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NOTE

Acaricidal and insecticidal activities of essential oils of *Cinnamomum zeylanicum* barks cultivated from France and India against *Dermatophagoides* spp., *Tyrophagus putrescentiae* and *Ricania* sp.

Ye-Jin Jeon[1] · Sang-Guei Lee[2] · Hoi-Seon Lee[1]

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Abstract The chemical composition of *Cinnamomum zeylanicum* bark oils cultivated from France and India was analyzed by GC–MS. The main components of *C. zeylanicum* oil were cinnamaldehyde (63.97 and 67.21%) and eugenol (6.84 and 19.79%) from France and India, respectively. Acaricidal and insecticidal activities of *C. zeylanicum* oils against *Dermatophagoides* sp, *T. putrescentiae* and *Ricania* sp. were evident. The LC_{50} values of *C. zeylanicum* oil were 123.77 and 93.06 mg/L in samples from France and India, respectively, against *Ricania* sp. adults in the spray bioassay. Using the leaf-dipping bioassay, the LC_{50} values of the oil from France and India were 80.99 and 57.44 mg/L, respectively, against *Ricania* sp. nymphs. Acaricidal activity of the *C. zeylanicum* oil from India in the fabric disk bioassay (LD_{50}, 0.64, 0.51 and 1.72 μg/cm^3, respectively) was greater than France oil (LD_{50}, 0.92, 0.81 and 1.82 μg/cm^3, respectively). In the filter paper bioassay, india oil (LD_{50}, 1.82, 1.55 and 3.08 μg/cm^2, respectively) was more potent than France oil (LD_{50}, 2.07, 1.94 and 6.20 μg/cm^2, respectively) against *D. farinae*, *D. pteronyssinus* and *T. putrescentiae*. The results indicate that the essential oils of *C. zeylanicum* barks could be an effective natural acaricide and insecticide for controlling house dust mites, stored food mites and fruit pests.

✉ Hoi-Seon Lee
hoiseon@jbnu.ac.kr

[1] Department of Bioenvironmental Chemistry, College of Agriculture and Life Sciences, Chonbuk National University, Jeonju 54896, Republic of Korea

[2] Crop Protection Division, National Academy of Agriculture Science, Wanju 54874, Republic of Korea

Keywords Acaricidal activity · *Cinnamomum zeylanicum* · *Dermatophagoides* sp. · Insecticidal activity · *Ricania* sp. · *Tyrophagus putrescentiae*

Introduction

In contrast to synthetic acaricides and insecticides, plant essential oils are environmentally benign, do not affect non-target organisms, are cost-effective and are convenient to handle and use [1]. Natural acaricides and insecticides are typically active against some species, are biodegradable, nonpoisonous and appropriate for use as mite and insect control agents [2–4]. Essential oils derived from plants could be important in protecting stored foods, fruit trees, crops and humans against mites and insects. Plant essential oils are potential sources of natural acaricides and insecticides [5].

Lauraceae is a plant family comprised mainly of evergreen shrubs and trees. The genus *Cinnamomum* includes about 250 species, which are generally located in Asia and Australia [6]. *Cinnamomum* provides diverse oils with various aromatic and chemical components, which include cinnamaldehyde, eugenol and camphor [7, 8]. *Cinnamomum zeylanicum* Blume is universally known as cinnamon. This economically important species is cultivated in India and Sri Lanka. *C. zeylanicum* leaves and barks are processed to yield a spice that has been commonly used worldwide for centuries by various cultures. *C. zeylanicum* has a history as a traditional medicine for gastritis, dyspepsia, inflammatory diseases and blood circulation issues [9]. *Cinnamomum* sp. oil has potential acaricidal and insecticidal activities against *Tetranychus cinnabarinus* [10] and *Callosobruchus maculatus* [11].

