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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)

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Plant essential oils from 29 plant species were tested for their insecticidal activities against the Japanese termite, *Reticulitermes speratus* Kolbe, using a fumigation bioassay. Responses varied with plant material, exposure time, and concentration. Good insecticidal activity against the Japanese termite was achived with essential oils of *Melaleuca dissitiflora*, *Melaleuca uncinata*, *Eucalyptus citriodora*, *Eucalyptus polybractea*, *Eucalyptus radiata*, *Eucalyptus dives*, *Eucalyptus globulus*, *Orixa japonica*, *Cinnamomum cassia*, *Allium cepa*, *Illicium verum*, *Evodia officinalis*, *Schizonepeta tenuifolia*, *Cacalia roborowskii*, *Juniperus chinensis* var*. horizontalis*, *Juniperus chinensis* var*. kaizuka*, clove bud, and garlic applied at 7.6 μL/L of air. Over 90% mortality after 3 days was achieved with *O. japonica* essential oil at 3.5 μL/L of air. *E. citriodora*, *C. cassia*, *A. cepa*, *I. verum*, *S. tenuifolia*, *C. roborowskii*, clove bud, and garlic oils at 3.5 μL/L of air were highly toxic 1 day after treatment. At 2.0 μL/L of air concentration, essential oils of *I. verum*, *C. roborowskik*, *S. tenuifolia*, *A. cepa*, clove bud, and garlic gave 100% mortality within 2 days of treatment. Clove bud and garlic oils showed the most potent antitermitic activity among the plant essential oils. Garlic and clove bud oils produced 100% mortality at 0.5 μL/L of air, but this decreased to 42 and 67% after 3 days of treatment at 0.25 μL/L of air, respectively. Analysis by gas chromatography−mass spectrometry led to the identification of three major compounds from garlic oil and two from clove bud oils. These five compounds from two essential oils were tested individually for their insecticidal activities against Japanese termites. Responses varied with compound and dose. Diallyl trisulfide was the most toxic, followed by diallyl disulfide, eugenol, diallyl sulfide, and β-caryophyllene. The essential oils described herein merit further study as potential fumigants for termite control.

KEYWORDS: Plant essential oils; *Reticulitermes speratus*; antitermitic activity; fumigant; garlic oil; clove bud oil; diallyl trisulfide; diallyl disulfide; eugenol

INTRODUCTION

Biological deterioration is a main factor that degrades the durability of wooden structures (*1*). Among all factors leading to biodegradation, damage by termites is the most serious to wooden structures worldwide. Termites cause damage to a variety of materials ranging from paper fabrics to even non-cellulosic materials such as asbestos, asphalt bitumen, lead, and metal foils (*2*). Damage to wooden structures and other cellulosic materials attributed to termites has been estimated to exceed \$3 billion annually worldwide (*3*).

Reticulitermes speratus Kolbe is the major termite species distributed in Korea, Japan, and China, and it has caused serious damage to wooden structures in Korea. Control of the Japanese termite is primarily dependent upon continued applications of synthetic pesticides or traditional wood preservatives (*4*). To avoid environmental pollution and health problems caused by the use of traditional wood preservatives or synthetic pesticides, there is a trend to search for naturally occurring toxicants in plants (*5*). Plant essential oils may provide potential alternatives to currently used termite control agents because they constitute a rich source of bioactive chemicals and are commonly used as fragrances and flavoring agents for foods and beverages (*6*, *7*).

This paper describes a laboratory study to assess the potential of plant essential oils for use as commercial termiticides. Insecticidal activities of essential oils from 29 plant species were assesed against Japanese termite adults using a fumigation bioassay.

