH). Specimens were evaluated for mold growth at 4 weeks and some tests were further evaluated at 6, 10, 12, 16, and 20 weeks. At each evaluation, individual specimens were rated on a scale of 0 to 5 with 0 indicating no growth and 5 indicating heavy mold growth. Rating ceased when test oils failed to substantially inhibit growth of test fungi.

Table. 1 Essential oils evaluated.

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

3. Results and discussion

Essential oils evaluated in this study were selected for their previously reported anti-microbial properties in pharmaceutical, food and packaging applications. Anti-fungal efficacy of essential oils on wood against spore suspension of three common air-born mold fungi was assessed by two different methods, dip treatment (Table 2) and vapor exposure (Table 3), and the results are presented as the average ratings of five specimens. Specimens were initially rated according to the ASTM standard after 4 wk incubation. Ratings continued periodically through 20 wks incubation or until test oils failed to substantially inhibit test fungi.

3.1 Dip treatment

Results of the dip treatment showed that geranium (Egyptian) and thyme oils completely inhibited all test fungi for at least 20 weeks, while control specimens showed 100% mold coverage at the initial 4 wk rating. Dill weed oil protected against *P. chrysogenum* and *A. niger,* but not *T.* viride, for up to 10 weeks. Ajowan, lemongrass, rosemary and tea tree showed approximately 80% surface coverage with mold growth at 6 wk and 100% coverage by 10 wk. Retention levels of concentrated oils, as well as controls, averaged approximately 0.04 g/cm^3.

Table 2. Inhibition of mold growth on dip-treated southern yellow pine[a].

Essential Oil	Time [wk]	T.viride	P. chrysogenum	A.niger
Ajowan	4	4.8	4.8	5
	6	5	4.2	5
	10	5	5	5
Dill Weed	4	0.4	0	0
	6	2.4	0	0
	10	2.4	0	0
	12	2.8	1.6	1.6
	16	3	1.2	1.2
	20	3.6	4.6	4.6
Geranium	4	0	0	0.2
	6	0	0	0
	10	0	0	0
	12	0	0	0
	16	0	0	0
	20	0	0	0
Lemongrass	4	2.2	4.8	5
	6	4.6	4.4	4.8
	10	4.8	4.8	4.8
Rosemary	4	2.2	4.8	4.8
	6	5	5	5
Tea Tree	4	2	0.4	0.6
	6	4.8	3.4	3.4
	10	5	5	5
Thyme	4	0	0	0
	6	0	0	0
	10	0	0	0
	12	0	0	0
	16	0	0	0
	20	0	0	0

[a]Value is the average rating of five specimens per treatment per test fungus. Rating system: 0=no growth; 1=20%; 2=40%; 3= 60%; 4=80%; and 5=100% coverage with test fungus.

Three active components of thyme oil, namely geraniol, thymol and carvone, are known to provide significant inhibition of mold growth and can serve as a broad-spectrum biocide against commonly occurring molds (Scheffer and Duncan 1946). Sporulation of several filamentous fungi, including *Aspergillus fumigatus* and *Penicillium expansum*, was reported to be suppressed on agar plates by gaseous contact with thyme oil, but not when thyme oil was applied as a solution (Inouye et al. 1998). Their report also suggested that the anti-sporulating effect seemed to correlate with inhibition of respiration rather than growth inhibition. Our results on wood

showed that while thyme oil surface treatment effectively inhibited the test fungi, vapor treatment did not inhibit growth of the test fungi on a wood substrate. This result in attributed to the active components of thyme oil on the wood substrate. Anti-fungal compounds frequently show varying levels of effectiveness on wood and agar against the same test fungi. Because anti-fungal compounds are usually more efficacious on agar than on wood, biocides intended for wood protection should be evaluated on wood.

3.2 Vapor treatment

3.2.1 ***Constant vapor exposure***

Test fungi showed a completely different response to volatiles of the test oils relative to the dip treatment (Table 3). The most effective mold inhibitor was dill weed 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. These findings suggest that ketone volatilization likely plays a key role in preventing spore germination for dill weed and rosemary oils. Lemongrass vapor retarded ***Penicillium*** growth for 12 weeks, but was ineffective against the other two test fungi. Ajowan and tea tree vapors did not inhibit growth of test fungi. Ajowan was not an effective mold inhibitor under the conditions of this study, which contradicts the observations of Sridhar *et al* (2003). Contrary to dip treatment results, geranium (Egyptian) and thyme oil vapors did not inhibit mold fungi in this study suggesting that the monoterpene components of these two oils act by inhibiting spore germination or vegetative growth upon contact.

