

Toxicity of hiba oil constituents and spray formulations to American house dust mites and copra mites

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Abstract

BACKGROUND: *Dermatophagoides farinae* and *Tyrophagus putrescentiae* are recognised as an important source of allergens. An assessment was made of the toxicity of hiba, *Thujopsis dolabrata* var. *hondai*, oil and 13 organic compounds and the control efficacy of four experimental spray formulations containing the oil (5–30 g L⁻¹ spray) against both mite species.

RESULTS: In a contact + fumigant mortality bioassay, (–)-thujopsene was the most toxic constituent against *D. farinae* and *T. putrescentiae* (24 h LC₅₀ 9.82 and 10.92 µg cm⁻²), and the toxicity of the compound was nearly identical to that of benzyl benzoate (9.33 and 10.14 µg cm⁻²). The toxicity was more pronounced in carvacrol, (+)-terpinen-4-ol, β-thujaplicin, (–)-terpinen-4-ol, cedrol and α-terpineol (LC₅₀ 12.05–15.20 and 12.74–16.48 µg cm⁻²) than in *N,N*-diethyl-3-methylbenzamide (LC₅₀ 35.53 and 38.42 µg cm⁻²) against both mite species. The hiba oil 30 g L⁻¹ spray and commercial permethrin (*cis:trans* 25:75) 2.5 g L⁻¹ spray treatment resulted in 100 and 11% mortality against the two mite species respectively. In vapour-phase mortality tests, the two compounds were consistently more toxic in closed versus open containers, indicating that toxicity was achieved mainly through the action of vapour.

CONCLUSION: Reasonable mite control in indoor environments can be achieved by a spray formulation containing the 30 g L⁻¹ hiba oil as a potential contact-action fumigant.

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Keywords: *Dermatophagoides farinae*; *Tyrophagus putrescentiae*; *Thujopsis dolabrata* var. *hondai*; botanical acaricide; natural fumigant; mode of delivery

1 INTRODUCTION

The prevalence and severity of allergic diseases such as asthma, atopic dermatitis, rhinitis and eczema caused by a number of arthropods, particularly pyroglyphid house dust mites (HDMs) and storage mites (SMs), are among the most serious global public health problems.^{1–3} HDMs usually make up 60–90% of the house dust acarofauna in temperate climate regions throughout the world.^{4,5} They are the most common cause of allergic symptoms in humans, affecting 65 million–1.2 billion people worldwide.^{5,6} HDMs, particularly the American house dust mite (AHDM), *Dermatophagoides farinae* Hughes (Acari: Pyroglyphidae), and the European house dust mite (EHDM), *Dermatophagoides pteronyssinus* Trouessart, and SMs such as the copra mite (CM), *Tyrophagus putrescentiae* Schrank (Acari: Acaridae), are recognised as an important source of allergens in certain occupational environments and in the domestic environment.^{1,5} CM acts as a carrier of bacteria and toxigenic fungi such as *Aspergillus* spp. and *Penicillium* spp. in stored grain kept under warm and moist conditions.^{7,8} In addition, cockroach exuviae are found to support large populations of EHDM, resulting in exacerbated cases of bronchial asthma.⁹

Increasing public concern about the environmental effects of acaricides, human health effects and undesirable effects on

non-target organisms intensifies when conventional contact acaricides such as benzyl benzoate, pirimiphos-methyl and pyrethroids^{10,11} are repeatedly used. In addition, the number of approved acaricides will probably be reduced in the near future in the United States by the US Environmental Protection Agency, as re-registration is required under the 1996 Food Quality and Protection Act.¹² The re-registration requirement is also a concern in other regions, including in the European Union, where it is regulated by the control of Commission Regulation (EC) No. 1048/2005.¹³ The removal of conventional acaricide products from markets owing to the increase in acaricide resistance or other concerns will have a serious impact on the proliferation of HDMs and SMs. There is, therefore, a critical need for the development of alternative acaricides with novel target sites to establish an

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efficient management strategy and tactics for these mites, particularly contact-action acaricides with fumigant activity, allowing effective application in occupational and domestic environments to reach deep refuges missed by many commercially available acaricides.