MATERIALS AND METHODS

Termites. Termites (*R. speratus* Kolbe) were collected at Suwon, Korea, from March to September 2004. Termite-infested wood

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Table 1. Plant Essential Oils Tested for Antitermitic Activity

plant species	family name	part	yield[a] (v/w, %)
Boswellia carterii	Burseraceae	resin	3.2
Cacalia roborowskii	Composite	whole plant	3.6
Chamaecyparis obtusa	Cupressaceae	leaf	0.66
Juniperus chinensis var. *horizontalis*		leaf	0.1
Juniperus rigida		leaf	0.072
Juniperus chinensis		leaf	0.35
Juniperus chinensis var. *Kaizuka*		leaf	0.41
Illicium verum	Illiciaceae	fruit	0.8
Schizonepeta tenuifolia	Labiatae	herba	1.6
Cinnamomum cassia	Lauraceae	cortex	
Amorpha fruticosa	Leguminosae	fruit	0.3
Allium cepa	Liliaceae	bulb	
Allium sativum		bulb	
Melaleuca dissitiflora	Myrtaceae	leaf	
Melaleuca uncinata		leaf	
Melaleuca linariifolia		leaf	
Melaleuca squamophloia		leaf	
Eucalyptus citriodora		leaf	
Eucalyptus smithii		leaf	
Eucalyptus polybractea		leaf	
Eucalyptus radiata		leaf	
Eucalyptus dives		leaf	
Eucalyptus globulus		leaf	
Eugenia caryophyllata		flos	
Rheum tanguticum	Polygonaceae	rhizome	0.2
Pinus densiflora	Pinaceae	leaf	0.37
Orixa japonica	Rutaceae	leaf	0.085
Evodia officinalis		fruit	0.057
Zingiber officinale	Zingiberaceae	rhizome	

[a] Yields of essential oils extracted by steam distillation are reported.

moistened with distilled water was kept in plastic cages (60 by 40 by 40 cm) at 25 ± 1 °C and 80% relative humidity.

Chemicals. Eugenol (purity = 95%), β-caryophyllene (purity = 80%), diallyl sulfide (purity = 99%), diallyl disulfide (purity = 70%, remainder mainly diallyl sulfide), and diallyl trisulfide (purity = 98%) were purchased from Wako (Osaka, Japan), Tokyo Kasei (Tokyo, Japan), Kanto Chemicals (Tokyo, Japan), Acros Organics (Fair Lawn, NJ), and MP Biomedical (Irvine, CA), respectively.

Sample Preparation. The plant essential oils used are listed in **Table 1**. *Cinnamomum cassia*, *Allium cepa*, *Melaleuca dissitiflora*, *Melaleuca uncinata*, *Melaleuca linaritifolia*, *Melaleuca squamophloia*, *Eucalyptus citriodora*, *Eucalyptus smithii*, *Eucalyptus polybractea*, *Eucalyptus radiata*, *Eucalyptus dives*, *Eucalyptus globulus*, *Zingiber officinale*, garlic, and clove bud oils were supplied from Shinill Science Co., Seoul, South Korea. The other plant essential oils were extracted by using a steam distillation method described by Park et al. (*8*). *Boswellia carterii*, *Cacalia roborowskii*, *Schizonepeta tenuifolia*, *Illicium verum*, *Roheum tanguticum*, and *Evodia officinalis* were purchased at Kyungdong medicinal market. The healthy, mature leaves of *Juniperus chinensis* var. *horizontalis*, *Juniperus rigida*, *Juniperus chinensis*, *J. chinensis* var. *kaizuka*, *Pinus densiflora*, *Chamaecyparis obtusa*, and *Orixa japonica* and the fruit of *Amorpha fruticosa* were obtained from mature plants growing in the Hongneung Arboretum, Korea Forest Research Institute (Seoul, South Korea) from October 2003 to August 2004. Plant samples were powdered in a blender, diluted with distilled water (800 mL) in a 2 L flask, and steam distilled (100 °C). Yields of essential oils are given in **Table 1**.

