Fumigant Toxicity of Essential Oils to *Reticulitermes flavipes*

Carol A. Clausen
Vina W. Yang
US Department of Agriculture, Forest Service
Forest Products Laboratory,
Madison Wisconsin

ABSTRACT

Subterranean termite infestations occur in every state in the contiguous United States and are responsible for damage to wooden structures in excess of two billion dollars (U.S.) annually. Essential oils have historically been used to repel insects. They have relatively low toxicity and some of them are exempt from regulation by the Federal Insecticide Fungicide and Rodenticide Act (FIFRA). Development of environmentally friendly wood protection systems may benefit from supplemental essential oils. Eight essential oils were evaluated for their ability to protect wood from attack by *Reticulitermes flavipes* (Kollar), either in the fumigant toxicity test or by surface treatment in laboratory tests. In a fumigant toxicity test, 5 μl of dill weed or rosemary oil and 10 μl lemongrass oil per 553 cm^3 air caused 100% mortality after 24 h in a closed container. The estimated LD_{50} for dill weed was 2.93 $\mu l/m^3$. In the surface treatment test, essential oils tested in concentrated form caused 100% mortality after 24 h. Geranium (Egyptian), lemongrass, and tea tree oils caused 95% to 100% mortality after 24 h when diluted 1:50. In light of fumigant toxicity findings, mortality in surface treatment tests can be attributed to the volatility of essential oils. These results suggest that dill weed, lemongrass, tea tree, rosemary, or geranium (Egyptian) essential oils could be used alone or as a co-termiticide fumigant for wood product applications to protect structures from termite attack.

Key words: essential oil, Reticulitermes flavipes, termite, fumigant

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.

INTRODUCTION

Essential oils exhibit various and variable antimicrobial activities, including antifungal, antiviral, antibacterial, insecticidal, and antioxidant properties (Prabuseenivasan et al. 2006). In the field of wood protection there is a continuing need to develop new, environmentally friendly fungitoxic and insecticidal chemicals. This need has resulted in a sharp rise in recent research activity on the antifungal and insecticidal properties of essential oils from woody plants, such as juniper, cypress, Melaleuca, eucalyptus, or yellow cedar (Park and Shin 2005, Sim et al. 2006; Yi et al. 2006, Mater et al. 2006). Wood from the *Meliaceae* family is well known for its insect repellent and antiviral properties (Carpinella et al. 2003).

Essential oils are presently used most often in the food industry for flavoring, the cosmetic industry for fragrances, and the pharmaceutical industry for their functional properties. However, dozens of herbaceous plant essential oils have been screened for fumigant toxicity against a variety of insect pests primarily for agricultural and food storage. They have been used occasionally as additives to coatings to introduce an insect-repellent nature to the dried film (Overman 2006), but overall, little work has been directed toward the protection of wood. Park and Shin (2005) evaluated essential oils from herbaceous and woody plants as potential fumigants against Japanese termites and reported that clove bud and garlic oils showed the most potent antitermitic activity among the plant essential oils. Kartal and others reported on the termiticidal properties of decanal, cinnamic acid, and its derivatives on *Coptotermes formosanus* (Kartal et al. 2006).

Well known essential oils with bioactivity, either as an insecticide or repellent, are clove, thyme, mint, lemongrass, cinnamon, rosemary, and oregano oils (Isman and Machial 2006). Bioactivity can vary greatly because of variability in chemical composition of the oils, dependent largely on the plant part extracted, time of the year, climatic and soil variations, and phenological state of the plant. Despite these variabilities, certain plant species, namely thyme, oregano, basil, rosemary, and mint, are consistently bioactive (Isman and Machial 2006). Essential oils are complex mixtures comprised of a large number of constituents in variable ratios (van Zyl et al. 2006). Whereas there are no regulations in America, AFNOR (Association French Normalization Organization Regulation) and ISO (International Standards Organization) certification standardizes chemical profile and principal constituents that differentiate therapeutic grade from lower Grade A essential oils. Figure 1 shows the major components identified by gas chromatography (GC) analysis for the therapeutic grade essential oils in this study that were found to be bioactive against subterranean termites.

