J. Microbiol. Biotechnol. (2004), **14**(4), 805–809

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Antimite Activity of Cumin Volatiles Against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae)

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Received: January 21, 2004
Accepted: March 2, 2004

Abstract The antimite activities of cumin seed oil-derived cuminaldehyde and eleven commercial components of *Cuminum cyminum* oil were examined against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* adults and compared with those of benzyl benzoate and *N,N*-diethyl-*m*-toluamide. Responses varied according to dose and mite species. On the basis of LD_{50} values, the compound most toxic to *D. farinae* adults was cuminaldehyde (2.40 μg/cm^2) followed by benzyl benzoate (9.32 μg/cm^2), thymol (9.43 μg/cm^2), DEET (36.84 μg/cm^2), and 3-carene (42.11 μg/cm^2). Against *D. pteronyssinus* adults, cuminaldehyde (1.94 μg/cm^2) was much more effective than benzyl benzoate (6.50 μg/cm^2), thymol (6.92 μg/cm^2), DEET (17.79 μg/cm^2), and 3-carene (39.85 μg/cm^2). These results indicate that the antimite activity of cumin seed oil could be caused by cuminaldehyde. Cuminaldehyde was about 3.9 and 3.4 times more toxic than benzyl benzoate against *D. farinae* and *D. pteronyssinus* adults, respectively. Therefore, further study is needed to confirm the findings of this study and the possibility of cuminaldehyde as a house dust mite control agent or a lead compound.

Key words: Antimite activity, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Cuminum cyminum*, cumin seed oil, cuminaldehyde

The most important pyroglyphid mites are *Dermatophagoides pteronyssinus* (Trouessart) and *Dermatophagoides farinae* (Hughes) for the following three reasons: (1) Their cosmopolitan occurrence and abundance; (2) They are a major source of multiple potent allergens; (3) Their causal association with sudden infant death syndrome [2, 6, 9, 22, 26]. Living environmental changes (such as a rise in the number of apartment households with central-heating, space-heating, tighter windows, and wall-to-wall carpeting) have improved conditions for the growth of dust mites [26]. Control of these mite populations has been principally through the use of chemicals such as benzyl benzoate and *N,N*-diethyl-*m*-toluamide (DEET) [26]. Although they are effective, their repeated use has sometimes resulted in the widespread development of resistance [19, 21, 26], had undesirable effects on nontarget organisms, and fostered environmental and human health concerns [8, 26]. These problems have highlighted the need for the development of new strategies for selective control of dust mites.

Plants may be an alternative to currently used antimite and insecticidal agents to control dust mites and specific pests [11, 12, 24, 25]. Since these are often active against a limited number of specific target species, biodegradable to nontoxic products, and potentially suitable for use in integrated management programs, they could lead to the development of new classes of possibly safer pest control agents. Therefore, much effort has been focused on plants or phytochemicals as potential sources of commercial mite-control agents [13, 15, 18]. In traditional Egyptian medicine, cumin oil has long been considered to have biological properties such as an antimicrobial activity, a food spice, a fungicide, and a tyrosinase inhibitor [3, 5, 16]. However, little work has been done to control house dust mites or restore their damage by using cumin seed oil in spite of its excellent biological actions [3, 5, 16]. I assessed *in vivo* the antimite activities of cumin seed oil-derived materials, the synthetic antimite benzyl benzoate and DEET, and eleven commercial components of cumin seed oil against *D. farinae* and *D. pteronyssinus* adults.

Materials and Methods

Chemicals

Benzyl benzoate and DEET were purchased from Aldrich (Milwaukee, WI, U.S.A.). 3-Carene, β-caryophyllene, cumic acid, cuminaldehyde, *p*-cymene, β-myrcene, α-phellandrene,

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α-pinene, β-pinene, sabinene, γ-terpinene, and thymol were supplied by Sigma (St. Louis, MO, U.S.A.). All other chemicals were of reagent grade.