*Dermatophagoides farina*e (Hughes), *Tyrophagus putrescentiae* (Schrank) and *D. pteronyssinus* (Trouessart) are the most important house dust and stored food mites. House dust mites are a main source of allergens related to asthma, atopic dermatitis and perennial allergic rhinitis [12]. Stored food mites are an etiological factor of allergic illness among workers and farmers handling polluted stored products. Ingestion of mites causes systemic anaphylaxis and acute enteritis [13]. Efforts to eradicate the mites by repeated application of synthetic acaricides have triggered the development of resistance, killing of non-target organisms and raised concern about human health [13]. *Ricania* sp. belongs to the planthopper family Ricaniidae. *Ricania* sp. is a pest of important economic plants in the agriculture and forestry sectors [14]. *Ricania* sp. was first described in 2010 [14] and was soon reported as a sporadic pest of some fruit plants, such as persimmon, plum, chestnut and blueberry [15]. The infestation area is increasing in the absence of an active insecticide for long-term control.

In seeking a solution, the potential acaricidal and insecticidal activities of *C. zeylanicum* bark oils cultivated from India and France against *Dermatophagoides* spp., *T. putrescentiae* and *Ricania* sp. attracted our attention. This paper reports the results of the chemical composition of the essential oils of *C. zeylamicum* obtained from bark gathered in India and France, and their acaricidal and insecticidal activities against *Dermatophagoides* spp., *T. putrescentiae* and *Ricania* sp.

Materials and methods

Plant material extraction

Cinnamomum zeylanicum bark (5 kg) cultivated from France and India was obtained from a medicinal herb market in Jeonju, South Korea. Samples were authenticated by Jeong-moon Kim at Chonbuk National University. Essential oils derived from *C. zeylanicum* barks were obtained by steam distillation. The concentrated volatile oils were stored at 4 °C before bioassay.

Test insects

Cultures of *Dermatophagoides* spp. and *T. putrescentiae* were maintained without exposure to any synthetic acaricides. The mites were reared on fry feed (Korea Special Feed-Meal Co. Ltd, Jeonju, South Korea) and dried yeast and housed in circular cages (15 × 12 × 6 cm) at 26 ± 1 °C and 75% relative humidity in the dark. The feed included protein (45.9%), fiber (4.1%), lipid (2.9%), phosphate (1.8%) and calcium (1.1%). *Ricania* sp. adults and nymphs were gathered from Wanju, South Korea, from May to October 2016 using an insect catcher. They were classified as the fourth instar stage of *Ricania* sp. nymphs.

Bioassays

Acaricidal activities of essential oils derived from *C. zeylanicum* bark against *Dermatophagoides* spp. and *T. putrescentiae* were measured with the fabric disk and filter paper bioassays, as modified [16]. In the fabric disk bioassay, various concentrations ranging from 80 to 1.2 μg/cm^3 in 10 μL acetone were applied to fabric disks (8 mm diameter and 1 mm thick). The fabric disks were dried in an air fume hood for 15 min and put in the cap of a microtube. Thirty adult mites were treated in the test and control tubes. In the filter paper bioassay, differing concentrations (80–1.2 μg/cm^2) of the sample were liquefied in 50 μL and applied to filter paper (50 mm diameter, 55 μm thick). After drying in the fume hood for 15 min, the treated filter paper was put in the base of a Petri dish (50 mm × 8 mm). Thirty mites were transferred to the dish, and the lid was completely sealed. Treatments for the fabric disk and filter paper bioassays were repeated three times at 26 ± 1 °C for 24 h. Dead mites were checked by microscopy examination (×20). Acetone and benzyl benzoate were used for the negative control and the positive control, respectively.

The insecticidal activities of *C. zeylanicum* oils against *Ricania* sp. nymph and adult with the spray and leaf-dipping bioassays were determined as previously described [3]. In the leaf-dipping bioassay, several concentrations of the sample (1000–50 mg/L) in distilled water of the test samples were dissolved and althea leaf (30 mm diameter) was dipped into each concentration. After drying in the fume hood for 6 min, the dried leaf was set into bottom of a Petri dish (60 × 15 mm) and 20 nymphs were added. In the spray bioassay, various concentrations (1000–50 mg/L) of the test sample were soluble in distilled water. The samples were sprinkled into a square cage (15 × 15 × 20 cm) which held 20 adults. The spray and leaf-dipping bioassays were maintained at 23 ± 2 °C for 48 and 72 h. All tests were done three times.

