

Fumigant toxicity of cassia bark and cassia and cinnamon oil compounds to *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae)

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Received: 7 November 2007 / Accepted: 11 January 2008 / Published online: 5 February 2008
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Abstract The toxicity to adults of the American house dust mite, *Dermatophagoides farinae*, and the European house dust mite, *Dermatophagoides pteronyssinus*, of cassia bark and cassia and cinnamon oil compounds was examined using residual contact and vapour-phase toxicity bioassays. Results were compared with those of the currently used acaricides: benzyl benzoate and dibutyl phthalate. The acaricidal principles of cassia bark were identified as (*E*)-cinnamaldehyde and salicylaldehyde. In fabric-circle residual contact bioassays with adult *D. farinae*, salicylaldehyde (17.3 mg/m²) and (*E*)-cinnamaldehyde (25.8 mg/m²) were 2.5 and 1.7 times more toxic than benzyl benzoate (43.7 mg/m²), respectively, based on 24-h LD₅₀ values. The acaricidal activity was more pronounced in benzaldehyde, menthol, α -terpineol, and thymol (70.8–234.3 mg/m²) than in dibutyl phthalate (281.0 mg/m²). Against adult *D. pteronyssinus*, salicylaldehyde (17.3 mg/m²) and (*E*)-cinnamaldehyde (19.3 mg/m²) were 2.4- and 2.2-fold more active than benzyl benzoate (41.9 mg/m²). The toxicity of benzaldehyde, menthol, α -terpineol, and thymol (75.3–179.2 mg/m²) was higher than that of dibutyl phthalate (285.1 mg/m²). In vapour-phase toxicity tests with adult *D. farinae*, the test compounds described were much more effective in closed—but not in open—containers, indicating that the effect of these compounds was largely a result of action in the vapour phase.

Keywords *Dermatophagoides farinae* · *Dermatophagoides pteronyssinus* · Natural acaricide · Natural fumigant · Cassia · Cinnamon

Introduction

Pyroglyphid mites usually account for >90% of the mite populations in houses. The American house dust mite, *Dermatophagoides farinae* Hughes (Acari: Pyroglyphidae), and the

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European house dust mite, *Dermatophagoides pteronyssinus* Trouessart, are two of the most important pyroglyphid mites because of their cosmopolitan occurrence and abundance in homes (Platts-Mills et al. 1992). The house dust mites are known to be causative agents of diseases that impact millions of people worldwide. They produce various kinds of allergens causing allergic symptoms to sensitive humans such as asthma, atopic dermatitis, conjunctivitis, and rhinitis (Stewart 1995; Arlian 2002). Changes in the living environment of humans, such as central heating, space heating, tighter windows and fitted carpets, have allowed increased dust mite growth (Pollart et al. 1987).

Plant preparations, in part because of their often multiple targets, minor adverse effects, low potentials to cause resistance, and few harmful effects on the environment, have been suggested as alternative sources for dust mite control products (Ahn et al. 2006). They can be applied to dust mite harborage such as beds, mattresses, carpets, and sofas in the same manner as the currently used contact or residual acaricides such as benzyl benzoate, organophosphates, or pyrethroids. Particularly plant essential oils are widely available and some are relatively inexpensive, compared with plant extracts (Isman 2001). Because certain plants and their constituents meet the criteria of reduced risk pesticides (US EPA 2004), much effort has been focused on them as potential sources of commercial acaricidal products. Applications of a 5% cassia oil containing spray to different space volumes and surface areas suggested that 50–60 mg of cassia oil was needed to control house dust mites in 3.4 m³ or in 1 m² (Kim et al. 2006). It was also reported that a single 100% cassia oil-based fumigant device with a 40 µm thick non-woven fabric cover resulted in substantial control in a space of 0.05 m³ and that two fumigant devices gave 88% mortality in a space of 1.73 m³ (Kim et al. 2006). Chemical constituents of essential oils from cassia and cinnamon, *Cinnamomum zeylanicum* Blume, were reported by Kubeczka and Formáček (2002) and Kong et al. (2007). A methanolic extract from the cassia bark was used to determine differences in the acaricidal constituents between the extract and two essential oils.

