

## **Inhibitory Effect of Essential Oils on Decay Fungi and Mold Growth on Wood**

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### **ABSTRACT**

Structural damage and potential health risks caused by wood decay and mold fungi in residential structures have been a major concern for homeowners, building contractors and insurance companies alike. The combined damage from decay fungi and mold claims exceeds several billion US dollars annually. Protection against decay and mold growth on wood is a critical economic concern for the building industry. An ideal compound for wood protection for interior applications must be nontoxic, hypoallergenic and able to provide long-term efficacy under high humidity. The objective of this study was to investigate the inhibitory effects of natural plant extracts, such as essential oils, on wood. Seven essential oils were evaluated for their ability to inhibit weight loss by brown-rot and white-rot fungi on southern pine in soil block tests. No weight loss occurred in specimens dip-treated with undiluted test oils. Essential oils were also evaluated for inhibition of mold growth in laboratory tests by two methods. Specimens dip-treated with thyme or geranium Egyptian oil inhibited growth of test mold fungi up to 22 weeks and specimens exposed to vapors of dill weed or rosemary oil also inhibited mold growth under laboratory conditions. We did not observe correlation between surface and vapor treatment for mold inhibition. These findings support the potential use of essential oils for natural wood protection against decay fungi or mold infestation for surface-treatment or fumigation of wood products.

### **INTRODUCTION**

Since the use of heavy metal-based wood preservatives such as chromated copper arsenate (CCA) was voluntarily withdrawn from housing markets in the U.S., research has been focused on developing new user friendly wood preservatives to protect wood against fungi, mold and insects (Kartal et al., 2004b). Potential health risks caused by mold growth in houses and building structures are a major concern for homeowners, builders, contractors and insurance companies alike. Lawsuits claiming health problems caused by indoor mold exposure exceeded 2.8 billion dollars in 2002 (Hartwig and Wilkinson, 2003). Chemical fungicides that are commonly used to control the growth of mold and decay fungi on wood are not environmentally suitable for many indoor applications. Searching for natural alternatives that are user friendly and exhibit negligible toxicity to human are most desired. Natural plant extracts such as essential oils and their derivatives are well known for their antimicrobial properties that are utilized in pharmaceutical, healthcare, food, and packaging applications (Adam et al., 1998; Cowan., 1999; Deferera et al., 2000; Hammer et al., 1999; Hoffman et al., 2004; Mau et al., 2001; Moretti et al., 1998; Muanza et al., 1994, 1995; Muller-Riebau et al., 1995; Rakotonirainy and Lavedrine, 2005; Scheffer and Duncan, 1946; Sivropoulou et al., 1995, 1997; Sridhar et al., 2003; Wang et al., 2005; Yang and Clausen, 2007)

The objective of this study was to investigate the antifungal effect of commercially available essential oils on wood against mold and wood decaying fungi by surface treatment and standard soil block test. The positive inhibitory effect of essential oils tested provides a promising wood protection alternative for construction and during storage.

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## MATERIALS AND METHODS

### *Essential oils*

Seven essential oils, ajowan, dill seed, geranium Egyptian, lemongrass, rosemary, tea tree, thyme used in this study were obtained from New Directions Aromatics Inc. (San Francisco, Calif.). All oils were used at full strength for mold and decay fungi tests. Diluted oils (1:10) were also tested for fungal inhibition. Linseed oil was used as diluent. Major components and functional groups of essential oils are shown in Table 1 (Edwards, V., 1999; Schnaubelt, K., 1998).

**Table 1.** List of essential oils tested

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

### *Test organisms*

Decay fungi, *Gloeophyllum trabeum* (Pers.:Fries) Murrill (MAD 617), *Postia Placenta* (Fr.) M. Lars. & Lomb. (MAD 698) and *Trametes versicolor* (L.Fr) Pilat (MAD 697), were maintained on 2% malt agar (Difco, Detroit, MI, USA), at 27°C and 70% relative humidity (RH).

