Toxicity of Plant Essential Oils to *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae)

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ABSTRACT A total of 53 plant essential oils were tested for their insecticidal activities against eggs, nymphs, and adults of *Trialeurodes vaporariorum* Westwood, using an impregnated filter paper bioassays without allowing direct contact. Responses varied according to oil type and dose, and developmental stage of the insect. Bay, caraway seed, clove leaf, lemon eucalyptus, lime dis 5 F, pennyroyal, peppermint, rosewood, spearmint, and tea tree oils were highly effective against *T. vaporariorum* adults, nymphs, and eggs at 0.0023, 0.0093, and 0.0047 μl/ml air, respectively. These results indicate that the mode of delivery of these essential oils was largely a result of action in the vapor phase. Significant correlations among adulticidal, nymphicidal, and ovicidal activities of the test oils were observed. The essential oils described herein merit further study as potential fumigants for *T. vaporariorum* control.

KEY WORDS natural insecticide, natural fumigant, essential oil, *Trialeurodes vaporariorum*, mode of action

THE WHITEFLY, *Trialeurodes vaporariorum* Westwood, was accidentally introduced into South Korea from Saudi Arabia and Japan in 1977 (Han 1998). This insect is now well established in the greenhouse ecosystem and is an economically important pest of various greenhouse vegetables, particularly tomatoes, cherry tomatoes, and cucumbers, as well as ornamentals. Control of *T. vaporariorum* populations worldwide is primarily dependent on repeated applications of conventional insecticides, such as organophosphates, carbamates, and pyrethroids. Although effective, their repeated use for decades has disrupted natural biological control systems and led to resurgence of this insect (Dittrich et al. 1990), sometimes resulted in the development of resistance (Dittrich et al. 1990, Omer et al. 1993), had undesirable effects on nontarget organisms, and fostered environmental and human health concerns (Hayes and Laws 1991). Furthermore, *T. vaporariorum* dwells on the undersurface of plant foliage, not easily reached by conventional spraying equipment (Dittrich et al. 1990). These problems have highlighted the need for the development of selective *T. vaporariorum* control alternatives with fumigant action but without phytotoxicity in greenhouses, where ventilation can be controlled.

Plant essential oils may be an alternative source for *T. vaporariorum* control because they constitute a rich source of bioactive chemicals and are commonly used as fragrances and flavoring agents in foods and beverages. Because of this, much effort has been focused on plant essential oils or phytochemicals as potential sources of commercial insect control agents. Little work has been done in relation to the control of *T. vaporariorum*, although insecticidal activity of plant essential oils has been well described by Isman (2000).

This paper describes a laboratory study aimed at assessing the potential of plant essential oils for use as commercial insecticides. Insecticidal activity of 53 essential oils was assessed against each developmental stage (eggs, nymphs, and adults) of *T. vaporariorum.* The insecticidal route of action of the test plant oils is also discussed.

Materials and Methods

Essential Oils. A total of 53 plant essential oils were purchased from Jin Aromatics, Anyang, Kyunggi Province, Korea, and are listed in Table 1.

Insects. Cultures of *T. vaporariorum* were maintained in the laboratory for 6 yr without exposure to any insecticide. Whiteflies were reared in acrylic cages (50 × 45 × 60 cm) on tobacco (*Nicotiana tabacum* L.) plants at 25 ± 2°C, 50–60% relative humidity (RH), and a photoperiod of 16:8 L:D. Under these conditions, longevity of eggs, nymphs, pupae, and adults was ≈6.3, 12.7, 2.6, and 7.3 d, respectively.

Bioassays. To synchronize the developmental stages for ovicidal, nymphicidal, and adulticidal bioassays,

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Table 1. List of essential oils from plants tested