Bioacaricides derived from essential oils mostly extracted by steam distillation or hydrodistillation from plants have been suggested as potential alternatives to conventional acaricides largely because the oils constitute a potential source of bioactive secondary substances that have been perceived by the general public as relatively safe and pose fewer risks to the environment, with minimal impacts on human and animal health.^{10,14,15} They can act at multiple and novel target sites,^{16–18} thereby reducing the potential for resistance.^{19,20} They are regarded as potential sources for developing commercial acaricides, as certain essential oil preparations and their constituents meet the criteria for minimum-risk acaricides.²¹ Previous studies have shown that essential oils from hiba, *Thujopsis dolabrata* var. *hondai* (Cupressaceae), possess good acaricidal activity against adult AHDMs and CMs. Many *Thujopsis* species, including hiba, are rich in terpenoids.^{22,23} No work has been done to assess the potential of the oil and its constituents for the control of AHDMs and CMs, although the oil has been shown to possess repellent²³ and insecticidal²⁴ activities.

In the present study, an assessment was made of the potential of hiba oil and its nine constituents and another four previously known hiba oil constituents^{22,25} against adult AHDMs and CMs. The toxicities of these materials were compared with those of two conventional acaricides benzyl benzoate and *N,N*-diethyl-3-methylbenzamide (deet). The efficacy of four experimental spray formulations containing hiba oil (5, 10, 20 and 30 g L⁻¹ sprays) against both mite species was evaluated using a spray bioassay to determine the most effective formulations and compared with that of commercially available permethrin (*cis:trans* 25:75) 2.5 g L⁻¹ spray. The route of acaricidal action of the oil constituents is also discussed.

2 MATERIALS AND METHODS

2.1 Materials

Hiba oil was obtained from Taiyo Kagaku (Yokkaichi, Mie, Japan). The nine commercially available organic pure compounds identified in the hiba oil and another four previously known hiba oil constituents^{22,25} are listed in Table 1, along with their sources. Benzyl benzoate ($\geq 99.0\%$ purity) and deet (97% purity) were purchased from Sigma-Aldrich (St Louis, MO). One commercially available acaricide examined in this study was permethrin (*cis:trans* 25:75) 2.5 g L⁻¹ spray [Dongsung Pharmaceuticals, Asan, Chungnam, Republic of Korea (ROK)]. Polyoxyethylene + polyoxypropylene (9:1) styrenated phenyl ether, a surfactant, was a gift from Hannong Chemical (Anyang, Gyeonggi, ROK). All of the other chemicals used in this study were of reagent-grade quality and available commercially.

2.2 Mites

The stock cultures of AHDMs²⁶ and CMs²⁷ had been separately maintained in temperature-controlled incubators to prevent cross-contamination, without exposure to any known acaricide. These mites were reared in plastic containers (12.5 × 10.5 × 5.0 cm) containing sterilised diet (ratio of fry feed No. 1 to dried yeast 1:1 by weight). The containers were maintained at 25 ± 1 °C and 70–80% relative humidity (RH) in darkness. The fry feed and dried

Table 1. The 13 organic pure compounds examined in this study

Compound	Source ^a	Compound	Source ^a
Carvacrol	WK	(+)-Terpinen-4-ol	S-A
(-)- α -Cedrene	S-A	(-)-Terpinen-4-ol	TCI
β -Cedrene	S-A	α -Terpineol	S-A
Cedrol	WK	β -Thujaplicin	WK
<i>p</i> -Cymene	S-A	(-)- α -Thujone	S-A
(-)-Isolongifolene	S-A	(-)-Thujopsene	S-A
(+)- α -Longipinene	S-A		

^a S-A, Sigma-Aldrich (St Louis, MO); TCI, Tokyo Chemical Industry (Tokyo, Japan); WK, Wako (Osaka, Japan).

Table 2. Four experimental spray formulations containing hiba oil

Spray formulation ^a	Content (%)			
	Hiba oil	Surfactant ^b	Ethanol	DW ^c
HBO-5	0.5	2	5	92.5
HBO-10	1	2	5	92
HBO-20	2	2	5	91
HBO-30	3	2	5	90

^a Hiba oil 5, 10, 20 and 30 g L⁻¹.

^b Polyoxyethylene + polyoxypropylene (9:1) styrenated phenyl ether.