This study was aimed at isolating acaricidal constituents of the bark from cassia towards adult *D. farinae* and *D. pteronyssinus*. The acaricidal activities of cassia bark and cassia and cinnamon oil compounds were compared with those of the currently used acaricides: benzyl benzoate and dibutyl phthalate. Also the acaricidal mode of delivery of these compounds is discussed.

Materials and methods

Chemicals

Twenty-three compounds were used in this study: camphene, camphor, 3-carene, (*E*)-cinnamaldehyde, cinnamyl alcohol, *p*-cymene, α -terpinene, and thymol purchased from Aldrich (Milwaukee, WI); benzaldehyde, α -caryophyllene, menthol, and salicylaldehyde purchased from Sigma-Aldrich (St. Louis, MO); linalool, α -phellandrene, (+)- α -pinene, (+)- β -pinene, and α -thujone purchased from Fluka (Buchs, Switzerland); β -caryophyllene, limonene, terpinen-4-ol, and terpinolene purchased from Tokyo Chemical Industry (Tokyo, Japan); and 1,8-cineole and α -terpineol purchased from Wako (Osaka, Japan). Benzyl benzoate ($\geq 99.0\%$ purity) and dibutyl phthalate were supplied by Sigma-Aldrich. All other chemicals were of reagent grade and available commercially.

Test mites

The stock cultures of *D. farinae* and *D. pteronyssinus* were maintained in the laboratory for more than 8 years without exposure to any known acaricide. Mites were separately reared in plastic containers (12.5 × 10.5 × 5.0 cm) containing 40 g of sterilized diet (fry feed no. 1/dried yeast, 1:1 by weight). Plastic containers were incubated at 25 ± 1°C and 70–80% relative humidity (RH) in darkness. The fry feed was purchased from Korea Special Feed Meal Co. (Inchon, Korea). The feed consisted of protein (44.0%), lipid (3.0%), cellulose (4.0%), Ca (1.0%) and P (1.8%).

Extraction and isolation

Air-dried bark (1 kg) of cassia was purchased from Boeun medicinal herb shop, Kyung-dong Market (Seoul). The bark was pulverized and extracted twice with methanol (3 l) at room temperature for 2 days and filtered. The combined filtrate was concentrated under vacuum at 40°C to yield ca. 6% (based on the weight of the dried bark). The extract (40 g) was sequentially partitioned into hexane (3.2 l, 12.8 g), chloroform (3.2 l, 3.0 g), ethyl acetate (3.2 l, 3.8 g), butanol (3.2 l, 4.6 g), and water soluble (1.6 l, 15.8 g) portions for subsequent bioassay. The organic solvent portions were concentrated to dryness by rotary evaporation at 40°C and the water portion was freeze-dried.

The most active hexane soluble fraction (10 g) was chromatographed on a 70 × 5.5 cm silica gel column (600 g, 0.063–0.2 mm, Merck, Darmstadt, Germany) and successively eluted with a gradient of hexane and ethyl acetate (99:1, 90:10, 80:20, 70:30, 50:50, 0:100 by volume) and finally with methanol. Column fractions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel plates (Kieselgel 60 F₂₅₄, Merck) with hexane. Fractions with similar *R_f* values on the TLC plates were pooled. Spots were detected by spraying with 10% H₂SO₄ and then heating on a hot plate. The bioactive 90:10 (4 g) and 80:20 (0.4 g) fractions were obtained. For further separation of the constituents from the active fractions, a Spectra System P2000 high-performance liquid chromatograph (HPLC) (Thermo Separation Products, San Jose, CA) was used. The column was a 250 × 5 mm i.d. μ Porasil Silica (Waters, Milford, MA) using a mobile phase of hexane and ethyl acetate (90/10 for 90:10 fraction and 100/1 for 80:20 fraction) at a flow rate of 3 ml/min. Chromatographic separations were monitored using a UV detector at 260 nm. Finally, two potent active principles **1** (3.3 g) from the 90:10 fraction and **2** (0.1 g) from the 80:20 fraction were isolated at retention time of 5.09 and 8.76 min respectively.

The structures of the active isolates were determined by instrumental analyses. ¹H- and ¹³C-NMR spectra were recorded in CD₃OD on a JNM-LA 400 F7 spectrometer (Jeol, Tokyo, Japan), using tetramethylsilane (TMS) as an internal standard, and chemical shifts are given in δ (ppm). UV spectra were obtained in methanol on a UVICON 933/934 spectrophotometer (Kontron Instrument, Milan, Italy) and mass spectra on a GSX 400 spectrometer (Jeol).

