LABORATORY EVALUATION OF BIOLOGICAL ACTIVITY OF *CINNAMOMUM CASSIA* TO FOUR SPECIES OF STORED-PRODUCT INSECTS¹

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ABSTRACT

Dry bark and oil of Cinnamonum cassia Blume were evaluated for their biological activity against the adults of Callosobruchus maculatus (F.), Sitophilus oryzae (L.), Lasioderma serricorne (F.) and Tribolium confusum Jacquelin du Val. The acetone extract of the bark provided practically no contact toxicity to C. maculatus and T. confusum up to 50 μ g/insect, but gave weak toxicity to S. oryzae and L. serricorne at 48 h after topical treatment, while the oil showed no toxicity to C. maculatus, very weak toxicity to T. confusum, and moderate toxicity to S. oryzae and L. serricorne at 30 and 50 μ g/insect, respectively.

Both the extract and the oil, applied to wheat at 0.2, 0.1 and 0.05% by weight, showed significant repellency against *S. oryzae* adults with the oil providing greater repellency than the extract. The repellency decreased as the concentration of treatment decreased. Both the extract and the oil provided the repellency to *T. confusum* when applied to paper. At the application rates of 600 and 400 μ g/cm², the extract showed a class III average repellency for 4 months. Repellency of the oil deteriorated at a faster rate, and showed only a class II average repellency for 4 months at the same dosages.

Key Words: Cinnamomum cassia, toxicity, repellency, Callosobruchus maculatus, Sitophilus oryzae, Lasioderma serricorne, Tribolium confusum.

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INTRODUCTION

Cinnamomum cassia Blume (Chinese cinnamon) and C. zeylanicum Nees in Wall. (Ceylon cinnamon) are members of the Lauraceae family, and have been used as spices since a very early period in history. C. cassia was first named "cassia," which should not be confused with the large genus Cassia in the Leguminosae or pea family from which senna leaves are obtained. In the United States, the Food, Drug, and Cosmetic Act of 1938 officially permits the term "cinnamon" to be used for both C. cassia and C. zeylanicum.

Cinnamonum cassia, native to Vietnam and Eastern Himalayas, is sometimes also known as Chinese cassia. Ordinarily, the steam bark and the branch bark are used as a spice, while the twigs and leaves are used to produce oil. The barks have carminative and astringent properties, and the oil has carminative as well as antiseptic properties. The powdered *C. cassia*, the oil, and the extracts are used extensively as flavoring ingredients in many major categories of food products, and are also used as a domestic spice in cooking, and for medicinal purposes.

¹ Mention of a commercial or proprietary product does not constitute a recommendation or an endorsement by the USDA.

The antibacterial properties of *C. cassia* oil were reported by Kellner and Kober (1954) and Goutam and Purohit (1974). Cimmamon oil as an ovicide for the screwworm, *Cochliomyia hominivorax* (Coquerel) (= americana C. and P.), was reported by Bushland (1939). Cinnamon oil has been used to formulate a cockroach repellent (Okada 1978a, b, c), and a plant-protecting insecticide (Sevelinges 1971). The toxicity of *C. cassia*, acetone extract and oil, to four species and repellency to two species of stored-product insects were investigated and are reported here.

MATERIALS AND METHODS

Materials

Dry bark of Chinese cinnamon (C. cassia) was obtained through the courtesy of the C. F. Sauer Co., Richmond, VA, and the cinnamon oil (cassia oil redistilled) produced by Gentry Corp., Fair Lawn, N. J., was purchased from LaPine Scientific Co., Chicago, IL.

The bark was ground in a CRC high speed micromill into fine powder of less than 250 μ m. The powder (24 g) in acetone (Reagent grade, 200 ml) at 40-50C was stirred for 30 min and filtered. The extraction process was repeated with the residue for three more times, and the combined filtrate was concentrated under reduced pressure to a small volume and then lyophilized to provide the extract.

Insects

Four species of stored-product insects, reared at 27 ± 1 C and $60 \pm 5\%$ RH at the Stored-Product Insects Research and Development Laboratory, Savannah, GA, were used. They were adults of cowpea weevils, *Callosobruchus maculatus* (F.) (< 5 h old); rice weevils, *Sitophilus oryzae* (L.) (< 24 h old); cigarette beetles, *Lasioderma serricorne* (F.) (< 72 h old); and confused flour beetles, *Tribolium confusum* Jacquelin du Val (7 - 14 days old).

Toxicity Study by Topical Treatment of Insects

A stock solution was obtained by dissolving 100 mg of the extract or oil in 1 ml of acetone (Reagent grade) to give a concentration of 100 mg/ml. Lower concentrations of 80, 60, 40, and 20 mg/ml were obtained by dilution of the stock solution with solvent.

Insects were anesthetized briefly with CO_2 and then picked up individually with a vacuum needle. Using a microapplicator, 0.5 μ l of the solution was applied to the dorsum of the thorax of the insect. Twenty insects of each species (unsexed except for 10 σ and 10 φ cowpea weevils) were treated with each dose. After treatment, insects were held in 100-mm-diam petri dishes, 10 insects/dish (10 kernels of undamaged soft winter wheat were placed in each dish together with rice weevils), in a room maintained at $27 \pm 1C$ and $60 \pm 5\%$ RH under alternating 12-h light and dark cycles. The insects were examined daily for one week. Those that did not move or respond to the gentle touch of a small probe were considered dead.