Gas chromatography–mass spectrometry (GC–MS)

Essential oils of *C. zeylanicum* barks cultivated in France and India were analyzed with GC–MS (HP 6890 and 5973 series; Agilent, Santa Clara, CA, USA) and were separated using a DB-5 column and HP-Innowax capillary column (0.25 mm i. d. × 3000 mm L × 0.25 μm thickness). Helium was used as the carrier gas at 0.76 mL/min. Ion source temperature was 220 °C, and column temperature was 50 to 210 °C at 2 °C/min. Electron ionization obtained mass spectra at 70 eV with a scan area of 20–400 amu. The constituents of *C. zeylanicum* bark oil were identified by

retention indices and mass spectra as compared to be spectra library (Table 1). The relative percentage (%) of constituents was measured by calculating with internal standards.

Statistical analysis

This study used probit analysis to handle mortality data, determining the lethal concentrations (LC_{50} and LC_{90}), the lethal doses (LD_{50} and LD_{90}) and 95% confidence intervals. SPSS version 12 software (SPSS Inc., Chicago, IL, USA) was used to analyze all data.

Results and discussion

Composition of *C. zeylanicum* essential oil

Essential oil extracted by steam distillation was obtained with a yield of 1.27% for bark from India and 1.05% for bark from France. Fourteen components were identified, accounting for 98.59% of the oil in samples from France, with 11 components obtained from samples from India, accounting for 97.90%. The chemical constituents of the essential oils of two countries are shown in Table 1. The two essential oils comprised monoterpene alcohol, monoterpene ether, monoterpene hydrocarbon, monoterpene ketone, phenolic, phenylpropanoid ester, phenylpropanoid ether, phenylpropanoid hydrocarbon and sesquiterpene hydrocarbon. The compounds derived from the *C. zeylanicum* oil from France were 2 monoterpene alcohols (linalool and α-terpineol), 1 monoterpene ether (eucalyptol), 4 monoterpene hydrocarbons (α-pinene, α-phellandrene, (±)-limonene and o-cymene), 1 monoterpene ketone (camphor), 1 phenolic (benzaldehyde), 1 phenylpropanoid ester (cinnamyl acetate), 1 phenylpropanoid ether (eugenol), 1 phenylpropanoid hydrocarbon (cinnamaldehyde) and 2 sesquiterpene hydrocarbons (α-humulene and β-caryophyllene). The main components of the *C. zeylanicum* oil from France were cinnamaldehyde

Table 1 GC–MS analyses of the essential oils derived from *Cinnamomum zeylanicum* cultivated from France and India by steam distillation extraction

No	Compounds	RI	% of total		Chemical formula	Molecular weight
			France	India		
1	α-Pinene	948	1.15	0.56	$C_{10}H_{16}$	136.23
2	Benzaldehyde	960	0.87	–	C_7H_6O	106.12
3	α-Phellandrene	1007	0.75	–	$C_{10}H_{16}$	136.23
4	(±)-Limonene	1033	0.68	0.52	$C_{10}H_{16}$	136.23
5	o-Cymene	1042	3.45	0.33	$C_{10}H_{14}$	134.22
6	Eucalyptol	1059	0.48	0.60	$C_{10}H_{18}O$	154.25
7	Linalool	1082	6.30	1.88	$C_{10}H_{18}O$	154.25
8	Camphor	1121	0.48	–	$C_{10}H_{16}O$	152.23
9	α-Terpineol	1143	1.40	1.13	$C_{10}H_{18}O$	154.25
10	Cinnamaldehyde	1189	63.97	67.21	C_9H_8O	132.16
11	Geraniol	1228	–	0.79	$C_{10}H_{18}O$	154.25
12	Cinnamyl acetate	1367	3.90	4.34	$C11H_{12}O_2$	176.21
13	Eugenol	1392	6.84	19.79	$C_{10}H_{12}O_2$	164.20
14	α-Humulene	1467	1.92	–	$C_{15}H_{24}$	204.35
15	β-Caryophyllene	1494	6.40	0.75	$C_{15}H_{24}$	204.35
	Major grouped compounds					
	Monoterpene alcohols		7.70	3.80		
	Monoterpene ethers		0.48	0.60		
	Monoterpene hydrocarbons		6.03	1.41		
	Monoterpene ketone		0.48	–		
	Phenolic		0.87	–		
	Phenylpropanoid esters		3.90	4.34		
	Phenylpropanoid ethers		6.84	19.79		
	Phenylpropanoid hydrocarbons		63.97	67.21		
	Sesquiterpene hydrocarbons		8.32	0.75		
Total (%)			98.59	97.90		