^c Distilled water.

yeast were purchased from Korea Special Feed Meal (Inchon, ROK) and Daeheung Pharmaceutical (Seoul, ROK) respectively.

2.3 Experimental spray formulations

Four experimental spray formulations containing hiba oil in 5 mL glass containers with a Homewell polypropylene pump spray nozzle (Seoul) were prepared to determine the effective acaricide products (Table 2). Single spray applications of 0.5, 1, 2 and 3% concentration of the oil preparations delivered ca 17.94, 35.87, 71.75 and 107.62 $\mu\text{g cm}^{-2}$ of total material to a 5 cm diameter black cotton-fabric circle.

2.4 Chromatographic analysis

An Agilent 7890A gas chromatography (GC) system (Palo Alto, CA), equipped with a splitless injector and a flame ionisation detection (FID) system, was used to separate and detect the constituents of hiba oil. Constituents were separated with an Agilent J&W Scientific 30 m × 0.32 mm i.d. ($d_f = 0.25 \mu\text{m}$) HP-5 capillary column (Folsom, CA). The flow rate of the nitrogen carrier gas was 1.0 mL min⁻¹. The oven temperature was kept at 50 °C (5 min isothermal) and programmed to 280 °C at a rate of 5 °C min⁻¹, then isothermal at 280 °C for 10 min. The injector temperature was 280 °C. Chemical constituents were identified by co-elution of authenticated samples following co-injection.

Gas chromatography–mass spectrometry (GC-MS) analysis was performed using a PerkinElmer Clarus 680T gas chromatograph–mass spectrometer (Fort Belvoir, VA). An Agilent 30 m × 0.25 mm i.d. ($d_f = 0.5 \mu\text{m}$) DB-5MS capillary column (Folsom, CA) was employed. The oven temperature was kept at 50 °C (5 min isothermal) and programmed to 280 °C at a rate of 5 °C min⁻¹, then isothermal at 280 °C for 10 min. The flow velocity of the helium carrier gas was 1.0 mL min⁻¹. The ion source

temperature was 250 °C. The interface temperature was kept at 260 °C, and mass spectra were obtained at 70 eV. The sector mass analyser was set to scan from 35 to 550 amu every 0.2 s. Chemical constituents were identified by comparison of mass spectra of each peak with those of authentic samples in a mass spectrum library.²⁸

2.5 Contact + fumigant mortality bioassay

A fabric-circle contact + fumigant mortality bioassay described by Yun *et al.*²⁹ was used to evaluate the toxicity of hiba oil and all compounds to adult AHDMs and CMs. Based upon the preliminary test results, 4–5 concentrations of each test material in 50 µL of ethanol were applied to 5 cm diameter black cotton-fabric circles. After drying in a fume hood for 1 min, each fabric circle was placed onto the bottom section of a disposable petri dish (5 cm diameter × 1 cm). Groups of 25–30 adult mites (including both sexes, 7–10 days old) were separately placed onto the treated fabric circles. Each petri dish was then sealed with the original tight-fitting lid and wrapped with Bemis Parafilm M (Neenah, WI). Benzyl benzoate and deet served as positive controls and were similarly formulated. Negative controls (i.e. no test material or acaricide) consisted of 50 µL of ethanol only.

Treated and control (ethanol only) mites were held under the same conditions as those used for colony maintenance. Mortalities were determined 24 h post-treatment under a dissecting microscope (×20). A mite was considered to be dead if its body and appendages did not move when prodded with a fine wooden dowel, as described previously.²⁹ Because not all bioassays could be conducted at the same time, treatments were blocked over time, with a separate control treatment included in each block.³⁰ Freshly prepared solutions were used for each block of bioassays. All treatments were replicated 3 times using 25–30 adults per replicate.