Three mold fungi, *Aspergillus niger* 2.242 provided by University of Virginia, *Penicillium chrysogenum* PH02 from Forest Products Laboratory, Madison, WI and *Trichoderma viride* ATCC 20476 were grown on 2% malt agar for 2 weeks. *Aureobasidium pullulans* MDX-18 was grown on 2% potato dextrose agar for 2 weeks explicitly for inoculation of the soil in the tank test chamber. Spore suspensions were prepared according to ASTM Standard D4445-91 (ASTM 1998). The spray bottle was adjusted to deliver 1 mL inoculum of  $3 \times 10^7$  spores/ml per spray.

### *Test Specimens and Methods*

#### **Decay fungi test**

Soil block culture bottles were prepared according to American Wood Preservers' Association (AWPA 2006) E-10-06 with a modification of wood block size to 1 x 1 x 1 cm. In soil block bottles, southern yellow pine feeders were inoculated with brown rot fungi, *G. trabeum* and *P. placenta*, and maple feeders were inoculated with the white-rot fungus, *T. versicolor*. Bottles were incubated at 27°C and 70% RH for 3 weeks until the fungi completely colonized each feeder. Preweighed southern yellow pine (SYP) blocks, conditioned at 27°C and 70% RH, were dip-treated for 15 seconds with each essential oil at 100% and 10%. Blocks were air dried overnight at room temperature and placed on feeders to incubate at 27°C, 70% RH for 12 weeks. Following incubation, surface mycelial growth was rated visually by percentage of surface

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coverage before mycelia were brushed off each block. Blocks were oven-dried at 60°C for 24 hr and reconditioned at 27°C, 70% RH for 2 weeks. Block weights were measured and average percent weight loss was calculated. Linseed oil served as control and diluent. Five replicas were used in each test.

### **Mold test specimens**

Southern yellow pine specimens (7x20 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 following tests:

#### ***Dip stake treatment***

Five random replicate specimens were dip-treated for 15 seconds in individual full strength (100%) essential oils. Vegetable oil served as a control. Specimens were held overnight at room temperature in a Petri dish test chamber (150x25 mm) (B-D Falcon, Los Angeles, Calif.) containing four layers of blotting paper that was saturated with 30 mL DI water and overlaid with a polyethylene mesh spacer to elevate specimens (ASTM D4445-91). Specimens were inoculated with 1 mL of mixed or individual mold spores 24 hours post-treatment. Petri dish test chambers were sealed in polyethylene bags to prevent drying, and incubated at 27°C, 70% RH. Specimens were evaluated for mold growth at 4, 6, 10, 12, 16, 20 and 22 weeks and rated on a scale of 0 to 5 with 0 indicating no growth and 5 indicating heavy mold growth. Specimen rating ceased when test oils failed to substantially inhibit mold growth.

Additionally, thyme and tea tree oils diluted 1:2, 1:4 and 1:8 were tested individually and in combination for 22 weeks for mold resistance.

#### ***Vapor exposure treatment***

In the Petri dish test chamber as described above, a small glass dish (4 cm diameter) containing individual test oil (3 ml) was placed next to the specimens 24 hr prior to inoculating with spores of mold fungi. The test oil remained in the Petri dish chamber for the entire test period (20 weeks). Vegetable oil served as a control. Specimens were sprayed with 1 mL of mixed or individual mold spore inoculum 24 hr post-treatment. Petri dish test chambers were incubated and specimens were rated as described above.

#### ***Leachability test***

Random replicate wood specimens were dip-treated with thyme oil and leached according to AWPA E1 1-06 (2006) standard method for determining the leachability of wood preservatives. Leaching occurred for a total of 14 days with water changes after 6, 24, 48 hours and thereafter at 48-hour intervals. Following leaching, specimens were placed in a Petri dish as described above and inoculated with mixed mold spores. Mold growth was evaluated after 3 and 6 months incubation.

## RESULTS

Essential oils were selected from various referenced literature for their antifungal properties. In the soil block test, mycelial growth on the wood surface was first evaluated visually. All specimens treated with full strength essential oils showed no growth except ajowan which showed approximately 20% growth on *P. placenta* blocks. On specimens treated with 1:10 dilutions of oils, 10-60% coverage by mycelial growth was observed on test blocks for all oils tested. Untreated control specimens showed extensive growth for the brown-rot fungi, but low weight losses occurred in *T. versicolor* control specimens.