Dust Mite

Cultures of *D. farinae* and *D. pteronyssinus* were maintained in the laboratory for 5 years without exposure to any known acaricide. They were reared in plastic containers (15×12×6 cm) containing 30 g of sterilized diet (fry feed no. 1/dried yeast, 1:1 by weight) at 25±1°C and 75% relative humidity in darkness. The fry feed was purchased from Korea Special Feed Meal Co. Ltd., Chonju, South Korea.

Extraction and Identification

The essential oil (yield 9.8%) of *C. cyminum* seeds (300 g) was extracted by steam distillation as previously described by Hwang and Lee [10]. A preparative HPLC (Spectra System P2000, Thermo Separation Products) was used for separation of the biologically active constituents from the oil (27 mg). The column was mPorasil (20 mm i.d.×500 mm, Waters, Milford, MA, U.S.A.) using hexane/ethyl acetate (7:3) at a flow rate of 1.5 ml/min and detection at 260 nm. In this step, five fractions (P1, P2, P3, P4, and P5) were obtained and bioassayed at 40 μg/cm^2 as described below. The active P3 fraction (8.9 mg, 100% mortality) was rechromatographed under the same condition. Finally, an active compound (7.2 mg) at the retention time of 9.8 min was isolated. The structure of the active isolate was determined by spectroscopic analyses. ^{1}H and ^{13}C NMR spectra were recorded in deuterochloroform with a JNM-LA 400F7 spectrometer at 400 and 100 MHz, respectively. UV spectra were obtained in methanol with a Jasco V-550 spectrometer and EI-MS spectra on a JEOL GSX 400 spectrometer.

Gas Chromatography-Mass Spectrometry

The oil of *C. cyminum* seeds was analyzed on a gas chromatograph (HP6890)-mass spectrometer (JMS-600W, JEOL) (GC-MS). The GC column was a 60 m×0.25 mm i.d. DB-WAX (0.25 μm film) fused silica capillary column (J&W Scientific, Folsom, CA, U.S.A.). The GC conditions were as follows: injector temperature, 210°C; column temperature, isothermal at 50°C for 15 min then programmed to 200°C at 2°C/min and held at this temperature for 15 min; ion source temperature, 200°C. Helium was used as a carrier gas at a rate of 0.8 ml/min. The 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 seconds. Compounds such as 3-carene, β-caryophyllene, cumic acid, cuminaldehyde, *p*-cymene, β-myrcene, α-phellandrene, α-pinene, β-pinene, sabinene, γ-terpinene, and thymol were identified by comparison with retention times and the mass spectra obtained with the authentic standards on the GC-MS system used for analysis. When an authentic sample was not available, the identification was carried out by comparison of mass spectra with those in the mass spectra library (*The Wiley Registry of Mass Spectral Data*, 6th Ed.).

Bioassays

An impregnated fabric disk bioassay was used for acaricidal activity of test materials. Various amounts (80, 40, 30, 20, 10, 5, 2.5, 2.0, 1.5, 1.25 mg/cm^2) of each test material dissolved in 20 μl of ethanol were applied to disks of black cotton fabric (5 cm diameter). Control fabric disks received 20 μl of ethanol. After drying in a fume hood for 30 sec, each piece was placed in the bottom of a Petri dish (5 cm diameter×1.2 cm). Then, 30 individuals of *D. farinae* (7-10 days old adults) and *D. pteronyssinus* (7-10 days old adults) were separately placed in each Petri dish and covered with a lid. Treated and control mites were held at 25±1°C and 75% relative humidity in darkness. Mortalities were determined 24, 48, and 72 h after treatment under a binocular microscope (20×). Mites were considered to be dead if appendages did not move when prodded with a pin. All treatments were replicated three times. The LD_{50} values were calculated by probit analysis [23].

Statistical Analysis

The percentage of mortality was determined and transformed to arcsine square-root values for analysis of variance (ANOVA). Treatment means were compared and separated by Scheffe's test at $P<0.05$ [23]. Means (±SE) of untransformed data are reported.