Repellency Study by Food Preference Method

Required amounts of the Chinese cinnamon extract or oil samples were dissolved in 1 - 2 ml of acetone (Reagent grade) and applied to wheat at 0.2, 0.1, and 0.05% by weight. These treated materials were evaluated using the modified

Loschiavo food preference apparatus as described by Laudani and Swank (1954). The apparatus consisted of a circular platform 50 cm in diameter, with a 5-cm metal rim. The platform had 12 holes, 8.75 cm in diameter, equally spaced along the outer edge to accommodate paper cups which were filled to the rim (ca. 80 g each) with treated or untreated grain samples. The center of the wheel cover had an opening, 1.25 cm in diameter, fitted with a plastic tube through which 120 adult rice weevils were introduced for a 24 h exposure period. For each experiment, three cups were filled with untreated wheat, and three each were filled with wheat treated with the Chinese cinnamon bark acetone extract or with cinnamon oil at 0.2, 0.1, and 0.05% by weight. The cups were arranged in different repeated sequence for each experiment (in A-B-C-D- for expt. 1, A-C-B-D- for expt. 2, and A-B-D-C- for expt. 3). At the end of each exposure period, the insects in each cup were collected and counted to determine their distribution. For each material, the test was repeated three times.

Data were analyzed by using analysis of variance and Duncan's multiple range (Duncan 1955) tests.

Another test was carried out with the food preference method when only one dose of treatment of wheat, either 0.2 or 0.1% by weight of wheat, was tested with the untreated. Six cups were filled with treated wheat and six cups with untreated wheat. The cups were arranged in an alternated sequence. At the end of the exposure period, the insects in the cups were collected and counted to determine their distribution.

Repellency Study by Treated Paper Method

The acetone extract was evaluated for its repellency against the confused flour beetle using the method described by Laudani et al. (1955) and McDonald et al. (1970). Strips of aluminum foil laminated to 40-lb kraft paper were treated on the paper side with acetone solutions of the extract or oil at concentrations of 600, 400, 200, and 100 μ g/cm² and then air dried. Each treated strip (10 × 20 cm) was attached lengthwise, edge to edge, to an untreated strip by cellulose tape on the reverse side. Two glass rings, 2.5 cm high and 6.4 cm ID, were placed over the two matched papers so that the joined edge bisected the ring. Ten adult confused flour beetles were exposed in each test arena inside the glass ring, and their numbers on the treated and untreated halves were recorded at 9 am and 4 pm each day for 5 consecutive days. The average of counts of each 5-day period was converted to percent repellency. The mean repellency from exposure at periods of 1 wk, 2 wk, 1 mo, 2 mo, and 4 mo after application was assigned a repellency class by using the following scale (class, percent repellency): 0, < 0.1; I, 0.1 - 20; II, 20.1 - 40; III, 40.1 - 60; IV, 60.1 - 80; V, 80.1 - 100.

Data of overall average repellency were analyzed as previously described.

RESULTS AND DISCUSSION

The acetone extraction of 24 g of pulverized Chinese cinnamon bark yielded 3.41 g (14.2%) of a dark red solid. This extract provided almost no topical toxicity to cowpea weevils and confused flour beetles, but exhibited weak toxicity to rice weevils at 30 μ g/insect and higher, and to cigarette beetles at 40 μ g/insect and higher (Table 1). The oil also provided no topical toxicity to cowpea weevils, very weak toxicity to confused flour beetles, but provided moderate toxicity to rice

Dose	Avg $\%$ mortality \pm SE*† at 48 h after application					
(μ g/insect)	CW	RW	CB	CFB		
		C. cassia extract				
50	1.25 ± 1.25	15.00 ± 4.56	12.50 ± 4.33	5.00 ± 3.53		
40	3.75 ± 1.25	11.25 ± 7.18	7.50 ± 3.23	3.75 ± 2.39		
30	1.25 ± 1.25	12.50 ± 7.22	2.50 ± 1.44	3.75 ± 1.25		
20	0	6.25 ± 2.39	2.50 ± 2.50	1.25 ± 1.25		
10	1.25 ± 1.25	3.75 ± 1.25	0	0		
Acetone only	0	0	0	0		
Untreated	0	0	1.25 ± 1.25	0		
		C. cassia oil				
50	1.25 ± 1.25	41.25 ± 3.75	30.00 ± 7.36	8.75 ± 7.18		
40	0	37.50 ± 4.33	7.50 ± 4.78	3.75 ± 2.39		
30	0	28.75 ± 5.54	2.50 ± 1.44	2.50 ± 1.25		
20	0	0	3.75 ± 1.25	1.25 ± 1.25		
10	0	0	0	1.25 ± 1.25		
Acetone only	0	0	0	0		
Untreated	0	0	0	0		

Table 1. Toxicity of C. cassia acetone extract and C. cassia oil applied topically to four species of adult stored-product insects (cowpea weevil - CW, rice weevil - RW, cigarette beetle - CB, and confused flour beetle -CFB).