Table 2 and Figure 1 show that no weight loss occurred in specimens treated with full strength essential oils. Ten percent dilutions of oils generally inhibited *G. trabeum* and *T. versicolor* (0-3% weight loss). *P. placenta* was able to cause weight loss in blocks treated with 5 and 7 dilute test oils. Only blocks treated with dilute thyme oil, showed no weight loss for all three decay fungi.

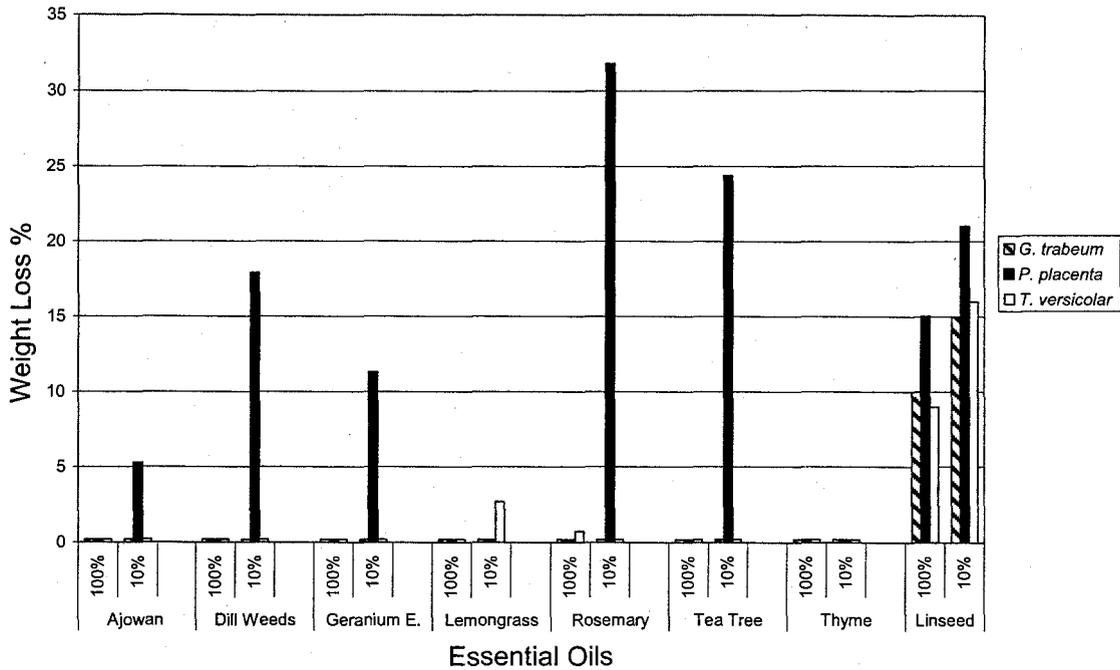
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Table 2. Essential oils inhibit fungal growth by soil bottle weight loss study.					
Essential oil	Concentrations	Fungal strain	Pre wts/s.d.	Post wts/s.d.	Wt loss %
Ajowan	100%	<i>G. trabeum</i>	0.555/0.082	0.584/0.015	0
		<i>P.placenta</i>	0.491/0.025	0.594/0.052	0
		<i>T.versicolor</i>	0.503/0.025	0.609/0.052	0
	10%	<i>G. trabeum</i>	0.524/0.04	0.616/0.03	0
		<i>P.placenta</i>	0.529/0.031	0.501/0.038	5.3
		<i>T.versicolor</i>	0.527/0.012	0.617/0.024	0
Dill Weed	100%	<i>G. trabeum</i>	0.51/0.056	0.568/0.046	0
		<i>P.placenta</i>	0.533/0.041	0.586/0.069	0
		<i>T.versicolor</i>	0.53/0.085	0.601/0.051	0
	10%	<i>G. trabeum</i>	0.508/0.039	0.599/0.045	0
		<i>P.placenta</i>	0.559/0.038	0.454/0.048	17.9
		<i>T.versicolor</i>	0.553/0.065	0.631/0.055	0
Geranium Egyptian	100%	<i>G. trabeum</i>	0.535/0.048	0.659/0.053	0
		<i>P.placenta</i>	0.502/0.032	0.684/0.096	0
		<i>T.versicolor</i>	0.56/0.074	0.691/0.073	0
	10%	<i>G. trabeum</i>	0.537/0.077	0.626/0.079	0
		<i>P.placenta</i>	0.53/0.036	0.47/0.064	11.3
		<i>T.versicolor</i>	0.538/0.043	0.602/0.045	0