Oil	Source plant	Family
Basil	*Ocimum basilicum*	Labiatae
Bay	*Pimenta racemosa*	Myrtaceae
Bergamot	*Citrus bergamia*	Rutaceae
Bitter orange	*Citrus aurantium*	Rutaceae
Black pepper	*Piper nigrum*	Piperaceae
Cade	*Juniperus oxycedrus*	Cupressaceae
Caraway seed	*Carum carvi*	Umbelliferae
Cardamone ceylon	*Elettaria cardamomum*	Zingiberaceae
Cedarwood	*Cedrus atlantica*	Cupressaceae
Chamomille roman	*Anthemis nobilis*	Compositae
Citronella java	*Cymbopogon nardus*	Gramineae
Clary sage	*Salvia sclarea*	Labiatae
Clove bud	*Eugenia caryophyllata*	Myrtaceae
Cloveleaf	*Eugenia caryophyllata*	Myrtaceae
Coriander	*Coriandrum sativum*	Umbelliferae
Cypress	*Cupressus sempervirens*	Cupressaceae
Eucalyptus	*Eucalyptus globulus*	Myrtaceae
Fir needle	*Abies alba*	Pinaceae
Frankinsence	*Boswellia carterii*	Burseraceae
Geranium	*Pelargonium graveolens*	Geraniaceae
Ginger	*Zingiber officinale*	Zingiberaceae
Grapefruit	*Citrus paradisi*	Rutaceae
Juniperberry	*Juniperus communis*	Cupressaceae
Lavender	*Lavendula officinalis*	Labiatae
Lemon 10-Fold	*Citrus limonum*	Rutaceae
Lemon eucalyptus	*Eucalyptus citriodora*	Myrtaceae
Lemongrass	*Cymbopogon citratus*	Gramineae
Lime dis 5F	*Citrus aurantifolia*	Rutaceae
Mandarine	*Citrus reticulata*	Rutaceae
Marjoram	*Origanum majorana*	Labiatae
Myrtle	*Myrtus communis*	Myrtaceae
Nutmeg	*Myristica fragrans*	Myristicaceae
Orange	*Citrus sinensis*	Rutaceae
Oregano	*Origanum vulgare*	Labiatae
Palmarosa	*Cymbopogon martinii*	Gramineae
Patchouli	*Pogostemon cablin*	Labiatae
Pennyroyal	*Mentha pulegium*	Labiatae
Peppermint	*Mentha piperita*	Labiatae
Petitgrain	*Citrus aurantium*	Rutaceae
Pimento berry	*Pimenta officinalis*	Myrtaceae
Rosemary	*Rosmarinus officinalis*	Labiatae
Rosewood	*Aniba rosaeodora*	Lauraceae
Sage	*Salvia officinalis*	Labiatae
Sandalwood	*Santalum album*	Santalaceae
Spearmint	*Mentha spicata*	Labiatae
Tagetes	*Tagetes glandulifera*	Compositae
Tangerine	*Citrus reticulata*	Rutaceae
Tea tree	*Melaleuca alternifolia*	Myrtaceae
Thyme red	*Thymus vulgaris*	Labiatae
Thyme white	*Thymus vulgaris*	Labiatae
Vetiver haiti	*Vetiveria zizaniodes*	Gramineae
Wormwood	*Artemisia absinthium*	Compositae
Ylang ylang	*Cananga odorata*	Annonaceae

adults were placed on tomato (*Lycopersicon esculentum* Mill.) plants with a fine brush and allowed to lay eggs for 24 h, after which time the adults were removed with an aspirator. The infested plants were held at 25 ± 2°C, 50–60% RH, and a photoperiod of 16:8 L:D. The stages tested consisted of eggs, nymphs, and adults. The numbers of each stage on each leaf were counted before treatment.

Toxic effects of the test essential oils on each stage were determined by an impregnated filter paper bioassay without allowing direct contact, and each treatment was replicated three times. In a bioassay with *T. vaporariorum* adults, amounts (2.3×10^{-3}, 9.3×10^{-4}, and 4.7×10^{-4} μl/ml air) of each test oil in 10 μl of ethanol were applied to filter papers (Whatman No. 2, 45-mm diameter). After drying in a fume hood for 1 min, each treated filter paper was placed on the bottom of a polyethylene cup (45-mm diameter, 95 mm high) and a wire sieve (45-mm diameter) was placed 30 mm above the filter paper to prevent direct contact by test insects. Adults (2–4 d old) were introduced into the cup over the wire sieve using an aspirator and tomato leaf petioles wrapped with water-soaked cotton were supplied. Then the cup was covered with a lid. For eggs (<24 h) and nymphs (10–11 d old), each filter paper, treated with 9.3×10^{-3}, 4.7×10^{-3}, and 2.3×10^{-3} μl/ml air of the test oils in 10 μl of ethanol, was placed on the bottom of the cup. Tomato leaves containing eggs and nymphs were cut and petioles were wrapped with water-soaked cotton. Each leaf was placed in a separate polyethylene cup (45 mm diameter, 95 mm high) as mentioned above and the cup was covered with a lid. Control filter papers received 10 μl of ethanol.