2.6 Vapour-phase mortality bioassay

The closed and open container treatment described by Kim *et al.*²⁶ was used to determine whether the lethality of hiba oil and eight selected constituents against adult AHDMs and CMs was attributable to contact or fumigant action. Groups of 25–30 adult mites (both sexes, 7–10 days old) were placed separately onto untreated cotton-fabric circles on the bottom section of polystyrene containers (5 cm diameter × 2 cm), and each container was sealed with the original tight-fitting lid which had a fine wire screen covering a 4.5 cm diameter central hole. Approximately 2.5-fold or fivefold quantities of the contact + fumigant LC₅₀ values of each test material were applied to 5 cm diameter filter papers, as stated in Section 2.5. Each treated filter paper was placed on top of the wire screen, which prevented direct contact of adult mites with the test material. Each container was sealed either with another solid lid (closed container treatment method) or with another solid lid with a 4.8 cm diameter central hole (open container treatment method) to investigate the potential vapour-phase toxicity of the test materials. Control filter papers received 50 µL of ethanol only. Mortalities were determined 24 h post-treatment, as stated in Section 2.5. All bioassays were replicated 3 times using 25–30 adults per replicate.

2.7 Spray bioassay

A spray bioassay described by Lee *et al.*³¹ was used to evaluate the efficacy of the four experimental spray formulations against adult AHDMs and CMs. Each test solution was sprayed 2 times

successively at 10 cm upwards onto the 5 cm diameter black cotton-fabric circle. After drying for 5 min, each fabric circle was placed onto the bottom section of a disposable petri dish (5 cm diameter × 1 cm). Groups of 40–50 adult mites (both sexes, 7–10 days old) were placed separately onto the treated fabric circles, and each dish was sealed with the original tight-fitting lid. Because permethrin (*cis:trans* 25:75) 2.5 g L⁻¹ spray has been approved by the Ministry of Food and Drug Safety (Osong, Chungbuk, ROK),³² the commercial acaricide served as positive controls. Negative controls consisted of the ethanol–polyoxyethylene + polyoxypropylene (9:1) styrenated phenyl ether solution in distilled water or water. Mortalities were recorded, as stated in Section 2.5. Each treatment was replicated 3 times using 40–50 adults per replicate.

2.8 Data analysis

Data were corrected for control mortality using Abbott's formula.³³ Mortality percentages were transformed to arcsine square root values for analysis of variance. The Bonferroni multiple-comparison method was used to test for significant differences among the treatments.³⁴ Student's *t*-test was used to test for significant differences between the two treatment methods.³⁴ Means ± standard error (SE) of untransformed data are reported. Concentration–mortality data were subjected to probit analysis.³⁴ The LC₅₀ values of each species and their treatments were considered to be significantly different from one another when 95% confidence limits (CLs) did not overlap. A compound having LC₅₀ > 500 µg cm⁻² was considered to be ineffective.

3 RESULTS

3.1 Chemical composition of hiba oil

Hiba oil was composed of four major (≥3.0%) and 15 minor constituents by comparison of mass spectral data and co-elution of authenticated samples following co-injection (Table 3). The four major constituents were (–)-thujopsene (peak 6), β-thujaplicin (peak 13), unknown (peak 12) and unknown (peak 10), and comprised 67.5, 9.3, 5.0 and 3.7% of the oil respectively.

3.2 Toxicity to American house dust mites

The toxicities of hiba oil, 13 commercially available organic pure compounds and two acaricides to adult AHDMs are listed in Table 4. As judged by 24 h LC₅₀ values, hiba oil was 13.5 and 3.6 times less toxic than benzyl benzoate (9.33 µg cm⁻²) and deet (35.53 µg cm⁻²) respectively. (–)-Thujopsene was the most toxic compound, followed by carvacrol. The toxicity of these compounds and benzyl benzoate did not differ significantly. High toxicity was also observed with (+)-terpinen-4-ol, β-thujaplicin, (–)-terpinen-4-ol, cedrol and α-terpineol (LC₅₀ 13.94–15.20 µg cm⁻²). These compounds were significantly more toxic than deet. The toxicity of (–)-α-thujone was nearly identical to that of deet. Low toxicity (LC₅₀ 104.60–287.90 µg cm⁻²) was obtained from the other five compounds. Mortality in the ethanol–surfactant–water-treated or water-treated controls was less than 2%.