Lemongrass	100%	<i>G. trabeum</i>	0.548/0.055	0.577/0.029	0
		<i>P.placenta</i>	0.521/0.046	0.578/0.022	0
		<i>T.versicolor</i>	0.513/0.023	0.566/0.038	0
	10%	<i>G. trabeum</i>	0.516/0.043	0.579/0.054	0
		<i>P.placenta</i>	0.516/0.034	0.579/0.038	0
		<i>T.versicolor</i>	0.59/0.067	0.574/0.023	2.7
Rosemary	100%	<i>G. trabeum</i>	0.532/0.059	0.542/0.061	0
		<i>P.placenta</i>	0.531/0.017	0.541/0.017	0
		<i>T.versicolor</i>	0.531/0.028	0.527/0.03	0.74
	10%	<i>G. trabeum</i>	0.526/0.044	0.549/0.076	0
		<i>P.placenta</i>	0.528/0.061	0.36/0.076	31.8
		<i>T.versicolor</i>	0.498/0.045	0.604/0.046	0
Tea Tree	100%	<i>G. trabeum</i>	0.592/0.055	0.596/0.052	0
		<i>P.placenta</i>	0.508/0.026	0.57/0.033	0
		<i>T.versicolor</i>	0.529/0.014	0.542/0.016	0
	10%	<i>G. trabeum</i>	0.537/0.048	0.592/0.037	0
		<i>P.placenta</i>	0.553/0.047	0.418/0.067	24.4
		<i>T.versicolor</i>	0.515/0.023	0.604/0.022	0
Thyme	100%	<i>G. trabeum</i>	0.532/0.032	0.561/0.036	0
		<i>P.placenta</i>	0.595/0.072	0.628/0.067	0
		<i>T.versicolor</i>	0.493/0.019	0.529/0.024	0
	10%	<i>G. trabeum</i>	0.528/0.034	0.568/0.025	0
		<i>P.placenta</i>	0.549/0.042	0.596/0.065	0
		<i>T.versicolor</i>	0.521/0.049	0.599/0.059	0
Linseed (Control)	100%	<i>G. trabeum</i>	0.524/0.022	0.614/0.025	0
		<i>P.placenta</i>	0.499/0.056	0.424/0.041	15.03
		<i>T.versicolor</i>	0.558/0.042	0.635/0.029	0
Untreated (Control)		<i>G. trabeum</i>	0.513/0.058	0.35/0.017	31.77
		<i>P.placenta</i>	0.547/0.056	0.354/0.026	35.28
		<i>T.versicolor</i>	0.534/0.034	0.49/0.035	9.34