RESULTS AND DISCUSSION

When cumin oil derived from *C. cyminum* seeds was bioassayed by direct contact, antimite activity of cumin oil was observed in various doses and exposure times (24, 48, and 72 h after treatment) against *D. farinae* and *D. pteronyssinus* adults (Table 1). Responses varied according to dose rather than exposure time. In a test with *D. farinae* and *D. pteronyssinus* adults, cumin oil gave 100% mortality at 40 and 20 μg/cm^2, respectively, 24 h after treatment. Furthermore, the antimite activity of cumin oil was 45 and 51% mortality at 10 μg/cm^2 against *D. farinae* and *D. pteronyssinus* adults, respectively. There was no mortality in the untreated controls.

The substances identified by GC-MS in cumin seed oil are presented in Table 2. Analysis led to identification of 14 volatiles from cumin seed oil. The main constituents were acoradiene (14.3%), 3-carene (5.3%), caryophyllene (4.6%), cumic acid (7.9%), cuminaldehyde (24.3%), *p*-cymene (19.1%), β-myrcene (0.3%), α-phellandrene (1.1%), α-

Table 1. Acaricidal activity of cumin seed oil against *D. farinae* and *D. pteronyssinus* adults.

Mite species	Dose μg/cm²	Mortality[a] (%, mean±SE)		
		24 h	48 h	72 h
D. farinae	80	100±0.0[a]	100±0.0[a]	100±0.0[a]
	40	100±0.0[a]	100±0.0[a]	100±0.0[a]
	20	100±0.0[a]	100±0.0[a]	100±0.0[a]
	10	45±3.4[b]	49±2.9[b]	50±2.2[b]
D. pteronyssinus	80	100±0.0[a]	100±0.0[a]	100±0.0[a]
	40	100±0.0[a]	100±0.0[a]	100±0.0[a]
	20	100±0.0[a]	100±0.0[a]	100±0.0[a]
	10	51±3.1[b]	55±2.6[b]	56±2.0[b]

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

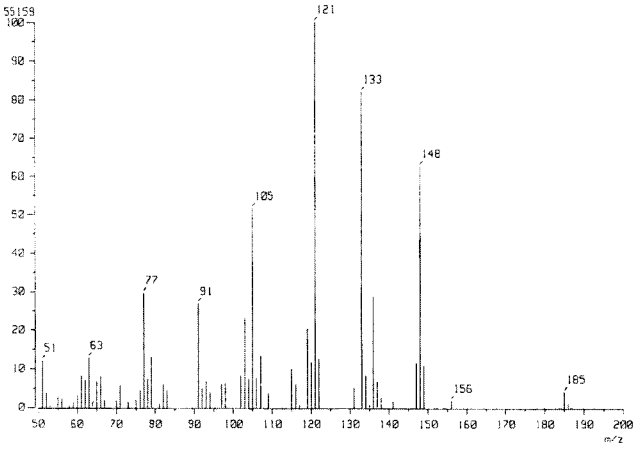

Fig. 1. Mass spectrum of cuminaldehyde isolated from cumin seed oil.

pinene (1.4%), β-pinene (7.9%), pulegone (0.5%), sabinene (1.5%), γ-terpinene (7.4%), and thymol (1.3%). The composition of cumin oil was extensively investigated many years ago. According to Anon's report [1], the main constituents of Egyptian cumin seed oil were cuminaldehyde, β-pinene, γ-terpinene, ρ-mentha-1,3-dien-7-al, ρ-mentha-1,4-dien-7-al, and *p*-cymene. The composition of cumin oil of Turkish origin was investigated by Borges and Pino [4], who found that Turkish cumin oil was characterized by high amounts of cuminaldehyde, ρ-mentha-1,3-dien-7-al, ρ-mentha-1,4-dien-7-al, γ-terpinene, *p*-cymene, β-pinene, and perillaldehyde [4].