Residual contact bioassay

A fabric-circle residual contact toxicity bioassay (Kim et al. 2007) was used to evaluate the toxicity of the test materials to adult *D. farinae* and *D. pteronyssinus* (7–10 days old) at 25 ± 1°C and 70–75% RH. Four to six concentrations of each test material in 20 μ l of ethanol were applied to black cotton-fabric circles (5 cm $\varnothing$). Control fabric circles received 20 μ l of ethanol. After drying in a fume hood for 20 s, each fabric circle was placed on the

bottom of a Petri dish (5×1 cm). Groups of 25 adult mites (including both sexes) were separately placed on the fabric and each Petri dish was sealed with a lid. Benzyl benzoate and dibutyl phthalate served as positive controls for comparison in residual contact toxicity tests.

Treated and control (ethanol only) mites were held at the same conditions as used for colony maintenance. Mortalities were evaluated 24 h post-treatment under a binocular microscope ($\times 20$). Mites were considered to be dead if appendages did not move when they were prodded with wooden dowels. All treatments were replicated four times.

Vapour-phase toxicity bioassay

The closed and open container treatment methods (Kim et al. 2007) were used to determine whether the lethal activity of the test compounds towards adult *D. farinae* (7–10 days old) was attributed to contact toxicity or fumigant action. Briefly groups of 25 adults were placed on the bottom of a Petri dish (5×1 cm) and covered with a lid that had a fine wire screen (200 mesh, 4 cm $\varnothing$) covering a central hole (3 cm $\varnothing$). Approximately twofold amounts of the contact LD_{50} values of each test compound in 20 μ l of ethanol were applied to black cotton-fabric circles (5 cm $\varnothing$). The treated fabric circle was then placed on top of the wire screen, which prevented direct contact of mites with the test compound. Each Petri dish was either sealed with a solid lid to investigate the potential vapour-phase toxicity of the test compounds (closed container treatment method) or sealed with a lid with a central hole (4.8 cm $\varnothing$) (open container treatment method). Control fabric circles in each format received 20 μ l of ethanol. Mortalities were determined 24 h post-treatment. All bioassays were replicated four times.

Data analysis

Mortality percentages were transformed to arcsine square root values for analysis of variance (ANOVA). The Scheffé's test was used to test for significant differences among the cassia bark-derived materials (SAS Institute 2004). A *t*-test was used to test for significant differences between two treatment methods (SAS Institute 2004). Means ($\pm$ SE) of untransformed data are reported. LD_{50} values were calculated by probit analysis (SAS Institute 2004). Acaricidal activity was considered to be significantly different when 95% confidence limits (CL) of the LD_{50} values failed to overlap. Relative toxicity (RT) was determined as the ratio of benzyl benzoate LD_{50} /the other compound LD_{50} . Susceptibility ratio (SR) was determined as the ratio of *D. farinae* LD_{50} /*D. pteronyssinus* LD_{50} as previously described (Kim et al. 2007).

Results

Bioassay-guided fractionation and isolation

Fractions obtained from the methanolic extract of cassia bark were bioassayed by the direct contact application (Table 1). Significant differences in lethal activity in fractions of the extract were observed, and they were used to identify peak activity fractions for the next step in the purification. At 509.6 mg/m², the hexane and chloroform soluble fractions showed 100% mortality towards adult *D. farinae* and *D. pteronyssinus*. There was no mortality in the ethanol-treated controls.

Table 1 Lethal activity of cassia bark-derived materials towards adults of two *Dermatophagoides* spp. using residual contact toxicity bioassay during a 24-h exposure at 509.6 mg/m²

Material	Mortality (mean $\pm$ SE) ^a , %	
	<i>D. farinae</i>	<i>D. pteronyssinus</i>
Methanolic extract	100 (0.0)a	100 (0.0)a
Hexane fraction	100 (0.0)a	100 (0.0)a
Chloroform fraction	100 (0.0)a	100 (0.0)a
Ethyl acetate fraction	5 (1.3)b	3 (2.7)b
Butanol fraction	7 (1.3)b	7 (1.3)b
Water fraction	4 (2.3)b	4 (0.0)b

^a Means within a column followed by the same letter are not significantly different ($P = 0.05$, Scheffé's test)