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**Figure 1.** Inhibitory effect of essential oils on wood decay fungi using soil block culture test method.

**Fig. 1. Inhibitory effect of essential oils on fungal growth**



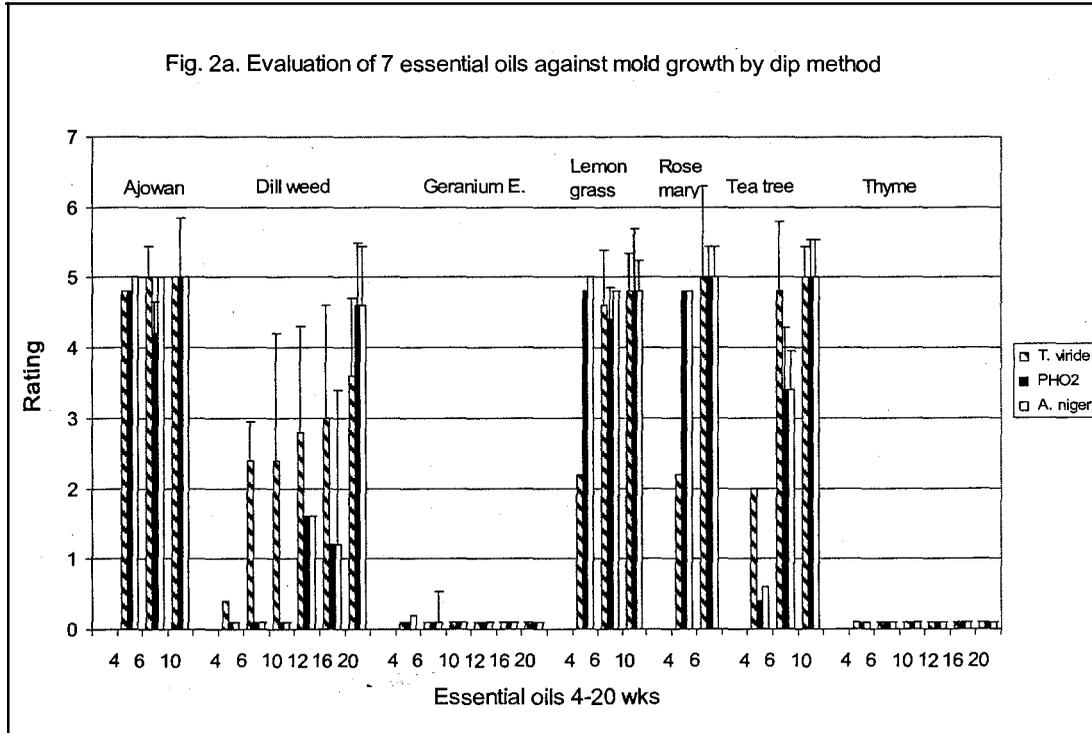
Inhibition of decay fungi by the soil block culture method demonstrated that essential oils inhibited test fungi. In order to evaluate essential oils' long term efficacy, an experiment to study the shelf life of the treatment is in progress. Wood treated with essential oils will be stored for 6 and 12 months before challenge with test fungi.

Antifungal effects of essential oils on wood against three common mold fungi were assessed by two different methods, dip stake and vapor exposure, and the results are presented as the average ratings of five specimens in Figure 2a and b. Specimens were initially rated after 4 wk incubation. Ratings continued periodically through 20 wks incubation 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. The inhibitory effect on the surface of wood specimens was low for these four essential oils using the dip stake method. Dill weed oil protected against *P. chrysogenum* PH02 and *A. niger*, but not *T. viride*, for up to 10 weeks. Geranium Egyptian and thyme oils completely inhibited all test fungi for at least 20 weeks (rated zero, but presented as 0.1 in Figure 2). 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, while tea tree oil (1:2) showed no mold growth 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 mold inhibitory property was reduced by tea tree oil (Table 3).

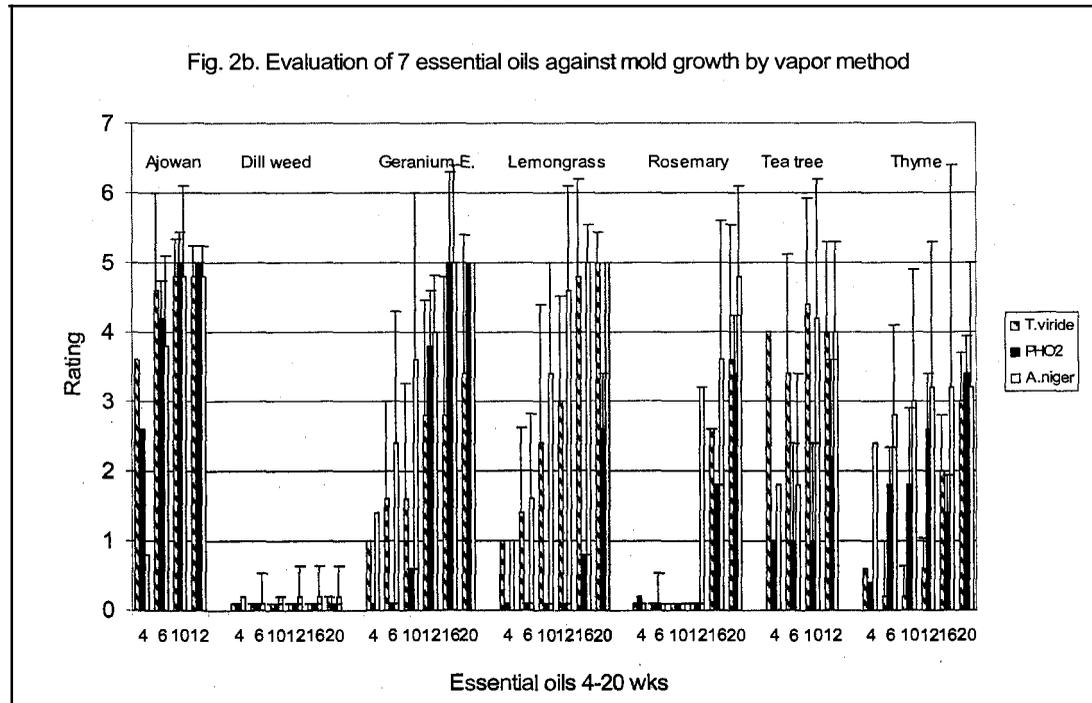
Leached thyme oil-treated specimens showed no mold growth at 3 and 6 months incubation (data not shown).