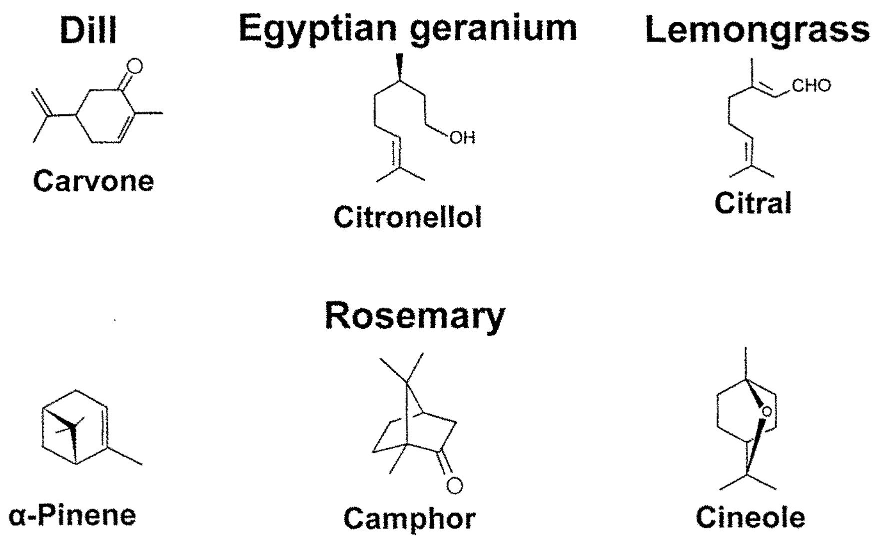

Fig. 1. Chemical structures of major components in essential oils exhibiting fumigant toxicity towards *Reticulitermes flavipes.*

Screening studies exist on a number of insect models, but there are relatively few reports of contact or fumigant toxicity of essential oils in termites. Vetiver (*Vetiveria zizanioides*) (Maistrello et al. 2003), patchouli (*Paogostemon cablin*) (Zhu et al. 2003), orange oil (Raina et al. 2007) and cinnamaldehyde (Chang and Cheng 2002) have previously been shown to exhibit varying degrees of effectiveness against *Coptotermes formosanus.*

The objective of this study was to evaluate essential oils for their contact and fumigant toxicities to *Reticulitermes flavipes* in laboratory termite tests.

MATERIALS AND METHODS

Essential Oils

Eight therapeutic grade essential oils were evaluated, including *Trachyspermum copticum* (ajowan), *Anethum graveolen* (dill weed), *Eucalyptus globulus* (eucalyptus), *Pelargonium graveolens* (geranium (Egyptian)), *Cymbopogon flexuosus* (lemongrass), *Rosmarinus officinalis* (rosemary), *Thymus zygis* (white thyme), and *Melaleuca alternifolia* (tea tree) (New Directions Aromatics, Inc., San Fransisco, CA, USA). Tea tree oil, well known for its insecticidal properties, served as a positive control. Vegetable oil served as a negative control. Additional controls included untreated southern pine and acetone, which was used as an oil diluent.

Termite tests

Termite tests were conducted according to AWPA E1-06 (2007) for effectiveness of test oils as a surface treatment. Southern pine sapwood specimens (25 x 25 x 5mm) were dip-treated (~15 s) in individual test oils (n = 5). Treated specimens were dried at 25°C over night in a chemical hood before being placed in the bottom of a cylindrical acrylic container (90 mm diameter x 60 mm height) with 50 g sand, 8.5 ml water, and one gram of *Reticulitermes flavipes* (Kollar) collected in Janesville, Wisconsin, USA. Containers were incubated at 27°C and 85% relative humidity (RH) and checked after 24-h for termite mortality.

Fumigant toxicity tests were conducted in glass containers (87 mm diameter x 93 mm height) with 50 g sand, 8.5 ml water, filter-paper food source, and 25 workers. Filter-paper disks (5 mm) impregnated with 1 to 50 µ1 of an individual essential oil (undiluted) were affixed to the lid of the container. Containers were incubated at 27°C and 85% RH and checked for 24-h mortality rate.