Fabric-circle residual contact toxicity bioassay-guided fractionation of cassia bark extract afforded two active principles identified by spectroscopic analysis, including MS and NMR. The bioactive constituents were characterized as the phenylpropanoid (*E*)-cinnamaldehyde (**1**) and the benzaldehyde salicylaldehyde (**2**). (*E*)-Cinnamaldehyde was identified based on the following evidence: EI-MS (70 ev), m/z (relative intensity): 132 [M]⁺ (76), 131 (100, base peak); ¹H-NMR (CD₃OD, 600 MHz): δ 6.72, 7.20–7.47, 7.49, 7.55, 9.70; ¹³C NMR (CD₃OD, 150 MHz): δ 128.9 d, 129.4 d, 130.1 d, 131.7 d, 134.7 s, 153.3 d, 194.2 d. Similarly structure of salicylaldehyde was determined.

Acaricidal activity of test compounds

The toxicity of 23 test compounds and two acaricides (benzyl benzoate and dibutyl phthalate) was evaluated by comparing the LD₅₀ values estimated from the fabric-circle residual contact toxicity bioassay to adult *D. farinae* (Table 2). As judged by 24-h LD₅₀ values, salicylaldehyde (17.3 mg/m²) was the most toxic compound and was 2.5 times more toxic than benzyl benzoate (43.7 mg/m²). (*E*)-Cinnamaldehyde (25.8 mg/m²) was 1.7-fold more toxic than benzyl benzoate. The acaricidal activity of benzaldehyde, cinnamyl alcohol, menthol, and thymol (70.8–234.3 mg/m²) were higher than that of dibutyl phthalate (281.0 mg/m²). The acaricidal activity of α -terpineol and α -caryophyllene was comparable to that of dibutyl phthalate, whereas β -caryophyllene, linalool, and terpinen-4-ol were slightly less active than dibutyl phthalate. Weak to no acaricidal activity were observed with the other 12 compounds.

Toxic effects on adult *D. pteronyssinus* of 23 test compounds and two acaricides were examined (Table 3). Based on 24-h LD₅₀ values, salicylaldehyde (17.3 mg/m²) and (*E*)-cinnamaldehyde (19.3 mg/m²) were 2.4- and 2.2-fold more toxic than benzyl benzoate (41.9 mg/m²). The acaricidal activity was more pronounced in benzaldehyde, linalool, menthol, terpinen-4-ol, α -terpineol, and thymol (75.3–179.2 mg/m²) than in dibutyl phthalate (285.1 mg/m²). The toxicity of α - and β -caryophyllene and cinnamyl alcohol was comparable to that of dibutyl phthalate. The other 12 compounds exhibited weak to no acaricidal activity.

Route of acaricidal action

The fumigant toxicity to adult *D. farinae* of 13 active compounds was examined using the vapour-phase toxicity bioassay in two formats (Table 4). After 24 h of exposure to 25.5

Table 2 Toxicity of 23 test compounds and two acaricides to adult *D. farinae* using residual contact toxicity bioassay during a 24-h exposure

Compound	No. mites	Slope ($\pm$ SE)	LD ₅₀ (95% CL), mg/m ²	χ^2	RT ^b
Benzaldehyde	300	6.1 (0.72)	75.9 (69.7–81.9)	0.72	0.58
Camphene	100		>2000		
Camphor	400	6.1 (0.54)	619.3 (580.9–657.5)	0.38	0.07
3-Carene	100		>2000		
1,8-Cineole	100		>2000		
α -Caryophyllene	300	2.1 (0.39)	259.1 (220.4–313.2)	0.39	0.17
β -Caryophyllene	500	2.9 (0.29)	388.2 (346.8–433.5)	0.29	0.11
(<i>E</i>)-Cinnamaldehyde ^a	300	5.7 (0.51)	25.8 (23.7–27.9)	0.44	1.69
Cinnamyl alcohol	300	2.7 (0.33)	221.2 (191.1–252.7)	0.90	0.20
<i>p</i> -Cymene	100		>2000		
Limonene	100		>2000		
Linalool	500	2.5 (0.24)	494.0 (416.2–628.6)	0.30	0.09
Menthol	500	4.5 (0.44)	113.5 (106.0–121.9)	0.11	0.39
α -Phellandrene	100		>2000		
(+)- α -Pinene	100		>2000		
(+)- β -Pinene	100		>2000		
Salicylaldehyde ^a	400	9.9 (1.30)	17.3 (12.1–19.7)	0.08	2.53
α -Terpinene	100		>2000		
Terpinen-4-ol	300	2.3 (0.40)	304.8 (261.8–377.1)	0.21	0.14
α -Terpineol	300	3.3 (0.36)	220.5 (195.9–246.5)	0.38	0.20
Terpinolene	100		>2000		
α -Thujone	300	7.4 (0.78)	816.6 (775.6–862.9)	0.21	0.05
Thymol	400	3.1 (0.37)	70.8 (63.2–81.1)	0.21	0.62
Benzyl benzoate	400	1.5 (0.20)	43.7 (29.8–55.2)	0.11	1.00
Dibutyl phthalate	300	4.4 (0.40)	281.0 (258.3–306.2)	0.93	0.16