Gas Chromatography−Mass Spectrometry. The essential oils of garlic and clove bud were analyzed on a a gas chromatograph (HP6890)−mass spectrometer (JMS-600W, JEOL) (GC-MS). The GC column was a 30 m × 0.32 mm i.d. (0.5 film) fused silica capillary column (J&W Scientific, Folsom, CA). The GC conditions were as follows: injector temperature, isothermal at 40 °C for 5 min, then programmed to 220 °C at 2 °C/min and held at this temperature for 5 min; ion source temperature, 200 °C. Helium was used as the carrier gas at the rate of 1.5 mL/min. Effluent of the GC column was introduced directly into the source of the MS. Spectra were obtained in the EI mode with 70 eV ionization energy. The sector mass analyzer was set to scan from 50 to 800 amu for 2 s. Compounds were identified by comparison of the mass spectrum of each peak with those of authentic samples in a mass spectra library (*The Wiley Registry of Mass Spectra Data*, sixth ed.) and confirmed by comparison of retention times obtained by GC with those of authentic samples.

Table 2. Fumigant Activity of Plant Essential Oils against Japanese Termite

plant species	concn (μL/L)	mortality[a] (mean ± SE, %) 24 h	48 h	72 h
B. carterii	7.6	30 ± 3.0bcde	100a	100a
	3.5	6 ± 3.0de	6 ± 3.0hij	6 ± 3.0gh
C. roborowskii	7.6	100a	100a	100a
	3.5	100a	100a	100a
C. obtusa	7.6	95.1de	24 ± 3.0efghij	27defgh
J. chinensis var. *horizontalis*	7.6	57.6 ± 6.3abcd	76 ± 3.0abcde	94 ± 6.0abc
	3.5	0e	0j	3 ± 3.0gh
J. rigida	7.6	57.6 ± 6.3abcd	67 ± 3.0abcdefg	88 ± 12.0abcd
	3.5	0e	0j	0h
J. chinensis	7.6	54.6 ± 5.4abcde	64 ± 5.1bcdefg	88 ± 3.0abcdef
	3.5	0e	0j	63.0gh
J. chinensis var. *kaizuka*	7.6	39 ± 3.0bcde	51.6 ± 3.3cdefgh	97 ± 3.0ab
	3.5	0e	0j	3 ± 3.0gh
S. tenuifolia	7.6	100a	100a	100a
	3.5	91 ± 9ab	97 ± 3.0ab	100a
C. cassia	7.6	100a	100a	100a
	3.5	100a	100a	100a
A. fruticosa	7.6	9 ± 5.1de	24 ± 3.0efghij	30 ± 3.0efgh
A. cepa	7.6	100a	100a	100a
	3.5	100a	100a	100a
A. sativum	7.6	100a	100a	100a
	3.5	100a	100a	100a
I. verum	7.6	100a	100a	100a
	3.5	100a	100a	100a
M. dissitiflora	7.6	88 ± 3.0abc	95 ± 6.0ab	95 ± 6.0abc
	3.5	12 ± 6.0de	27 ± 5.1efghij	61 ± 3.0abcdefg
M. uncinata	7.6	21 ± 3.0cde	85 ± 3.0abcd	97 ± 3.0ab
	3.5	0e	3 ± 3.0ij	18fgh
M. linariifolia	7.6	42 ± 3.0bcde	79 ± 6.0abcde	79 ± 6.0abcdef
	3.5	12 ± 6.0de	18fghij	33 ± 3.0bcdefgh
M. squamophloia	7.6	6 ± 3.0de	64 ± 5.1bcdefg	85 ± 3.0abcdef
	3.5	0e	3 ± 3.0ij	24 ± 6.0defgh
E. citriodora	7.6	100a	100a	100a
	3.5	100a	100a	100a
E. smithii	7.6	15 ± 3.0cde	73abcdef	85 ± 3.0abcdef
	3.5	0e	0j	6 ± 3.0gh
E. polybractea	7.6	30 ± 3.0bcde	91 ± 5.1abc	100a
	3.5	3 ± 3.0de	15 ± 3.0ghij	21 ± 3.0efgh
E. radiata	7.6	39 ± 3.0bcde	67 ± 3.0abcdefg	91 ± 5.1abcde
	3.5	9 ± 5.1de	27 ± 5.1efghij	36bcdefgh
E. dives	7.6	21 ± 3.0cde	67 ± 3.0abcdefg	97 ± 3.0ab
	3.5	15 ± 6.0cde	36 ± 5.1defghi	61 ± 6.0abcdefg
E. globulus	7.6	15 ± 3.0cde	67 ± 3.0abcdefg	97 ± 3.0ab
	3.5	0e	6 ± 3.0hij	63 ± 3.0gh
E. caryophyllata	7.6	100a	100a	100a
	3.5	100a	100a	100a
R. tanguticum	7.6	6 ± 6.0de	100a	100a
	3.5	0e	27 ± 5.1efghij	82 ± 5.1abcdef
P. densiflora	7.6	21 ± 3.0cde	27efghij	27defgh
O. japonica	7.6	24 ± 3.0cde	100a	100a
	3.5	3 ± 3.0de	48 ± 3.0cdefgh	100a
E. officinalis	7.6	30 ± 3.0bcde	100a	100a
	3.5	6 ± 6.0de	51.6 ± 3.3cdefgh	88 ± 7.9abcdef
Z. officinale	7.6	36 ± 5.1bcde	36 ± 5.1defghi	42 ± 3.0abcdefgh