Treated and control insects were held under the same conditions used for colony maintenance. Toxicity of the test oils to the eggs was based on color change of the eggs. *Trialeurodes vaporariorum* eggs are yellowish when freshly laid but turn black after 1 or 2 d and hatch after 7 d at 25°C (Vet et al. 1980). In a preliminary test, the color of eggs treated with the test oils remained yellowish, leading to death without hatching. After 72 h of incubation, changes in egg color were observed under a stereomicroscope (40×), at which time yellowish eggs were considered as dead. Nymphal mortality was assessed on the basis of failure to complete the next ecdysis. Evaluation of adulticidal activity was made 24 h after treatment. Insects were considered dead if appendages did not move when prodded with a fine camel's hair brush. Data were corrected for control mortality using Abbott's (1925) formula.

Statistical Analysis. Percentage mortality was determined and transformed to arcsine square-root values for analysis of variance. Treatment means were compared and separated by Scheffe test at $P = 0.05$ (SAS Institute 1996). Means ± SEM of untransformed data are reported. Relationships between toxicity of essential oils to each life stage of *T. vaporariorum* were estimated by regression analysis (SAS Institute 1996).

Results

When 53 essential oils were bioassayed, significant differences were observed in toxicity to *T. vaporariorum* adults (Table 2). Of these, 23 essential oils gave >80% mortality 24 h after treatment at 2.3×10^{-3} μl/ml air. Pennyroyal and peppermint oils gave 89 and 83% mortality at 9.3×10^{-4} μl/ml air, respectively. However, adulticidal activity of the 23 essential oils was significantly lower at 4.7×10^{-4} μl/ml air. Mortality in untreated controls was below 6%.

Toxicities of the test essential oils to *T. vaporariorum* nymphs are shown in Table 3. Responses varied ac-

Table 2. Toxicity of test essential oils to *T. vaporariorum* adults using the impregnated filter paper bioassay

Essential oil[a]	Mortality (24 h) (mean ± SEM), %					
	Concentration, μl/ml air					
	n	2.3×10^{-3}	*n*	9.3×10^{-4}	*n*	4.7×10^{-4}
Basil	108	92 ± 6.0ab	116	33 ± 4.7def		–[b]
Bay	135	100 ± 0.0a	91	20 ± 6.5f		–
Cade	111	60 ± 5.0c	74	19 ± 6.5ef		–
Caraway seed	78	100 ± 0.0a	76	14 ± 5.5ef		–
Cardamonc ceylon	129	85 ± 5.2abc	165	32 ± 5.9def		–
Citronella java	123	88 ± 5.6abc	116	31 ± 4.9def		–
Clary sage	126	76 ± 6.8bc	70	19 ± 2.3ef		–
Clove bud	132	90 ± 5.1abc	81	32 ± 2.6def		–
Clove leaf	117	90 ± 5.5abc	114	58 ± 5.5bcd	96	17 ± 5.3a
Coriander	102	92 ± 5.2abc	75	19 ± 4.5ef		–
Geranium	70	92 ± 6.9abc	72	25 ± 4.3def		–
Lavender	122	78 ± 6.9abc	79	9 ± 1.4f		–
Lemon eucalyptus	72	100 ± 0.0a	77	12 ± 6.3f		–
Lemongrass	75	100 ± 0.0a	73	23 ± 6.5def		–
Lime dis 5F	122	90 ± 5.5abc	73	30 ± 6.5def		–
Oregano	83	100 ± 0.0a	120	76 ± 5.8abc	75	24 ± 5.1a
Palmarosa	127	100 ± 0.0a	78	19 ± 5.9ef		–
Pennyroyal	74	100 ± 0.0a	85	89 ± 4.9a	90	18 ± 5.0a
Peppermint	83	100 ± 0.0a	149	83 ± 4.2ab	86	29 ± 6.2a
Petitgrain	118	75 ± 5.7bc	76	12 ± 3.9ef		–
Pimento berry	75	100 ± 0.0a	115	18 ± 5.0ef		–
Rosewood	110	93 ± 5.1ab	117	28 ± 5.5def		–
Sage	121	67 ± 6.1bc	78	14 ± 5.3ef		–
Spearmint	113	100 ± 0.0a	117	28 ± 4.7def		–
Tea tree	107	91 ± 4.6abc	102	28 ± 1.1def		–
Thyme red	114	91 ± 4.4abc	77	22 ± 4.5def		–
Thyme white	71	100 ± 0.0a	73	43 ± 4.1ede	76	8 ± 4.2a
Wormwood	115	100 ± 0.0a	83	16 ± 4.6ef		–