RESULTS

Table 1 summarizes select essential oils tested for 24-h fumigant toxicity to essential oil vapor exposure. Twenty-four hour termite mortality was 100% at 5 µl per 553 cm^3 (~9.04 ml/m^3) air for dill weed and rosemary oil based on the volume of the test container. The LD_{50} for dill weed was estimated at 1.62 µl per 553 cm3 (~2.93 $\mu l/m^3$) using the probit analysis model. Model fitting and estimation of 95% fiducial (inverse confidence) limits were calculated with SAS ® V9.1 proc PROBIT (SAS, 2004). However, LD_{50} could not be estimated with this model for geranium, rosemary or lemongrass oils because of the all or nothing dose response. The LD_{50} falls between 25 and 35 µl for geraniium oil, between 2.5 and 5 µl for rosemary oil, and between 5 and 10 µl for lemongrass oil per 553 cm^3 air.

Table 1. Twenty-four hour kill rate for *Reticulitermes flavipes* in a laboratory fumigant toxicity test.

Essential oil	Volume (µl)	24-h mortality (%)
Dill weed	0.05	0
	0.1	20
	2.5	76
	5	100
	10	100
Geranium	5	0
	10	0
	25	0
	50	100
Rosemary	2.5	0
	5	100
	10	100
	25	100
Lemongrass	1	0
	5	0
	10	100

Rapid onset of toxicity from essential oils in insects may indicate a neurological mode of action. Using the American cockroach as a model, Enan provided evidence that essential oil constituents poison insects by blocking octopamine receptors (2005a). Octopamine is a neuromodulator and neurotransmitter in arthropods. Enan's results on *Drispohila* and the American cockroach (Enan 2005a, 2005b) suggest that essential oils' eugenol, cinnamic alcohol, and trans-anethole bind to and activate G-protein-coupled neurological and olfactory receptors, which trigger a metabolic disruption that may kill the insect. Disruption of cell membranes or blockage of the tracheal system in insects may be alternative mechanisms of rapid-onset toxicity.

Table 2 summarizes the efficacy of southern pine that has been surface-treated with eight essential oils to *R. flavipes.* Oils were tested in concentrated form and diluted 1:50 with acetone. In concentrated form, all oils tested caused 100% termite mortality in 24 h. Response (i.e., mortality) was either all or nothing for the dilute oils. Only geranium (Egyptian), lemongrass, and tea tree oils::aused 95% to 100% mortality after 24 h at 2% v/v concentration. Vegetable oil (control) killed 25% of the termites after 24 h. Because of the closed nature of the test container and results from the fumigant toxiciiy tests, deaths from surface-treated wood are likely attributable to volatiles from the test oils, although termites were in physical contact with the oil-treated wood in this test. The 25% mortality observed in controls that were surface-treated with vegetable oil may be due to a physical respiratory blockage and suffocation. Advantages offered by surface treatment of wood with essential oil include water repellency, increased penetration resulting in higher retention, and direct protection from termites, which are desirable characteristics for in-service building materials.

Table 2. Twenty-four hour kill rate for *Reticulitermes flavipes* exposed to southern pine surface-treated with essential oil.

Treatment	Essential oil	Concentration	24-h mortality (%)	Dilution	24-h mortality (%)
Surface	Ajowan	Undiluted	100	1:50	0
	Dill weed	"	100	"	0
	Eucalyptus	"	100	"	0
	Geranium	"	100	"	95
	Lemongrass	"	100	"	100
	Rosemary	"	100	"	0
	Tea tree	"	100	"	100
	Thyme	"	100	"	0
Control	Vegetable oil	"	25	"	0
	Acetone diluent	"	0	"	0
Untreated		N/A	0		