RI Retention indices determined on a DB-5 column

– Not detected

(63.97%), eugenol (6.84%), β-caryophyllene (6.40%), linalool (6.30%), cinnamyl acetate (3.90%), o-cymene (3.45%), α-humulene (1.92%), α-terpineol (1.40%), α-pinene (1.15%), benzaldehyde (0.87%), α-phellandrene (0.75%), (±)-limonene (0.68%), eucalyptol (0.48%) and camphor (0.48%). Most of the oil for France was phenylpropanoids (74.71%) and only 14.69% monoterpenoids and 8.32% sesquiterpenoids.

The composition of *C. zeylanicum* oil from India included 3 monoterpene alcohols (linalool, α-terpineol and geraniol), 1 monoterpene ether (eucalyptol), 3 monoterpene hydrocarbons (α-pinene, (±)-limonene and o-cymene), 1 phenylpropanoid ester (cinnamyl acetate), 1 phenylpropanoid ether (eugenol), 1 phenylpropanoid hydrocarbon (cinnamaldehyde) and 1 sesquiterpene hydrocarbon (β-caryophyllene). The main composition of *C. zeylanicum* oil from India included cinnamaldehyde (67.21%), eugenol (19.79%), cinnamyl acetate (4.34%), linalool (1.88%), α-terpineol (1.13%), geraniol (0.79%), β-caryophyllene (0.75%), eucalyptol (0.60%), α-pinene (0.56%), (±)-limonene (0.52%) and o-cymene (0.33%). Most of the Indian oil was phenylpropanoids (91.34%), with only 5.81% monoterpenoids and 0.75% sesquiterpenoids. The oil from India was richer in phenylpropanoids (91.34%) than that from France (74.71%). Eugenol comprised 19.79% of the oil from India and 6.84% of the oil from France. *C. zeylanicum* bark oil constituents differ according to the tree chemotype, climate, year and region. Chalchat and Valade [7] reported that the main compounds of *C. zeylanicum* oil from Madagascar were cinnamaldehyde and camphor. Jirovetz et al. [8] reported that eugenol (85%) was the main component of the oil from Cameroon.

Insecticidal activities of *C. zeylanicum* oil

The insecticidal activities of *C. zeylanicum* oils from France and India against *Ricania* sp. adults and nymphs were analyzed using spray and leaf-dipping bioassays (Table 2). In the spray bioassay, the LC_{50} values against *Ricania* sp. adults of *C. zeylanicum* oil from France were 160.39 mg/L at 48 h and 123.77 mg/L at 72 h. The respective values from the oil from India were 109.72 and 93.06 mg/L. Using the leaf-dipping bioassay, the LC_{50} values against *Ricania* sp. nymphs of *C. zeylanicum* oil from France were LC_{50} 104.95 mg/L at 48 h and 80.99 mg/L at 72 h. The respective values of the oil from India were 76.89 and 57.44 mg/L. The activities of *C. zeylanicum* oil from India were about 1.33–1.46 times greater than that of the oil from France. The negative control displayed no activity against *Ricania* sp. Lee et al. [4] reported that eugenol had great insecticidal activity (143.24 and 124.44 mg/L, respectively) against *Ricania* sp. adults and nymphs. Taken together with GC–MS data of *C. zeylanicum* oils, it is assumed that the difference in insecticidal activity between the oils cultivated from France and India was related to the chemical content of eugenol. In addition, variation in the insecticidal activities of essential oil from different region may reflect chemical composition, phytochemicals and the size and weight of *Ricania* sp. adults and nymphs [17]. Santos et al. [17] described different activities of *Copaifera reticulata* oils from Acre and Para against bacteria.