Means within a column followed by the same letter are not significantly different ($P = 0.05$, Scheffe test [SAS Institute 1996]).
[a] Essential oils producing <40% mortality are not presented.
[b] Mortality at 4.7×10^{-4} μl/ml air was not evaluated for test oils exhibiting <40% mortality at 9.3×10^{-4} μl/ml.

cording to test oil and dose. After 24 h of exposure, 25 essential oils exhibited >80% mortality at 9.3×10^{-3} μl/ml air. At 4.7×10^{-3} μl/ml air, >80% mortality was observed for peppermint and rosewood oils only. Mortality in untreated controls was below 9%.

Table 4 shows the toxic effects of 53 essential oils against *T. vaporariorum* eggs. After 24 h of exposure, 20 essential oils caused >80% mortality at 9.3×10^{-3} μl/ml air. At 2.3×10^{-3} μl/ml air, >80% mortality was achieved with caraway seed, lemongrass, lime dis 5 F, oregano, and spearmint oils. Mortality in untreated controls was below 6%.

Linear relationships among egg, nymphal, and adult mortalities by the test oils were determined (Fig. 1). Mortalities for each developmental stage by the test oils were positively correlated. The relationship between egg and nymphal mortalities is described by the formula: NM = 37.36 + 0.52 EM (Shapiro-Wilks W = 0.98, $P = 0.80$, $n = 53$, $r^2 = 0.44$); between egg and adult mortalities as AM = 25.59 + 0.75 EM (Shapiro-Wilks W = 0.95, $P = 0.07$, $n = 49$, $r^2 = 0.89$); and between nymphal and adult mortalities as AM = 8.37 + 0.85 NM (Shapiro-Wilks W = 0.97, $P = 0.25$, $n = 53$, $r^2 = 0.70$), where NM, EM, and AM are nymphal, egg, and adult mortalities, respectively.

Discussion

Plant essential oils are potentially useful for *T. vaporariorum* control because many of them are selective to pests, and have little or no harmful effects on nontarget organisms and the environment (Isman 2000). They act in many ways on various types of pests and can be applied to plants or stored products in the same way as other conventional insecticides (Desmarchelier 1994, Isman 2000). Many essential oils are known to possess ovicidal, repellent, and insecticidal activities against various insect species (Saxena 1989, Desmarchelier 1994, Isman 2000).

Additionally, some plant-derived compounds can be highly effective against insecticide-resistant insect pests (Lindquist et al. 1990, Ahn et al. 1997). In the current study, potencies varied according to oil type and dose, and developmental stage of the insect. Potent insecticidal activity against all stages of *T. vaporariorum* was observed with bay, caraway seed, clove leaf, lemon eucalyptus, lime dis 5 F, pennyroyal, rosewood, spearmint, and tea tree oils. These essential oils might be good candidates for naturally occurring *T. vaporariorum* control agents.

Elucidation of the mode of action of chemicals is of practical importance for insect control because it may

Table 3. Toxicity of test essential oils to *T. vaporariorum* nymphs using the impregnated filter paper bioassay