Bioassay-guided fractionation of cumin oil afforded an active constituent identified by spectroscopic analyses including EI-MS and NMR, and by direct comparison with the authentic compound. As a result, the active constituent was identified as cuminaldehyde (Figs. 1-3). The spectral analyses of cuminaldehyde are identical to the data of cuminaldehyde isolated from cumin oil [4, 16]. The antimite activities of cuminaldehyde, 3-carene, caryophyllene, cumic acid, *p*-cymen, β-myrcene, α-phellandrene, α-pinene, β-pinene, sabinene, γ-terpinene, and thymol observed from cumin oil was compared with those of benzyl benzoate and DEET against *D. farinae* and *D. pteronyssinus* adults (Table 3). The commonly used benzyl benzoate and DEET served as a standard of comparison for the antimite activity. On the basis of LD_{50} values, the most toxic compound against *D. farinae* and *D. pteronyssinus* adults was cuminaldehyde followed by benzyl benzoate, thymol, DEET, and 3-carene. However, no activity was observed for caryophyllene, cumic acid, *p*-cymene, β-myrcene, α-phellandrene, α-pinene, β-pinene, sabinene, and γ-terpinene (not shown). These results indicate that the antimite activity of cumin oil could be caused by cuminaldehyde. Cuminaldehyde was about 3.9 and 3.4 times more

Table 2. Volatile compounds in cumin seed oil identified by gas chromatography-mass spectrometry.

Peak number	Compound	Mass spectral data[a]	Retention time (min)	Relative (%)
1	Cumic acid	77, 103, 105, 119, 149	1:30	7.9
2	α-Pinene	93, 77, 41, 27, 121	6:96	1.4
3	β- Pinene	69, 93, 121, 136	9:54	7.9
4	β–Myrcene	93, 41, 69,27, 53	10:98	0.3
5	α-Phellandrene	93, 77, 136, 41, 27	11:79	1.1
6	*p*-Cymene	93, 119, 121, 134, 154	13:92	19.1
7	γ-Terpinene	93, 121, 136	17:99	7.4
8	Pulegone	77, 82, 109, 137, 152	30:01	0.5
9	Cuminaldehyde	77, 91, 105, 121, 148	33:88	24.3
10	3-Carene	77, 79, 91, 93	36:92	5.3
11	Caryophyllene	204, 176, 148, 133, 107	42:86	4.6
12	Thymol	77, 91, 115, 135, 150	45:96	1.3
13	Sabinene	93, 77, 41, 27, 121	47:41	1.5
14	Acoradiene	41, 55, 93, 119, 147	49:32	14.3

[a]Major fragmentation ions, base peak (listed first), and other ions in decreasing order of relative abundance.

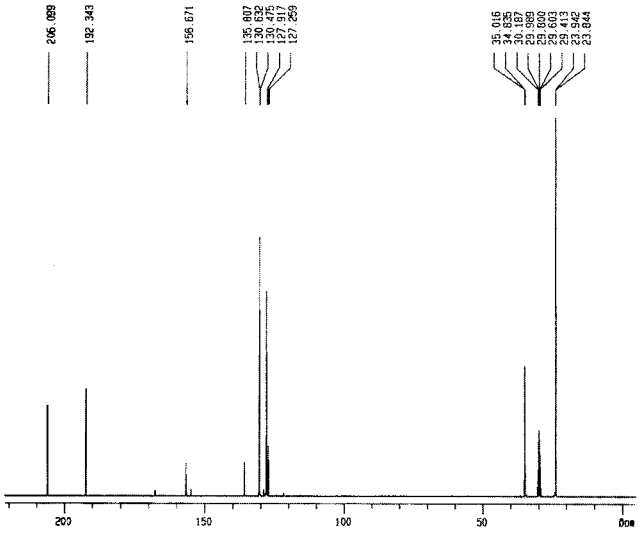

Fig. 2. ^{13}C NMR spectrum of cuminaldehyde isolated from cumin seed oil.