^a Cassia bark compounds identified in this study. The other compounds were derived from cassia and cinnamon oil reported by Kubeczka and Formáček (2002) and Kong et al. (2007)

^b Relative toxicity

$\mu\text{g}/\text{cm}^3$, there was a significant difference in acaricidal activity of benzaldehyde between exposure in a closed (100% mortality) versus in an open container (0% mortality) to adult *D. farinae*. Similar differences in the response of adult *D. farinae* to the other 12 compounds in closed and open container treatments were likewise observed.

Discussion

Mono and sesquiterpenoids and related phenols exist in essential oils (Isman 2000, 2001; Lawless 2002) and jointly or independently they contribute to a variety of biological activities, including repelling or attracting dust mites and acaricidal activity (Ahn et al. 2006). Many plant preparations and their constituents manifest acaricidal activity towards *D. farinae* and *D. pteronyssinus* (Ahn et al. 2006). Additionally certain plant preparations or their constituents can be highly effective against insecticide resistant insect pests (Lindquist et al. 1990; Ahn et al. 1997). Much effort has been focused on determining the distribution, nature, and practical use of plant-derived substances that have acaricidal activity. Naturally occurring acaricidal compounds against the house dust mites have been well described by Ahn et al. (2006). Additionally it has been reported that susceptibility to six Lauraceae plant leaf oils was greater in adult *D. farinae* than adult *D. pteronyssinus* (Furuno et al.

Table 3 Toxicity of 23 test compounds and two acaricides to adult *D. pteronyssinus* using residual contact toxicity bioassay during a 24-h exposure

Compound	No. mites	Slope ($\pm$ SE)	LD ₅₀ (95% CL), mg/m ²	χ^2	RT ^b	SR ^c
Benzaldehyde	400	5.4 (0.56)	75.3 (69.0–80.8)	0.25	0.56	1.0
Camphene	100		>2000			
Camphor	300	6.2 (0.71)	607.7 (568.6–652.3)	0.13	0.07	1.0
3-Carene	100		>2000			
1,8-Cineole	100		>2000			
α -Caryophyllene	300	3.0 (0.35)	320.8 (283.8–368.9)	0.15	0.13	0.8
β -Caryophyllene	500	2.2 (0.24)	375.1 (309.9–433.7)	0.71	0.11	1.0
(<i>E</i>)-Cinnamaldehyde ^a	300	7.9 (0.78)	19.3 (18.2–20.3)	0.65	2.17	1.3
Cinnamyl alcohol	300	4.3 (0.50)	292.5 (265.9–330.0)	0.42	0.14	0.8
<i>p</i> -Cymene	100		>2000			
Limonene	100		>2000			
Linalool	300	3.2 (0.41)	446.4 (391.8–531.0)	0.42	0.09	1.1
Menthol	400	4.7 (0.54)	158.9 (148.4–171.8)	0.11	0.26	0.7
α -Phellandrene	100		>2000			
(+)- α -Pinene	100		>2000			
(+)- β -Pinene	100		>2000			
Salicylaldehyde ^a	400	9.9 (1.71)	17.3 (12.0–19.8)	0.07	2.42	1.0
α -Terpinene	100		>2000			
Terpinen-4-ol	300	2.3 (0.40)	303.2 (259.9–376.2)	0.40	0.14	1.0
α -Terpineol	300	2.7 (0.40)	256.9 (226.1–295.8)	0.68	0.16	0.9
Terpinolene	100		>2000			
α -Thujone	300	8.3 (0.86)	821.5 (783.6–863.2)	0.45	0.05	1.0
Thymol	300	3.6 (0.45)	75.4 (65.0–84.5)	0.21	0.56	0.9
Benzyl benzoate	500	4.2 (0.38)	41.9 (36.5–46.3)	0.11	1.00	1.0
Dibutyl phthalate	500	2.5 (0.23)	285.1 (254.9–318.8)	0.16	0.15	1.0