Essential oil[a]	Mortality (24 h) (mean ± SEM), %			
	Concentration, μl/ml air			
	n	9.3×10^{-3}	*n*	4.7×10^{-3}
Basil	195	96 ± 3.8abc	166	73 ± 5.1ab
Bay	84	91 ± 2.9abcdef	103	36 ± 5.4abc
Caraway seed	112	85 ± 3.5abcdef	120	73 ± 6.1abc
Cardamone ceylon	77	83 ± 3.9abcdef	72	23 ± 5.1abc
Chamomille roman	162	64 ± 6.8ef	84	18 ± 1.5bc
Citronella java	136	79 ± 6.9abcdef	78	54 ± 5.5abc
Clary sage	103	98 ± 1.6ab	144	55 ± 7.0abc
Clove leaf	175	98 ± 1.8ab	132	55 ± 5.3abc
Coriander	94	90 ± 4.7abcdef	70	71 ± 5.3abc
Eucalyptus	194	92 ± 4.6abcde	110	24 ± 2.1abc
Fir needle	153	63 ± 6.9ef	70	28 ± 6.0abc
Geranium	190	87 ± 5.6abcdef	131	36 ± 3.6abc
Lavender	344	97 ± 3.2abc	79	56 ± 6.8abc
Lemon eucalyptus	286	98 ± 1.5abc	65	62 ± 5.0abc
Lemongrass	107	67 ± 6.0def	122	26 ± 6.9abc
Lime dis 5F	279	93 ± 4.9abcde	98	56 ± 3.6abc
Marjoram	78	75 ± 4.7bcdef	96	41 ± 5.9abc
Oregano	129	100 ± 0.0a	91	34 ± 5.4abc
Palmarosa	160	70 ± 5.7cdef	114	54 ± 6.5abc
Pennyroyal	93	100 ± 0.0a	118	74 ± 6.3ab
Peppermint	104	98 ± 2.4abc	150	80 ± 6.2ab
Petitgrain	138	95 ± 4.6abcd	154	60 ± 6.9abc
Pimento berry	124	99 ± 1.3ab	134	60 ± 6.2abc
Rosemary	228	77 ± 5.6bcdef	76	8 ± 4.2c
Rosewood	145	99 ± 0.8ab	75	89 ± 5.7a
Sage	128	94 ± 5.5abcde	70	69 ± 2.7abc
Spearmint	151	99 ± 0.8ab	76	36 ± 2.0abc
Tea tree	153	97 ± 3.1abc	158	66 ± 4.7abc
Thyme red	180	90 ± 5.0abcde	128	38 ± 6.9abc
Thyme white	76	88 ± 5.1abcdef	100	70 ± 5.1abc
Wormwood	150	91 ± 5.5abcde	130	72 ± 5.7ab
Ylang Ylang	74	50 ± 2.1f	73	27 ± 3.8abc

Means within a column followed by the same letter are not significantly different ($P = 0.05$, Scheffe test [SAS Institute 1996]).

[a] Essential oils producing <40% mortality are not presented.

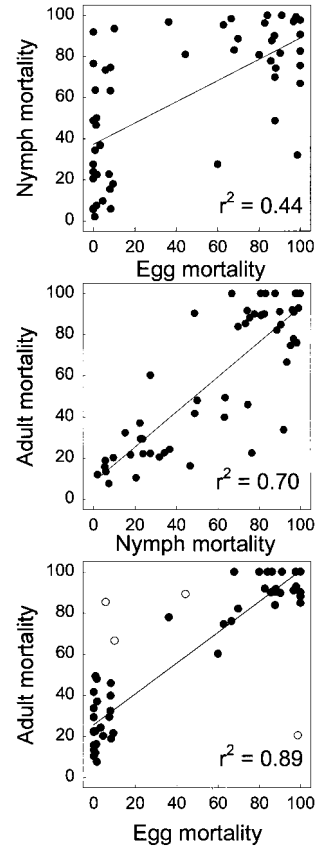

Fig. 1. The linear relation between toxicity of essential oils to each developmental stage (egg, nymph, and adult) of *T. vaporariorum*. Open circles indicate data excluded from the regression analysis because of abnormally high residuals.

give useful information on the appropriate formulation types. Volatile compounds of many plant extracts and essential oils consist of alkanes, alcohols, aldehydes, and terpenoids, especially monoterpenoids, and exhibit fumigant activity (Coats et al. 1991, Ahn et al. 1998, Kim and Ahn 2001, Kim et al. 2003). Fumigant activity of plant essential oils against some storage insects has been reported (El-Nahal et al. 1989, Shaaya et al. 1997, Ahn et al. 1998, Kim et al. 2003). Our study demonstrated that many of the test essential oils were effective against all stages of *T. vaporariorum* without direct contact. These results indicate that the mode of delivery of the oils was largely caused by action in the vapor phase: they may be toxic through penetration via the respiratory system.

Understanding the susceptibility to chemicals for different developmental stages of *T. vaporariorum* is important for whitefly control. Differential susceptibility of developmental stages of insects to chemicals has been well described by Harris (1972) and Eda (1985). The insensitivity might be attributable to an increase in body weight, a decrease in penetration, or biochemical and physiological changes in insect itself (Harris 1972, Eda 1985). *Trialeurodes vaporariorum* eggs were found to be more susceptible to phytoecdysteroids than nymphs in an in vitro bioassay (Melé et al. 1992). In our study, the stage most susceptible to the test essential oils was the adult followed by the egg and nymphal stages. The presence of wax spines or filaments on *T. vaporariorum* nymphs (Gerling 1990) may provide some protection against fumigants.