3.3 Toxicity to copra mites

The toxic effects of hiba oil, all compounds and two acaricides on adult CMs were likewise compared (Table 5). Based upon 24 h LC₅₀ values, hiba oil was 17.2 and 4.5 times less toxic than benzyl benzoate (10.14 µg cm⁻²) and deet (38.42 µg cm⁻²) respectively. (–)-Thujopsene was the most

Table 3. Chemical composition of hiba oil

Peak number	Compound	RT ^a (min)	Percentage area in GC ^b FID	Identification	
				CI ^c	MS ^d
1	Terpinen-4-ol	19.38	1.05	O ^e	O
2	Isolongifolene	25.23	1.34	O	O
3	β -Elemene	25.46	0.33	X ^f	O
4	α -Cedrene	25.83	1.91	O	O
5	β -Cedrene	26.04	0.95	O	O
6	(-)-Thujopsene ^g	26.31	67.46	O	O
7	Unknown	26.97	0.41		
8	Longipinene	27.09	1.08	O	O
9	Unknown	27.25	2.59		
10	Unknown ^g	27.43	3.65		
11	Unknown	27.62	0.35		
12	Unknown ^g	27.97	5.02		
13	β -Thujaplicin ^g	28.07	9.29	O	O
14	Unknown	28.28	0.10		
15	δ -Cadinene	28.42	0.79	X	X
16	Unknown	28.67	0.60		
17	Unknown	28.96	0.61		
18	Unknown	29.66	0.63		
19	Cedrol	30.33	0.96	O	O

^a Retention time.^b Gas chromatography.^c Co-injection with authentic samples.^d Mass spectrometry.^e Identified by co-injection or mass spectrometry.^f Unidentified by co-injection or mass spectrometry.^g Major constituent (>3%).

toxic compound, followed by (+)-terpinen-4-ol and carvacrol (Table 5). The toxicity of these compounds and benzyl benzoate did not differ significantly. High toxicity was also observed with cedrol, β -thujaplicin, (-)-terpinen-4-ol, α -terpineol and (-)- α -thujone (LC₅₀ 15.82–21.76 $\mu\text{g cm}^{-2}$). The toxicity of these compounds was more pronounced than that of deet. Low (LC₅₀ 96.90–203.84 $\mu\text{g cm}^{-2}$) or no toxicity (>500 $\mu\text{g cm}^{-2}$) was obtained from the other four compounds and one compound respectively. Mortality in the ethanol–surfactant–water-treated or water-treated controls was less than 2%.

3.4 Route of acaricidal action

Because of their good acaricidal activity, the fumigant toxicity of hiba oil and eight selected compounds to adult AHDMs was examined using a vapour-phase mortality bioassay in two formats (Table 6). After 24 h of exposure to 34.85 mg cm^{-3} of (+)-terpinen-4-ol there was a significant difference ($P < 0.0001$) in lethality between exposure in a closed container, which resulted in 100% mortality, and exposure in an open container, which resulted in 11% mortality against AHDMs. Similar differences in the response of adult AHDMs to hiba oil and the other seven compounds in closed versus open container treatments were also observed.

Against adult CMs there was a significant difference ($P < 0.0001$) in toxicity of 31.85 mg cm^{-3} of carvacrol between closed (100% mortality) and open containers (11% mortality) (Table 7). Similar differences in the response of adult CMs to hiba oil and the other seven compounds in closed versus open container treatments were also observed.

3.5 Efficacy of experimental spray formulations

The control efficacy of four experimental spray formulations containing hiba oil and a commercial permethrin (*cis:trans* 25:75) 2.5 g L^{-1} spray against adult AHDMs and CMs was evaluated using a spray bioassay (Table 8). Hiba applied as 30 g L^{-1} spray (HBO-30) provided 100% mortality against adult AHDMs and CMs, whereas the 20 g L^{-1} spray (HBO-20) resulted in $\approx 97\%$ mortality against both mite species. The lethalties of the 10 g L^{-1} (HBO-10) and 5 g L^{-1} sprays (HBO-5) were 87 and 79%, and 47 and 43% against adult AHDMs and CMs respectively. A commercial permethrin spray treatment resulted in 13 and 11% mortality against adult AHDMs and CMs respectively. There was no significant difference in toxicity of the five spray formulations between AHDMs and CMs. There was no mortality for the ethanol–surfactant–water-treated or water-treated dust mites in the spray bioassay.