[a] Means within a column followed by the same letters are not significantly different (P = 0.05, Scheffe's test).

Antitermitic Activity. Antitermitic activity was evaluated using a fumigation bioassay. A filter paper (Whatman no. 2, 4.5 cm diameter) treated with essential oils was placed in the bottom lid of a glass cylinder (5 cm diameter × 10 cm) with a wire sieve fitted at 3.5 cm above the bottom, after which the lid was sealed. Ten workers and one soldier were placed on the sieve. This prevented direct contact of the termite with the test plant oils and compounds. The termites were exposed for

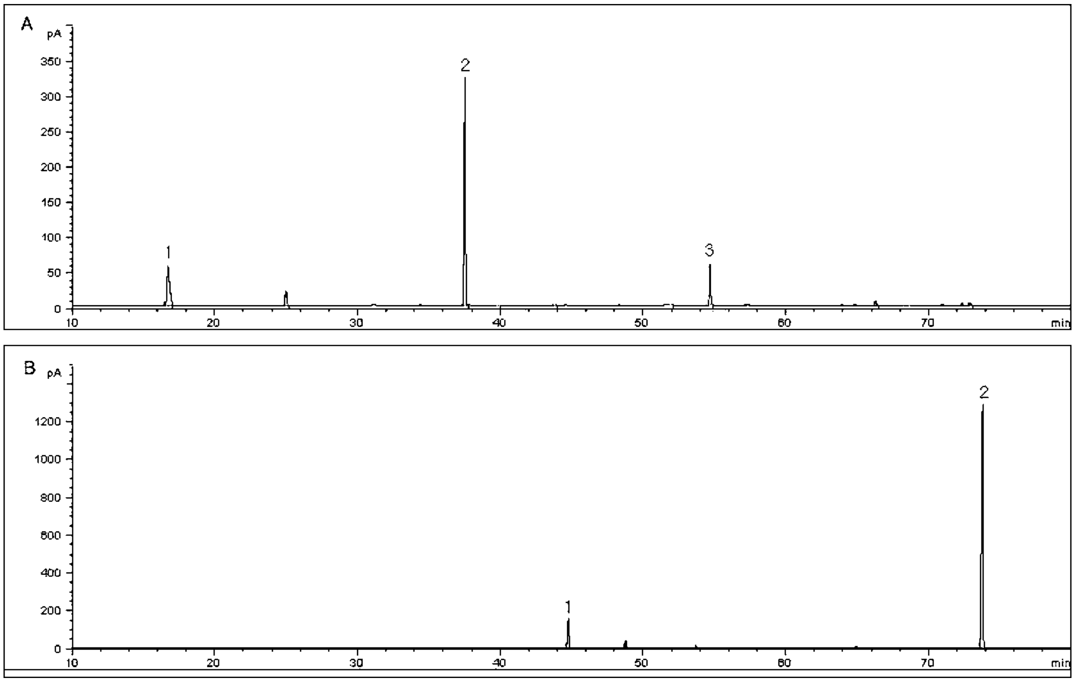

Figure 1. Gas chromatogram of garlic (**A**) and clove bud oils (**B**). Peaks: (A) 1, diallyl sulfide (21.3%); 2, diallyl disulfide (59.7%); 3, diallyl trisulfide (10.9%); (B) 1, β-caryophyllene (11.1%); 2, eugenol (86.1%).

1−3 days. Filter paper soaked with water was supplied for food. Treated insects were held at at 25 ± 1 °C and 80% relative humidity. Cumulative mortalities were determined 1, 2, and 3 days after treatment. All treatments were replicated three times.

Statistical Analyses. Mortality of termites was transformed to arcsine square root values for analysis of variance (ANOVA). Treatment means were compared and separated by Scheffe's test (*9*).

RESULTS

Antitermitic Activity of Plant Essential Oils. When 29 plant essential oils were bioassayed, mortalities varied according to oil type and dose (**Table 2**). Of these, 20 essential oils gave >90% morality 3 days after treatment at 7.6 μL/L of air. At 3.5 μL/L of air, *E. citriodora*, *O. japonica*, *C. cassia*, *A. cepa*, *I. verum*, *A. sativum*, *S. tenuifolia*, *C. roborowskii*, garlic, and clove bud oils gave >90% mortality. Plant essential oils showing >90% mortality at 3.5 