Results of this and earlier studies indicate that some essential oils could be useful as fumigants for *T. vaporariorum*, provided that a carrier producing a slow-release effect can be selected or developed. For practical use of these oils as novel fumigants, further research should be performed on several issues, including safety of these oils for human health, changes in the quality of crops treated with the oils (e.g., color, flavor, odor, and texture), effects on natural enemies such as *Encarsia formosa* Gahan and *E. versicolor* Girault, and formulations for improving the insecticidal potency and stability.

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Table 4. Toxicity of test essential oils to *T. vaporariorum* eggs using the impregnated filter paper bioassay

Essential oil[a]	Mortality (24 h) (mean ± SEM), %					
	Concentration, μl/ml air					
	n	9.3×10^{-3}	*n*	4.7×10^{-3}	*n*	2.3×10^{-3}
Basil	74	83 ± 6.1abcde	190	12 ± 6.21		–[b]
Bay	77	100 ± 0.0a	188	98 ± 1.1ab	144	14 ± 3.4g
Cade	134	60 ± 6.9e	236	24 ± 6.7hi		–
Caraway seed	175	93 ± 5.4abcde	173	80 ± 5.1abcd	346	87 ± 5.0ab
Citronella java	128	100 ± 0.0a	266	56 ± 6.9defgh	186	14 ± 2.5g
Clary sage	88	67 ± 6.6cde	105	56 ± 6.8defgh	209	14 ± 5.9g
Clove bud	249	94 ± 3.8abcde	171	88 ± 6.1abcd	366	58 ± 6.7bcdef
Clove leaf	217	91 ± 4.5abcde	108	86 ± 3.7abcde	238	69 ± 6.5bcd
Coriander	157	70 ± 6.9bcde	124	34 ± 6.8ghi		–
Geranium	118	88 ± 6.7abcde	232	43 ± 6.7efghi	152	16 ± 2.4g
Lemon 10-Fold	352	99 ± 0.8a	356	78 ± 6.4bcdefg	179	15 ± 2.2g
Lemon eucalyptus	140	98 ± 2.4ab	200	95 ± 3.0abc	228	43 ± 5.4cdefg
Lemongrass	88	100 ± 0.0a	167	100 ± 0.0a	133	98 ± 1.1a
Lime dis 5F	70	100 ± 0.0a	166	94 ± 3.6abc	213	79 ± 6.7abc
Oregano	74	84 ± 4.0abcde	205	78 ± 4.6abcdefg	218	82 ± 5.8ab
Palmarosa	191	96 ± 2.0abc	190	88 ± 6.3abcd	294	67 ± 6.7bcd
Pennyroyal	114	91 ± 5.3abcde	164	83 ± 5.9abcdef	275	68 ± 6.6bcd
Peppermint	76	100 ± 0.0a	289	70 ± 6.7bcdefg	187	63 ± 6.7bcde
Petitgrain	192	63 ± 4.5de	84	62 ± 4.2cdefgh	233	20 ± 6.0g
Pimento berry	146	79 ± 6.6abcde	307	68 ± 6.7bcdefg	188	27 ± 6.6efg
Rosewood	89	98 ± 2.1a	153	83 ± 3.4abcdef	197	38 ± 6.3defg
Spearmint	275	94 ± 3.9abcd	81	90 ± 6.6abcd	270	86 ± 4.8ab
Tea tree	71	97 ± 2.0abc	233	88 ± 6.1abcd	256	22 ± 4.8fg
Thyme red	76	87 ± 6.7abcde	266	80 ± 5.9abcdef	340	23 ± 5.4fg
Thyme white	209	86 ± 6.5abcde	222	42 ± 6.5fghi	125	28 ± 6.5efg

Means within a column followed by the same letter are not significantly different ($P = 0.05$, Scheffe test [SAS Institute 1996]).
[a] Essential oils producing <40% mortality are not presented.
[b] Mortality at 2.3×10^{-3} μl/ml air was not evaluated for test oils producing <40% mortality at 4.7×10^{-3} μl/ml air.

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