4 DISCUSSION

Certain essential oil preparations containing the active constituents could be developed as commercial acaricide products suitable for integrated mite management because they can be selective, have few harmful effects on non-target organisms and are environmentally non-persistent.^{10,15} They can also be used in conjunction with biological control.¹⁵ A mixture of complex hydrocarbons (usually terpenoids) and oxygenated compounds (alcohols, aldehydes, esters, ketones, oxides and phenols) exists in essential oils,¹⁴ and jointly or independently the oil constituents contribute to both behavioural efficacy (e.g. repellence and feeding deterrence) and physiological efficacy (e.g. acute toxicity and developmental disruption) against various types of pest complex.¹⁵ Essential oil-derived acaricide products can also be applied to habitats of HDMs and SMs in the same manner as other conventional acaricides.¹⁰ Naturally occurring acaricidal constituents against HDMs have been well documented by Ahn et al.¹⁰

In the present study, the acaricidal principles of hiba oil were determined to be the monoterpenoids terpinen-4-ol and β -thujaplicin and the sesquiterpenoids cedrol and (-)-thujopsene. The toxicity of (-)-thujopsene and carvacrol did not differ from that of benzyl benzoate, and cedrol, (+)-terpinen-4-ol, (-)-terpinen-4-ol, α -terpineol and β -thujaplicin were more toxic than deet. In addition, the 20 and 30 g L^{-1} sprays containing hiba oil resulted in potent control efficacy compared with permethrin (*cis:trans* 25:75) 2.5 g L^{-1} spray. This original finding indicates that hiba oil preparations containing the active constituents described may hold promise for the development of novel and effective products for the control of HDMs and SMs.

Investigations of the modes of action and route of acaricidal action of bioacaricides provide important practical information for mite control, such as the most appropriate formulations and delivery means to be adopted for their future commercialisation and for the development of selective control alternatives with novel target sites.^{10,15} Essential oil volatile constituents, such as alcohols, aldehydes, alkanes, esters, ketones, oxides, phenols and terpenoids, particularly monoterpenoids,¹⁴ primarily act as fumigants with additional contact action against HDMs.¹⁰ Fumigant toxicity to adult AHDMs and CMs has been reported for the phthalide butyridenephthalide^{27,35} and the aryl ketone paeonol.^{36,37} In a separate study, the sesquiterpenoid bisabolangelone was found to be largely toxic through contact action.³⁸

Table 4. Toxicity of hiba oil, 13 organic pure compounds and two acaricides to adult American house dust mites using a contact + fumigant mortality bioassay with 24 h exposure

Compound	LC ₅₀ (µg cm ⁻²) (95% CL)	Slope ± SE	χ ^{2a}	P-value
Hiba oil	126.26 (106.08–150.79)	2.2 ± 0.25	1.66	0.998
(-)-Thujopsene ^{b,c}	9.82 (7.97–12.27)	1.6 ± 0.15	6.89	0.907
Carvacrol ^c	12.05 (10.30–14.01)	2.5 ± 0.38	1.60	0.998
(+)-Terpinen-4-ol ^{b-d}	13.94 (11.97–16.69)	2.2 ± 0.27	4.17	0.989
β-Thujaplicin ^b	14.47 (12.98–16.27)	3.4 ± 0.42	3.13	0.978
(-)-Terpinen-4-ol ^{b-d}	14.51 (12.34–17.69)	2.1 ± 0.26	4.70	0.981
Cedrol ^{b,c}	14.68 (13.32–16.29)	3.9 ± 0.44	3.32	0.972
α-Terpineol ^d	15.20 (13.84–16.85)	3.3 ± 0.34	2.70	0.987
(-)-α-Thujone ^d	33.34 (28.17–39.60)	2.4 ± 0.35	4.25	0.934
(-)-Isolongifolene ^b	104.60 (92.98–117.73)	2.3 ± 0.19	3.55	0.995
β-Cedrene ^b	110.00 (94.15–129.48)	1.9 ± 0.20	4.67	0.912
(+)-α-Longipinene ^{b,c}	139.91 (116.03–169.38)	1.4 ± 0.23	6.27	0.935
(-)-α-Cedrene ^{b,c}	191.52 (169.23–217.12)	2.6 ± 0.23	2.66	0.988
p-Cymene ^d	287.90 (223.41–403.19)	1.4 ± 0.18	2.91	0.998
Benzyl benzoate	9.33 (7.86–10.96)	1.9 ± 0.17	3.55	0.995
Deet	35.53 (30.61–41.61)	2.0 ± 0.17	2.64	0.998