3.2.2 ***Twenty-four hour vapor exposure***

Exposing specimens to dill weed vapor for only 24 hours prior to inoculation did not provide protection from mold spore germination. The average rating for the ten specimens was 1.8 after 2 weeks and 3.1 after 3 weeks incubation. Based on the passive evaporation rate of dill weed oil in the 295 cm^3 test apparatus, specimens were exposed to an average 0.60 mg/cm^3 of dill weed vapor during the 24-hr exposure period.

Table 3. Inhibition of mold growth southern yellow pine exposed to constant vapor source[a].

Essential Oil	Time [wk]	T.viride	P. chrysogenum	A.niger
Ajowan	4	3.6	2.6	0.8
	6	4.6	4.2	3.8
	10	4.8	5	4.8
Dill Weed	4	0	0	0.2
	6	0	0	0
	10	0	0	0.2
	12	0	0	0.2
	16	0	0	0.2
	20	0	0	0.2
Geranium	4	1	0	1.4
	6	1.6	0	2.4
	10	1.6	0.6	3.6
	12	2.8	3.8	4
	16	2.8	5	5
	20	3.4	5	5
Lemongrass	4	1	0	1
	6	1.4	0	1.6
	10	0	0	3.4
	12	3	0	4.6
	16	4.8	0.8	5
	20	5	2.6	5
Rosemary	4	0	0.2	0
	6	0	0	0
	10	0	0	0
	12	0	0	3.2
	16	2.6	1.8	3.6
	20	3.6	3.4	4.8
Tea Tree	4	4	1	1.8
	6	3.4	1	1.8
	10	4.4	1	4.2
	12	4	2.2	4
Thyme	4	0.6	0.4	2.4
	6	0.2	1.8	2.8
	10	0.2	1.8	3
	12	0.6	2.6	3.2
	16	2	1.4	3.2
	20	3	3.4	3.2

[a]Value is the average rating of five specimens per treatment per test fungus. Rating system: 0=no growth; 1=20%; 2=40%; 3= 60%; 4=80%; and 5=100% mold coverage.

3.2.3 Minimal inhibitory concentration

The minimal inhibitory concentration has not been determined. Even the lowest volume of dill weed oil tested, 0.1 µl, inhibited 100% mold spores when the oil was

placed on a filter paper disc and allowed to remain in the test jar for the duration of the test. Less than 0.22 g dill oil vapors /m^3 air (extrapolated from the volume of the test apparatus) provided continuous protection to test specimens for at least 4 weeks, while the controls were nearly 100% covered with mold (average rating 4.7).

The application of fungistatic vapor to control mold growth was explored as early as 1946 by Scheffer and Duncan. The tendency for high volatilization combined with mold inhibitory properties may broaden the range of useful applications for certain 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.

4. Conclusions

Three of seven essential oils tested, thyme, geranium (Egyptian) and dill weed, are efficacious against *T. viride, P. chrysogenum* and *A. niger.* Southern yellow pine dip treated with thyme or geranium (Egyptian) oils provided complete protection against the test fungi for at least 20 wk, while dill weed vapor prevented growth of the test fungi for at least 20 wk. Naturally occurring anti-fungal compounds may be useful for inhibition of mold fungi on wood and wood products in service, particularly if lower concentrations are effective as dip treatments. On the other hand, anti-fungal vapors may be better suited as fumigants for protection of stored building materials, such as framing lumber, millwork or truss systems.

5. 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, 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 Microbiological 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 *Penicillium digitatum. Journal of Agricultural and Food Chemistry,* **48,** 2576-2581.

Edwards, V. 1999. *The aromatherapy companion,* Storey Books, Pownal, Vermont pp. 55-62.

Hammer, K.A., Carson, C.F., Riley, T.V. 1999. Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology,* **86,** 985-990.

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.

Inouye, S., Watanabe, M., Nishiyama, Y., Takeo, K., Akao, M, Yamaguchi, H. 1998. Antisporulating and respiration-inhibitory effects of essential oils on filamentous fungi. *Mycoses* **41,** 403-410.

Maruzzella, J.C., Ligouri, L. 1958. The *in vitro* antifungal activity of essential oils. *Journal of the American Pharmacological Association (Science Edition),* **47,** 250-254.

Maruzzella, J.C., Sicurella, N.A. 1960. Antibacterial activity of essential oil vapors. *Journal of the American Pharmacological Association (Science Edition),* **49,** 692-694.

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 Pharmacogy,* **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 Pharmacogy,* **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.

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.* 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,** 6 19-62 1.

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,** 3 197-320 1.

Sridhar, S.R., Rajagopal, RV., 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.

Figure legends

Fig. 1. Vapor exposure method: Glass Petri dish containing 5 specimens and glass dish containing essential oil after 4 wk incubation. Untreated control samples show mold growth (right) at week 3 and no growth in the presence of dill oil vapor (left).