μL/L of air were bioassayed at lower concentrations (**Table 3**). Garlic and clove bud oils produced 100% mortality at 0.5 μL/L of air, but this decreased to 42 and 67% morality 3 days after treatment at 0.25 μL/L of air, respectively. Antitermitic activities of *I. verum* and *C. roborowskik* essentail oils were 94 and 88% at 0.5 μL/L of air, but no mortality was observed with either oil at 0.25 μL/L of air. The essential oils of *S. tenuifolia*, *C. cassia*, and *A. cepa* produced 100% insecticidal activity at 2 μL/L of air, but only moderate activity at 1 μL/L of air.

Chemical Components of Clove Bud and Garlic Oils. Figure 1 shows chromatograms of clove bud and garlic oils using a DB-Wax fused silica capillary column. The main components of clove bud oil were β-caryophyllene (11.1%) and eugenol (86.1%). Diallyl sulfide (21.3%), diallyl disulfide (59.7%), and diallyl trisulfide (10.9%) were identified as major components of garlic oil.

Antitermitic Activity of Individual Compounds. The antitermitic activities of five compounds from clove bud and garlic oils are shown in **Table 4**. Potenticies varied according to compound and dose. The compound most toxic to the Japanese termite was diallyl trisulfide, followed by diallyl disulfide, eugenol, diallyl sulfide, and β-caryophyllene. At 0.125 μL/L of air, diallyl trisulfide gave 100% mortality, but mortality was reduced to 75 and 15% at 0.05 and 0.025 μL/L of air, respectively. Diallyl disulfide produced 100% mortality at 0.25 μL/L of air, but only 48 and 21% at 0.125 and 0.05 μL/L of air, respectively. Very strong antitermitic activity was observed with eugenol at 1 and 0.5 μL/L of air. However, activity was weak at 0.125 μL/L of air. The antitermitic activity of diallyl sulfide and β-caryophyllene was very weak at 5 μL/L of air.