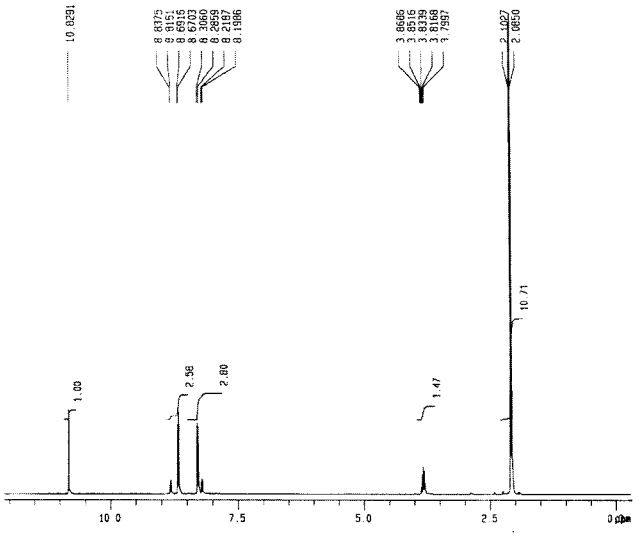

Fig. 3. ^{1}H NMR spectrum of cuminaldehyde isolated from cumin seed oil.

toxic than benzyl benzoate against *D. farinae* and *D. pteronyssinus* adults, respectively. In addition, the antimite activity of thymol was comparable to that of benzyl benzoate.

Plants are potential products for dust mite control because many of them are selective to pests, have no or little harmful effects on nontarget organisms and the environment [11, 12], and may be applied to dust mite nests such as beds, carpeted floors, furniture, and sofas in the same way as other conventional antimite agents [22]. Moreover, many plant extracts and phytochemicals are known to possess antimite activity against dust mites [7, 14, 17, 20]. The reported naturally occurring antimite compounds against dust mites include anisaldehyde and perillaldehyde derived from perilla [3] and acryophyllene oxide, α-cadinol, and isosericenine from the leaves from *Neolitsea sericea* [5]. Also, it has been reported that susceptibility to some plants such as almond bitter, caraway, and perilla was greater in *D. farinae* adults than in *D. pteronyssinus* adults [14]. However, *D. farinae* adults are found to be more tolerant to the wood of *Thuga heteropylla* Sarg. and *Cryptomeria japonica* D. Don than *D. pteronyssinus* adults [20]. No significant difference in antimite activity of either *trans*-cinnamaldehyde, cinnamyl alcohol, or salicylaldehyde between *D. farinae* and *D. pteronyssinus* has been observed [14]. Similar results in butylidenephthalide have been also reported [17]. Results of the findings of this study and earlier studies indicate that cumin seed oil-derived cuminaldehyde could be useful as antimite agents against *D. farinae* and *D. pteronyssinus*. Further research should be done on safety issues of this compound for human health, antimite mode of action, and formulations improving the acaricidal potency and stability.

Table 3. Acaricidal activity of commercial constituents derived from cumin seed oil and acaricides against *D. farinae* and *D. pteronyssinus* adults[a].

Compound	Mite species	Slope (±SE)	LD_{50} (μg/cm^2)	95% Confidence limit	RT[b]
Cuminaldehyde	*D. farinae*	5.02±0.58	2.40	2.26-2.51	3.9
	D. pteronyssinus	8.45±1.86	1.94	1.82-2.09	3.4
3-Carene	*D. farinae*	2.72±0.48	42.11	39.8-45.2	0.2
	D. pteronyssinus	3.56±0.74	39.85	37.09-42.02	0.2
Thymol	*D. farinae*	6.85±0.81	9.43	9.46-9.95	1.0
	D. pteronyssinus	5.88±0.86	6.92	6.71-7.15	0.9
Benzyl benzoate	*D. farinae*	6.48±0.84	9.32	8.79-9.92	1.0
	D. pteronyssinus	3.32±0.51	6.50	5.49-7.53	1.0
DEET	*D. farinae*	5.07±0.81	36.84	34.75-40.88	0.3
	D. pteronyssinus	3.01±0.47	17.79	14.89-21.89	0.4

[a]Exposed for 24 h.
[b]Relative toxicity=LD_{50} value of benzyl benzoate/LD_{50} value of each chemical.

Acknowledgment

This work was supported by Korea Research Foundation Grant (KRF-2003-041-F20010).

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