Aromatic compounds of dill have been reported to retain 40 constituents that were identified as essential volatiles after more than 35 years storage (Jirovetz et al. 2003). Jirovetz and others (2003) found that the two primary aromatic impact compounds identified from dill, D-carvone (50.1 %) and D-limonene (44.1 %), retained their bioactivity against the mold fungus, *Aspergillus niger*. Previous findings of Yang and Clausen (2006, 2007, 2008) described two distinct categories of mold-inhibiting properties of essential oils on wood contact (surface treatment) and vapor phase (fumigant). In laboratory tests on wood, geranium and thyme oils demonstrated mold-inhibitory properties as a surface treatment for wood, whereas dill weed and rosemary oils demonstrated mold inhibition in the vaporous phase. Utilizing essential oils that exhibit fungicidal and insecticidal properties as a component in wood-protection systems could have broad applications especially if the volatile component(s) retain long-term bioactivity. Klaric et al. (2006) characterized essential oil of thyme and pure thymol, the primary constituent of thyme oil, for the minimum inhibitory concentration needed to inhibit mold isolated from damp dwellings of Slovakia. They concluded that the vaporous phase of thyme oil exhibited long-lasting suppressive activity on molds (60-d exposure), suggesting that thymol or thyme essential oil could be used to disinfect mold on walls at low concentration. Pure thymol exhibited approximately three times stronger inhibition of molds than thyme oil. If major components of essential oils evaluated in this study are found to be termiticidal, then purified components might be suitable substitutes for the essential oils. Purified components may be more economical and readily available than therapeutic essential oils.

Essential oils may also act synergistically with existing preservatives, thereby enabling low preservative concentrations to be equally effective and more environmentally friendly. Newly developed wood protection systems that inhibit decay and mold fungi as well as exhibit insecticidal properties would have broader application to the field of forest products. Potential applications include additives to coatings and sealants, fumigants for warehouse storage, additives to engineered composites, or surface treatment for framing lumber during construction.

ACKNOWLEDGEMENTS

The authors thank Rachel Arango for her assistance with the termite tests.

CONCLUSIONS

Dill weed and rosemary oil caused 100 % mortality in 24 h against *Reticulitermes flavipes* at a rate of ~9mL per m^3 air. The 100 % effective dose of lemongrass for termite-fllmigant toxicity was twice that of dill weed and rosemary oils. Surface dip-treatments with diluted lemongrass, geranium (Egyptian), and tea tree oils were equally effective, but surface treatment with essential oil would not be as cost effective as fumigant treatment. The high price of therapeutic grade plant essential oils (~$40/kg) is offset by the low effective dose of dill weed, rosemary, and lemongrass oils as fumigants.

REFERENCES

1. American Wood Preservers' Association Standards. 2007. Standard method for laboratory evaluation to determine resistance to subterranean termites. E1-06. *In:* Annual Book of AWPA Standards, Birmingham, AL. pp. 300-303.
2. Carpinella, M.C., L.M. Giorda, C.G. Ferrayoli, and S.M. Palacios. 2003. Antifungal effects of different organic extracts from *Melia azedarach* L. on phytopathogenic fungi and their isolated active components. Journal of Agricultural and Food Chemistry **51**: 2506-2511.
3. Chang, S., and S. Cheng. 2002. Antitermitic activity of leaf essential oils and components from *Cinnamomum osmophleum.* Journal of Agricultural and Food Chemistry **50**(6): 1389-1392.
4. Enan, E.E. 2005a. Molecular and pharmacological analysis of an octopamine receptor from American cockroach and fruit fly in response to plant essential oils. Archives of Insect Biochemistry and Physiology **59**(3): 161-171.
5. Enan, E.E. 2005b. Molecular response of *Drisophila melanogaster* tyramine receptor cascade to plant essential oils. Insect Biochemistry and Molecular Biology **35**(4): 309-321.
6. Isman, M.B., and C.M. Machial. 2006. Pesticides based on plant essential oils: from traditional practice to commercialization. Chapter 3, In: Advances in phytomedicine 3: naturally occurring bioactive compounds. M. Rai and M.C. Carpinella, Eds. Elsevier, New York, NY.
7. Jirovetz, L., G. Bubhbauer, A.S. Stoyanova, E.V. Geogiev, and S.T. Danianova. 2003. Composition, quality control, and antimicrobial activity of the essential oil oflong-time stored dill (*Anethum graveolens* L.) seeds from Bulgaria. Journal of Agricultural and Food Chemistry **51**: 3854-3857.