Acaricidal activity of *C. zeylanicum* oil

The acaricidal activities of *C. zeylanicum* oil from France and India against house dust and stored food mites were tested using fabric disk and filter paper bioassays and compared with that of the benzyl benzoate positive control (Tables 3). In the fabric disk bioassay, the LD_{50} values against *D. farinae*, *D. pteronyssinus* and *T. putrescentiae* of the essential oil from France were 0.92, 0.81 and 1.82 μg/cm^3, respectively, and those of the essential oil from India were 0.64, 0.51 and 1.72 μg/cm^3, respectively. The LD_{50} values against *D. farinae*, *D. pteronyssinus* and *T. putrescentiae* of *C. zeylanicum* oil from France in the filter paper bioassay were 2.07, 1.94 and 6.20 μg/cm^2, respectively, and those of the oil from India were 1.82, 1.55 and 3.08 μg/cm^2, respectively. Relative toxicity (RT) was expressed as the ratio of benzyl benzoate LD_{50} value/each test material LD_{50} value. In the fabric disk bioassay, the RT values for *D. farinae*, *D. pteronyssinus* and *T. putrescentiae* of *C. zeylanicum* oils from bark obtained from India (14.78, 16.57 and 6.73) were greater than France oil (10.92, 10.41 and 6.36). In the filter paper bioassay, the RT values of *C. zeylanicum* india oils (4.26, 4.22 and 3.28 times) were higher than France oil (3.75, 3.37 and 1.63 times) against *D. farinae*, *D. pteronyssinus* and *T. putrescentiae*, respectively. Based on the LD_{50} values, *C. zeylanicum* oil from India was more sensitive than the oil from France against house dust and stored food mites. The acaricidal activities of *C. zeylanicum* oils are impacted by type and proportion of constituent from plant oil, detoxification enzyme activity and biological conditions of mites [16]. In a previous study, the acaricidal activities of eugenol and cinnamaldehyde, which is the main component of *C. zeylanicum* oil, were potent against house dust and stored food mites [18, 19].

Cinnamon oil and eugenol may deleteriously affect cell membrane permeability and inhibit growth due to disruption of intracellular enzymes [20]. Cinnamon oil is safe when ingested and has been granted GRAS (generally recognized as safe) status by the United States Food and Drug Administration. Cinnamon displays varied pharmacological activities including antiallergic [21] and antimicrobial [22] activities. The present findings implicate the

Table 2 Insecticidal activities of the essential oil of *C. zeylanicum* cultivated from France and India against *Ricania* sp. adults and nymphs[a]

Samples	Stage	Time (h)	LC_{50} (95% CI) (mg/L)	LC_{90} (95% CI) (mg/L)	Slope ± SE	χ^2 (df, *p*)
France	Nymph	48	104.95 (92.83–116.79)	198.01 (173.46–237.81)	4.65 ± 0.53	7.452 (8, 0.489)
		72	80.99 (59.03–91.63)	158.90 (138.14–194.44)	4.38 ± 0.58	7.535 (7, 0.375)
	Adults	48	160.39 (139.89–178.49)	271.92 (242.30–318.88)	4.59 ± 0.73	8.876 (6, 0.181)
		72	123.77 (104.83–140.31)	226.35 (199.76–266.91)	4.89 ± 0.62	9.466 (7, 0.221)
India	Nymph	48	76.89 (66.71–86.55)	156.47 (136.21–188.77)	4.15 ± 0.46	10.623 (9, 0.302)
		72	57.44 (48.49–65.45)	121.59 (103.48–157.21)	3.92 ± 0.56	4.409 (8, 0.818)
	Adults	48	109.72 (92.10–125.17)	206.32 (180.99–235.30)	4.67 ± 0.60	9.049 (7, 0.249)
		72	93.06 (80.52–104.19)	173.48 (152.16–209.94)	4.74 ± 0.63	6.248 (7, 0.511)
Negative control	Nymph	48	–[a]	–	–	–
	Adults	48	–	–	–	–