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**Figure 2a.** Mold resistance of southern yellow pine specimens dip-treated with seven individual essential oils and challenged with three mold fungi in a Petri dish test chamber.



**Figure 2b.** Mold resistance of southern yellow pine specimens exposed to vapors of seven individual essential oils and challenged with three mold fungi in a Petri dish test chamber.



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**Table 3.** Mold inhibition of diluted tea tree and thyme oils by dip stake method.

Essential Oil	Dilution	Weeks	Rating
Tea Tree	1:2	4	0
	1:2	6	0
	1:2	10	3.2
	1:2	12	4.8
	1:4	4	4.2
	1:4	6	5
	1:8	4	4
	1:8	6	5
Thyme	1:2	4	0
	1:2	6	0
	1:2	10	0
	1:2	12	0
	1:2	16	0
	1:2	22	0
	1:4	4	0
	1:4	6	0
	1:4	10	0
	1:4	12	0
	1:4	16	0
	1:4	22	0
	1:8	4	0
	1:8	6	4.6
Tea Tree + Thyme (1:1)	Undiluted	4	0
	Undiluted	6	0
	Undiluted	10	0
	Undiluted	12	0
	Undiluted	16	0
	Undiluted	22	0
	1:2	4	0.6
	1:2	6	1.8
	1:2	10	4.4
	1:4	4	1.6
1:4	6	4	

From this study, it is clear that thyme and geranium Egyptian oils can play an important role in wood protection from molds by dip treatment. Three active components of thyme oil, geraniol, thymol and carvone, provide significant inhibition of mold growth and can serve as broad-spectrum biocides against commonly occurring molds (Scheffer et al., 1946) and decay fungi. 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). The major component of dill oil is carvone and the vapor provides significant retaliation of mold spore attachment on the wood.

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### CONCLUSIONS

Essential oils are known for their natural components, such as monoterpenes, diterpenes and hydrocarbons with various functional groups. Their constituents and derivatives have long been studied for their applications as antimicrobial and antifungal properties in the area of food preservation and pharmaceutical industries. In the past ten years, more interest has focused on the potential applications of natural plant extracts as wood protection agents to prevent mold and fungal growth on in-service wood. Two of seven essential oils tested, thyme and geranium Egyptian, are efficacious against *T. viride*, *P. chrysogenum* and *A. niger* by dip treatment on SYP. At the time for this report, thyme oil diluted 1:4 provided protection from mold growth for at least 20 weeks. Longer efficacy is anticipated. Thyme oil also exhibited inhibition of three wood decay fungi. Dill weed oil vapor has demonstrated inhibition of mold growth. The two treatment methods, surface treatment and vapor exposure, demonstrated different inhibitory effects of essential oils.

These natural antimycotic compounds are useful to inhibit mold and decay fungi on wood in service or during storage of building materials, such as framing lumber, millwork, or truss systems. Application of essential oils, such as thyme oil and dill weed oil vapor, can protect wood from mold and fungal attack, thereby preserving lumbers' economic value and improving product durability.

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