^a Pearson χ², goodness-of-fit test.
^b Constituents identified in this study.
^c Constituents of hiba oil reported by Mehmeti.²⁵
^d Constituents of hiba oil reported by Yatagai *et al.*²²

Table 5. Toxicity of hiba oil, 13 organic pure compounds and two acaricides to adult copra mites using a contact + fumigant mortality bioassay with 24 h exposure

Compound	LC ₅₀ (µg cm ⁻²) (95% CL)	Slope ± SE	χ ^{2a}	P-value
Hiba oil	174.76 (148.37–211.83)	2.0 ± 0.22	1.35	0.999
(-)-Thujopsene ^{b,c}	10.92 (8.71–14.01)	1.5 ± 0.15	4.16	0.989
(+)-Terpinen-4-ol ^{b-d}	12.74 (11.23–14.67)	2.3 ± 0.23	4.56	0.983
Carvacrol ^c	13.60 (11.73–15.95)	2.3 ± 0.36	4.03	0.945
Cedrol ^{b,c}	15.82 (14.27–17.84)	3.4 ± 0.39	4.57	0.917
β-Thujaplicin ^b	15.88 (14.31–17.88)	3.7 ± 0.45	3.04	0.980
(-)-Terpinen-4-ol ^{b-d}	15.90 (13.60–19.37)	2.2 ± 0.23	4.46	0.985
α-Terpineol ^d	16.48 (14.82–18.37)	2.9 ± 0.29	1.82	0.997
(-)-α-Thujone ^d	21.76 (18.89–25.05)	2.5 ± 0.29	4.48	0.922
(-)-Isolongifolene ^b	96.90 (84.98–111.16)	2.3 ± 0.23	1.51	0.998
β-Cedrene ^b	122.10 (100.71–147.20)	1.5 ± 0.16	6.83	0.910
(+)-α-Longipinene ^{b,c}	160.13 (133.27–195.00)	1.3 ± 0.14	6.92	0.906
(-)-α-Cedrene ^{b,c}	203.84 (180.12–231.53)	2.6 ± 0.23	2.18	0.994
p-Cymene ^d	>500			
Benzyl benzoate	10.14 (8.58–11.88)	1.9 ± 0.17	5.64	0.958
Deet	38.42 (33.19–44.95)	2.1 ± 0.17	2.10	0.999

^a Pearson χ², goodness-of-fit test.
^b Constituents identified in this study.
^c Constituents of hiba oil reported by Mehmeti.²⁵
^d Constituents of hiba oil reported by Yatagai *et al.*²²

In the present study, hiba oil and the eight selected compounds described were consistently more effective in closed versus open containers. These results indicate that the route of acaricidal action of these constituents was largely a result of vapour action. The dual contact + fumigant action of hiba oil and its constituents described, as demonstrated through the present vapour-phase mortality and spray bioassays, is of practical importance because it allows the essential oil or its volatile constituents to reach deep refuges in a closed space such as

a building, wardrobe or storage bin, resulting in good control of HDMs and SMs. This dual contact + fumigant action system has advantages over the contact system because exposure to volatile constituents can be easily controlled in a closed space using an appropriate automatic control facility.³⁹ Because of the high volatility of the hiba oil constituents described, the binary mixtures of hiba oil or these constituents and compounds with contact action (e.g. bisabolangelone)³⁸ could be useful in a space where a window is open. Detailed tests are needed

Table 6. Fumigant toxicity of hiba oil and eight selected compounds to adult American house dust mites using a vapour-phase mortality bioassay with 24 h exposure