[a] No activity in the negative control

Table 3 Acaricidal activities of *C. zeylanicum* oils cultivated from France and India against *Dermatophagoides* spp. and *Tyrophagus putrescentiae*[a]

Samples	Bioassays	Mite species	LD_{50} (95% CI) (μg/cm^3)	LD_{90} (95% CI) (μg/cm^3)	Slop ± SE	χ^2 (d*f*, *p*)	RT[b]
France	Fumigant	*D. farinae*	0.92 (0.78-1.06)	2.09 (1.77-2.26)	3.76 ± 0.48	3.064 (7, 0.879)	10.92
		D. pteronyssinus	0.81 (0.68-0.95)	1.89 (1.66-2.24)	3.48 ± 0.45	3.884 (7, 0.793)	10.41
		T. putrescentiae	1.82 (1.65-2.10)	3.66 (3.38-3.88)	4.22 ± 0.53	4.594 (6, 0.597)	6.36
	Contact	*D. farinae*	2.07 (1.55-2.54)	6.19 (4.88-9.03)	2.82 ± 0.44	3.045 (6, 0.803)	3.75
		D. pteronyssinus	1.94 (1.31-2.46)	7.17 (5.50-12.76)	2.26 ± 0.43	4.648 (7, 0.703)	3.37
		T. putrescentiae	6.20 (5.13-7.15)	12.89 (10.70-17.85)	4.03 ± 0.69	7.536 (5, 0.184)	1.63
India	Fumigant	*D. farinae*	0.64 (0.54-0.75)	1.54 (1.27-2.01)	3.39 ± 0.37	3.876 (9, 0.919)	14.78
		D. pteronyssinus	0.51 (0.42-0.61)	1.32 (1.07-1.68)	3.12 ± 0.37	3.578 (8, 0.893)	16.57
		T. putrescentiae	1.72 (1.45-1.90)	3.64 (3.43-3.83)	3.94 ± 0.49	4.71 (6, 0.581)	6.73
	Contact	*D. farinae*	1.82 (1.33-2.34)	8.73 (6.03-12.53)	1.88 ± 0.30	10.689 (7, 0.153)	4.26
		D. pteronyssinus	1.55 (1.13-1.97)	6.42 (4.70-10.64)	2.08 ± 0.31	6.536 (7, 0.479)	4.22
		T. putrescentiae	3.08 (2.39-3.61)	6.45 (5.45-8.60)	3.99 ± 0.72	3.848 (5, 0.571)	3.28
Benzyl benzoate	Fumigant	*D. farinae*	9.46 (8.30-10.72)	19.97 (16.90-23.20)	3.95 ± 0.43	4.306 (9, 0.890)	1.00
		D. pteronyssinus	8.43 (7.25-9.69)	19.76 (16.97-22.99)	3.46 ± 0.39	4.681 (9, 0.861)	1.00
		T. putrescentiae	11.58 (10.07-13.17)	25.57 (21.58-28.45)	3.73 ± 0.42	8.706 (9, 0.465)	1.00
	Contact	*D. farinae*	7.76 (6.54-9.09)	20.06 (17.38-23.52)	3.11 ± 0.34	7.507 (9, 0.584)	1.00
		D. pteronyssinus	6.54 (5.55-7.63)	16.12 (13.22-19.20)	3.28 ± 0.36	3.935 (9, 0.916)	1.00
		T. putrescentiae	10.09 (8.75-11.50)	22.18 (19.75-24.01)	3.75 ± 0.42	8.447 (9, 0.490)	1.00

[a] Exposed for 24 h

[b] Relative toxicity, LD_{50} value of benzyl benzoate/LD_{50} value of each test material

essential oils of *C. zeylanicum* bark as an effective natural acaricide and insecticide for controlling house dust mites, stored food mites and sporadic pests.

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