8. Kartal, S.N., W.J. Hwang, Y. Imamura, and Y. Sekine, Y. 2006. Effect of essential oil compounds and plant extracts on decay and termite resistance of wood. Holz Roh Werbt. **64**(6): 455-461.
9. Klaric, M.S., I. Kosalec, K.J. Mastelic, E. Pieckova, and S. Pepeljnak. 2006. Antifungal activity of thyme (*Thymus vulgaris* L.) essential oil and thymol against moulds from damp dwellings. Letters in Applied Microbiology **44**: 36-42.
IO.Maistrello, L.G., G. Henderson, and R.A. Laine. 2003. Comparative effects of vetiver, nootkatone, and disodium octaborate tetrahydrate on *Coptotermes formosanus* and its symbiotic fauna. Pest Management Science **59**(1): 58-68.
I I. Mater, D.K., J.J. Karchesy, and RG. Kelsey. 2006. The sporicidal activity of yellow-cedar heartwood, essential oil and wood constituents towards *Phytophthora ramorum* in culture. Forest Pathology **36**(5): 297-308.
12. Overman, G.R. 2006. A method for admixing plant essential oils to coatings (paints, stains, etc.) for the purpose of repelling insects during coating application and introducing an insect repellent nature to the dried film. U.S. Patent 2006/0029630A1.
. 13. Park, I.K., and S.C. Shin. 2005. Fumigant activity of plant essential oils and components from garlic (*Allium sativum*) and clove bud *(Eugenia caryophyllata)* oils against the Japanese termite (*Reticulitermes speratus* Kolbe). Journal of Agricultural and Food Chemistry **53**(11): 4388-4392.
14. Prabuseenivasan, S., M. Jayakumar, and S. Ignacimuthu. 2006. *In vitro* antibacterial activity of some plant essential oils. BMC Complementary and Alternative Medicine **6**(1): 39.
I5. Raina, A., J. Bland, M. Doolittle, A. Lax, R Boopathy, and M. Folkins. 2007. Effect of orange oil extract on Formosan subterranean termite (Isoptera: Rhinotermitidae). Journal of Economic Entomology **100**(3): 880-885.
16. SAS Institute, Inc. 2004. SAS/STAT® 9.1 User's Guide. SAS Publishing. SAS Institute Inc. Cary, North Carolina. 5136 pp.
17. Sim, M.l, D.R. Choi, and YJ. Abu. 2006. Vapor phase toxicity of plant essential oils to *Cadra cautella* (Lepidopera: Pyralidae). Journal of Economic Entomology **99**(2): 593-598.
18. van Zyl, R.L., ST. Seatlholo, and S.F. van Vuuren. 2006. The biological activities of 20 nature identical essential oil constituents. Journal of Essential Oil Research **18**: 129-133.
19. Yang, V.W., and C.A. Clausen. 2006. Moldicidal properties of essential o:ls. International Research Group on Wood Protection, Stockholm, Sweden. IRG/WP/06-30404. 12p.
20. Yang, V.W., and c.A. Clausen. 2007. Antifungal effect of essential oils on southern yellow pine. International Biodeterioration & Biodegradation **59**: 302-306.
21. Yang, V.W., and C.A. Clausen. 2008. Inhibitory effect of essential oils on decay fungi and mold growth on wood. Proceedings for American Wood Protection Society, Birmingham, AL. 103: 62-70.
22. Yi, C.G., B.R. Choi, H.M. Park, C.G. Park, and A. Yj. 2006. Fumigant toxicity of plant essential oils to *Thrips palmi* (Thysanoptera: Thripidae) and *Orilus strigicollis* (Heteroptera: Anthrocoridae). Journal of Economic Entomology **99**(5): 1733-1738.
23. Zhu, B.C.R, G. Henderson, Y. Yu, and R.A. Laine. 2003. Toxicity and repellency of patchouli oil and patchouli alcohol against Formosan subterranean termites *Coptotermes formosanus* Shiraki (Isotera: Rhinotermiditidae). Journal of Agricultural and Food Chemistry **51**(16): 4585-4588.

PROCEEDINGS

One Hundred Fourth Annual Meeting

of the

AMERICAN WOOD PROTECTION ASSOCIATION

Portland Marriott Downtown Waterfront
Portland, Oregon
May 18-20, 2008

VOLUME 104

AMERICAN WOOD PROTECTION ASSOCIATION
POBOX 361784 • BIRMINGHAM, ALABAMA 35236-1784 • USA

ISSN 0066-1198

AMERICAN WOOD PROTECTION ASSOCIATION
P.O. BOX 361784
BIRMINGHAM, ALABAMA 35236-1784
USA

Colin McCown, *Editor*
Beth Williams, *Assistant Editor*