Compound	Concentration ($\mu\text{g cm}^{-3}$)	Mortality (%) ($\pm$ SE)		P-value ^a
		Vapour in closed container	Vapour in open container	
Hiba oil ^b	315.65	100	13 $\pm$ 1.2	<0.0001
(-)-Thujopsene ^c	49.1	69 $\pm$ 1.5	24 $\pm$ 3.5	<0.0001
Carvacrol ^b	30.13	74 $\pm$ 1.3	7 $\pm$ 4.5	<0.0001
(+)-Terpinen-4-ol ^b	34.85	100	11 $\pm$ 2.2	<0.0001
(-)-Terpinen-4-ol ^b	36.27	100	12 $\pm$ 1.6	<0.0001
β -Thujaplicin ^c	72.34	85 $\pm$ 2.4	20 $\pm$ 3.5	<0.0001
Cedrol ^c	73.4	61 $\pm$ 3.3	18 $\pm$ 1.7	<0.0001
α -Terpineol ^b	38.0	100	7 $\pm$ 2.4	<0.0001
(-)- α -Thujone ^c	166.7	97 $\pm$ 2.8	35 $\pm$ 3.1	<0.0001

^a According to Student's *t*-test.^b Treatment with ca 2.5-fold LC₅₀.^c Treatment with ca fivefold LC₅₀.**Table 7.** Fumigant toxicity of hiba oil and eight selected compounds to adult copra mites using a vapour-phase mortality bioassay with 24 h exposure

Compound	Concentration ($\mu\text{g cm}^{-3}$)	Mortality (%) ($\pm$ SE)		P-value ^a
		Vapour in closed container	Vapour in open container	
Hiba oil ^b	436.9	90 $\pm$ 2.3	11 $\pm$ 2.3	<0.0001
(-)-Thujopsene ^c	54.6	70 $\pm$ 3.9	38 $\pm$ 2.4	<0.0001
Carvacrol ^b	31.85	100	11 $\pm$ 1.9	<0.0001
(+)-Terpinen-4-ol ^b	39.75	100	14 $\pm$ 1.4	<0.0001
(-)-Terpinen-4-ol ^b	34	71 $\pm$ 2.2	9 $\pm$ 2.3	<0.0001
β -Thujaplicin ^c	79.1	69 $\pm$ 1.5	29 $\pm$ 1.8	<0.0001
Cedrol ^c	39.7	62 $\pm$ 5.9	2 $\pm$ 1.2	<0.0001
α -Terpineol ^b	41.2	99 $\pm$ 1.3	8 $\pm$ 2.1	<0.0001
(-)- α -Thujone ^c	108.8	97 $\pm$ 1.7	21 $\pm$ 2.4	<0.0001

^a According to Student's *t*-test.^b Treatment with ca 2.5-fold LC₅₀.^c Treatment with ca fivefold LC₅₀.

to understand fully the mode of action of these active compounds, although the octopaminergic and γ -aminobutyric acid receptors have been suggested as novel target sites for some monoterpenoid essential oil constituents in *Periplaneta americana*¹⁶ and *Helicoverpa armigera*¹⁷ and *Drosophila melanogaster*¹⁸ respectively.

In conclusion, hiba oil preparations containing (-)-thujopsene, carvacrol and terpinen-4-ol could be useful as contact-action fumigants for protection of humans from various allergic diseases caused by HDMs and SMs. An average of 4000 t sawdust year⁻¹ is produced as a byproduct of the timber industry, because hiba is an important timber species in Japan.⁴⁰ There is, therefore, a potential source of crude hiba oil available as an eco-product.⁴¹ For the practical use of hiba-oil-derived materials as novel acaricides to proceed, further research is needed to establish their human safety. In addition, detailed tests are needed to understand how to

Table 8. Effectiveness of four experimental spray formulations containing hiba oil and commercial permethrin against adult American house dust mites and copra mites using a spray bioassay with 24 h exposure

Spray treatment (g L ⁻¹)	Mortality ^a (%) ($\pm$ SE)		P-value ^d
	AHDM ^b	CM ^c	
HBO-5	47 $\pm$ 3.1 c	43 $\pm$ 2.6 c	0.33
HBO-10	87 $\pm$ 1.8 b	79 $\pm$ 3.1 b	0.075
HBO-20	97 $\pm$ 1.5 a	98 $\pm$ 1.3 a	0.90
HBO-30	100 a	100 a	
Permethrin 2.5 ^e	13 $\pm$ 2.7 d	11 $\pm$ 2.6 d	0.69

^a Means within a column followed by the same letter are not significantly different (*P* = 0.05, Bonferroni method).^b American house dust mite.^c Copra mite.^d According to Student's *t*-test.^e Mixture (*cis:trans* 25:75).

improve acaricidal potency and stability for eventual commercial development.

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