Table 3. Fumigant Activity of 10 Selected Plant Essential Oils against Japanese Termite

plant species	concn (μL/L)	mortality[a] (mean ± SE, %) 24 h	48 h	72 h
C. roborowskik	2	91 ± 5.1abc	100a	100a
	1	55abcd	100a	100a
	0.5	0e	27de	88 ± 3.0bc
	0.25	0e	0f	0f
S. tenuifolia	2	51 ± 6.3abcd	100a	100a
	1	42 ± 6.3bcde	48 ± 3.3bc	61 ± 3.0de
C. cassia	2	91 ± 5.1abc	100a	100a
	1	3 ± 3.0de	21 ± 3.0e	54 ± 5.4de
A. cepa	2	94 ± 6ab	100a	100a
	1	33 ± 3.0cde	51 ± 3.3bc	64cde
A. sativum	0.5	100a	100a	100a
	0.25	33 ± 3.0cde	36bcde	42 ± 3.0de
	0.125	0e	24 ± 3.0e	33 ± 3.0e
I. verum	2	54 ± 5.4abcd	100a	100a
	1	45 ± 5.4bcde	100a	100a
	0.5	6 ± 6.0de	36 ± 5.1cde	94 ± 3.0ab
	0.25	0e	0f	0f
E. citriodora	2	6 ± 6.0de	33 ± 3.0cde	55de
	1	3 ± 3.0de	21 ± 3.0e	33 ± 3.0e
E. caryophyllata	2	100a	100a	100a
	1	94 ± 3.0abc	100a	100a
	0.5	85 ± 7.9abc	100a	100a
	0.25	0e	0f	67 ± 3.0cd
	0.125	0e	0f	33.0f
O. japonica	2	6 ± 3.0de	33 ± 3.0cde	55de
E. officinalis	2	3 ± 3.0de	45bcd	61 ± 3.0de

[a] Means within a column followed by the same letters are not significantly different (P = 0.05, Scheffe's test).

Table 4. Fumigant Activity of Consituents from Clove Bud and Garlic Oils against Japanese Termite

compound	concn (μL/L)	mortality[a] (mean ± SE, %)		
		24 h	48 h	72 h
diallyl sulfide	5	9bcd	15 ± 3.0cde	36 ± 5.1cd
	2.5	3 ± 3.0cd	6 ± 3.0ef	15 ± 3.0de
diallyl disulfide	0.25	100a	100a	100a
	0.125	0d	33 ± 3.0bcd	48 ± 3.0bc
	0.05	0d	9cdef	21 ± 3.0cde
diallyl trisulfide	0.25	100a	100a	100a
	0.125	36b	100a	100a
	0.05	15 ± 3.0bc	39 ± 3.0bc	75 ± 3.0b
	0.025	6 ± 3.0cd	9 ± 5.1def	15 ± 3.0de
eugenol	1	100a	100a	100a
	0.5	97 ± 3.0a	100a	100a
	0.25	0d	606.0b	69 ± 3.0b
	0.125	0d	0f	6 ± 3.0e
β-caryophyllene	5	0d	0f	6 ± 6.0e

[a] Means within a column followed by the same letters are not significantly different ($P = 0.05$, Scheffe's test).

DISCUSSION

Many plant essential oils and phytochemicals are known to possess antitermitic or repellent activity. Reported naturally occurring antitermitic compounds include cinnamaldehyde from *Cinnanmomum osmophleum* (*10*), cedrol and α-cadinol from *Taiwania cryptomerioides* heartwood oil (*5*), chamaecynone from *Chamaecyparis pisifera* (*11*), and 7-methyljuglone from *Diopyros virginiana* (*12*). Other studies confirmed that some essential oils, such as that extracted from cedarwood (*13*), *Litsea cubeba* (*14*), and *Cinnamomum* spp. (*15*), are naturally repellent to termites. In our study, a total of 20 plant essential oils showed anitermitic activity at 7.6 μL/L of air. Among them, garlic and clove bud oils were the most toxic. *E. citriodora, O. japonica, C. cassia, A. cepa, I. verum, A. sativum, S. tenuifolia, E. caryophyllata*, and *C. roborowskii* were also effective at 3.5 μL/L of air.