^a For explanation, see table 2^b Relative toxicity^c Susceptibility ratio**Table 4** Fumigant toxicity of 13 active compounds to adult *D. farinae* using vapour-phase toxicity bioassay during a 24-h exposure

Compound	Mortality (mean $\pm$ SE), %		
	Dose, μ g/cm ³	Closed containers	Open containers
Benzaldehyde	25.5	100 (0.0)	0 (0.0) ^a
Camphor	152.7	100 (0.0)	0 (0.0) ^a
α -Caryophyllene	50.9	100 (0.0)	0 (0.0) ^a
β -Caryophyllene	101.8	100 (0.0)	0 (0.0) ^a
(<i>E</i>)-Cinnamaldehyde	5.1	100 (0.0)	0 (0.0) ^a
Cinnamyl alcohol	50.9	100 (0.0)	22 (3.8) ^a
Linalool	101.8	100 (0.0)	0 (0.0) ^a
Menthol	25.5	100 (0.0)	0 (0.0) ^a
Salicylaldehyde	5.1	100 (0.0)	0 (0.0) ^a
Terpinen-4-ol	101.8	100 (0.0)	13 (2.5) ^a
α -Terpineol	50.9	100 (0.0)	1 (1.0) ^a
α -Thujone	152.7	100 (0.0)	0 (0.0) ^a
Thymol	25.5	100 (0.0)	16 (1.6) ^a

^a Significant at $P < 0.0001$, according to a *t*-test

1994). However, adult *D. farinae* is found to be more tolerant to eugenol, isoeugenol, and methyleugenol than adult *D. pteronyssinus* (Kim et al. 2003). No significant difference in toxicity of either butylidenephthalide (Kwon and Ahn 2002) or paeonol and benzoic acid (Kim et al. 2004) between *D. farinae* and *D. pteronyssinus* were reported. In the present study, the acaricidal principles of cassia bark were the phenylpropanoid (*E*)-cinnamaldehyde and the benzaldehyde salicylaldehyde. Of the test compounds, (*E*)-cinnamaldehyde and salicylaldehyde were more toxic than benzyl benzoate against both mite species. The acaricidal activity was more pronounced in benzaldehyde, menthol, and thymol than in dibutyl phthalate. Additionally there was no significant difference in toxicity of the test compounds between *D. farinae* and *D. pteronyssinus*. These compounds described might be good candidates for naturally occurring dust mite control products.

Investigation on acaricidal mode of action of natural acaricidal compounds and acaricides is of practical importance for dust mite control because it may give useful information on the most appropriate formulations and delivery means. Volatile compounds of many plant extracts and essential oils are composed of alkanes, alcohols, aldehydes and terpenoids, particularly monoterpenoids (Visser 1986). Many of them exhibit fumigant activity (Ahn et al. 2006). For example, fumigant toxicity to adult *D. farinae* and *D. pteronyssinus* has been reported for butylidenephthalide (Kwon and Ahn 2002); eugenol, isoeugenol and methyleugenol (Kim et al. 2003); paeonol (Kim et al. 2004); and atractylon and atractylonolide III (Kim et al. 2007). However, bisabolangelone was found to be largely toxic through contact action (Kang et al. 2006). In the present study with adult *D. farinae*, the 14 active compounds, including (*E*)-cinnamaldehyde and salicylaldehyde, were much more effective in closed but not open containers. These results indicate that the mode of delivery of these compounds was largely caused by action in the vapour phase.

Acknowledgements This work was supported by grants from Plant Diversity Research Center of 21st Century Frontier Research Program (M106KD010024–07K0401–02410) funded by Ministry of Science and Technology of Korean Government and the Ministry of Education & Human Resources Development for Brain Korea 21 Project of the Korean Government to YJA.

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