Various compounds, including alcohols, aldehydes, fatty acid derivatives, terpenoids, and phenolics, exist in plant essential oils. Jointly or independently, they contribute to insecticidal, ovicidal, repellent, and antifeeding activities (*16*). In this study, the antitermitic constituents of oils were identified as diallyl disulfide and diallyl trisulfide from garlic and eugenol from clove bud oil by GC-MS analysis. The compound most toxic to the Japanese termite was diallyl trisulfide, followed by diallyl disulfide and eugenol, whereas diallyl sulfide and β-caryophyllene showed weak antitermitic activity.

Elucidation of the mode of action of oils and their constituents is of pratical importance for insect control because it may give useful information on the most appropriate formulation and delivery means. In our study, garlic and clove bud oils, diallyl trisulfide, diallyl disulfide, and eugenol were very effective via fumigation. These results indicate that the mode of delivery of these oils and compounds was by vapor action, likely via the respiratory system.

Structure−activity relationships of certain plant compounds against arthropod pests have been well studied. Rice and Coats (*17*) and Tsao et al. (*18*) attrempted to enhance the potency of selected monoterpenes and phenols through derivatization of the hydroxyl group. They found that enhanced bioactivity of the derivatives appeared to result from increased vapor pressure, leading to better penetration and bioavailability in the insect's body. Regnault-Roger and Hamraoui (*19*) studied the structure−activity relationship between monoterpenoids and fumigant activity against *Acanthoscelides obtectus* (Say) adults: the oxygenated structures proved to be the most active, especially carvacrol, linalool, and terpineol. In our study, there was a strong relationship between sulfur number and antitermitic activity among constituents from garlic oils. Diallyl trisulfide is the most toxic followed by diallyl disulfide. Diallyl sulfide was the least effective, although the chemical structure is very similar to those of diallyl trisulfide and diallyl disulfide.

The medicinal value of garlic was well-known to the ancient Arab, Jewish, Greek, and Roman civilizations. Garlic also enjoys wide use as an important condiment and flavoring in various cuisines of the world and is also known as an anticancer, antiseptic, tonic, bactericide, vermifuge, expectorant, stomachic, and antihypertensive (*20*). Garlic essential oils are reported to consist primarily of diallyl, dimethyl, and allylmethyl mono-, di-, trisulfides and a few minor components. Diallyl disulfide accounts for 30−50% of the total mixture (*20*). In our study, the main compounds from garlic oils were diallyl disulfide (59.7%), followed by diallyl sulfide (21.3%) and diallyl trisulfide (10.9%). Organosulfur compounds from garlic such as diallyl mono-, di-, and trisulfide show antimicrobial activity (*21*), inhibit arylamine *N*-acetyltransferase (NAT) activity in a human colon tumor cell line (*22*), and induce apoptosis of human colon tumor cells (*23*). Clove has also long been considered to have medicinal properties such as a stimulant against digestive disorders and diarrhea. Clove bud oil has insecticidal activity against some stored product insects (*24*), the American house dust mite, and the European house dust mite (*25*). In our study, the main compounds of clove bud oils were eugenol (86.1%) and β-caryophyllene (11.1%), and antitermitic activity was mainly attributed to the action of eugenol.

Our results indicate that garlic and clove bud oils and their components could be useful as fumigants for Japanese termites. For the practical use of garlic and clove bud oils and their constituents as novel fumigants, further study is necessary on the safety of these materials to humans and on development of formulations to improve the efficacy and stability and to reduce cost.

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