* Average of four replicates, 20 insects/replicate.

[†] Standard error of the mean.

weevils at a dosage of 30 μ g/insect and higher, and to cigarette beetles at 50 μ g/ insect.

Both the acetone extract and oil when applied on wheat showed good repellency to rice weevils when evaluated by the food preference wheel method (Table 2). The overall average repellency for the three dosage levels of the extract or oil treatment were significantly different (P = 0.05) from that of the untreated. The repellency decreased as the concentration of the treatment decreased. The oil at dosage levels of 0.2 and 0.1% was very effective in repelling rice weevils with none and 1.95% of the insects being present, respectively, in the treated wheat. The repellent effect decreased at the 0.05% concentration, but it is still significantly different from that of the untreated control.

The cinnamon extract was less effective as compared with the oil. At 0.2, 0.1, and 0.05% dosage levels, there were 5, 14.33, and 24.72% of the insects present. At the 0.2% level, the effect was not significantly different from that of 0.1%, but was significantly different from that at the 0.05% dosage level. The 0.1% dosage level was not significantly different from that of the 0.05% level.

When the repellency of the extract or oil at the 0.2 or 0.1% dosage levels was compared separately with the untreated wheat, the repellent effect was still very high at 0.2% treatment, but decreased considerably at 0.1% dosage level (Table 3). Both treated samples gave significant repellent effect (P = 0.05) when compared with the untreated.

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Distribution of insects Dose $(\% \pm SE)^*$ in expt no.						Overall			
(% wt. of grain)	1	avg. (%)†‡							
	C. cassia extract								
0.2	$5.00 \pm$	0.58	$5.00 \pm$	1.15	5.00 ± 1.15	5.00 ± 0.50	ab		
0.1	10.00 \pm	0	$10.00 \pm$	1.53	23.33 ± 2.85	14.33 ± 1.32	bc		
0.05	$25.00 \pm$	1.53	$22.50~\pm$	2.08	26.67 ± 2.60	24.72 ± 1.09	с		
Untreated	$60.00 \pm$	4.16	$62.50 \pm$	10.26	45.00 ± 2.52	55.83 ± 3.46	d		
			C. cassic	ı oil					
0.2	0		0		0	0	а		
0.1	0		$1.67~\pm$	0.33	4.17 ± 0.33	1.95 ± 0.28	а		
0.05	$7.50~\pm$	1.73	$21.67 \pm$	2.33	16.67 ± 1.20	15.28 ± 1.23	bc		
Untreated	92.50 ± 1	0.79	$76.66 \pm$	6.44	79.16 ± 5.24	82.77 ± 4.05	е		

Table 2. Distribution of S. oryzae adults present in wheat with C. cassia acetone extract or C. cassia oil, and untreated wheat after a 24-h exposure in a repellency wheel.

* Distribution of 120 insects with three samples of each treatment in each experiment. Standard error of the mean. Average of three experiments.

[‡] Values followed by the same letter within a column are not significantly different at the 0.05 level according to Duncan's multiple range test.

Table 3. Distribution of S. oryzae adults present in wheat with C. cassia acetone extract or C. cassia oil, and untreated wheat after a 24-h exposure in a repellency wheel.

	Dis	tribution of ins	ects		
Dose	(% :	\pm SE)*† in expt	no.	Overall	
(% wt. of grain)	1	2	avg. (%)‡		
	(C. cassia extract	t		
0.2	5.83 ± 0.40	2.50 ± 0.19	6.67 ± 0.41	5.00 ± 0.20 a	
Untreated	94.17 ± 2.17	97.50 ± 2.44	93.33 ± 2.93	95.00 ± 1.38 f	
0.1	20.83 ± 0.66	16.67 ± 0.60	16.67 ± 0.77	18.06 ± 0.38 b	
Untreated	79.17 ± 2.28	83.33 ± 2.18	83.33 ± 1.97	81.94 ± 1.17 e	
		C. cassia oil			
0.2	1.67 ± 0.28	2.50 ± 0.19	0.50 ± 0.19	2.22 ± 0.12 a	
Untreated	98.33 ± 2.28	97.50 ± 3.48	97.50 ± 3.35	$97.78 \pm 1.67 ~{ m f}$	
0.1	31.67 ± 1.07	29.17 ± 1.63	28.33 ± 1.30	29.72 ± 0.73 c	
Untreated	68.33 ± 0.60	70.83 ± 2.24	71.67 ± 1.25	70.28 ± 0.83 d	

* Distribution of 120 insects with six samples of each treatment in each experiment.

[†] Standard error of the mean.

[‡] Average of three experiments. Values followed by the same letter within a column are not significantly different at the 0.05 level according to Duncan's multiple range test.

The acetone extract-treated paper showed good repellency against confused flour beetles, but the oil showed only moderate repellency (Table 4). At application rates of 600 and 400 μ g/cm², the extract gave an average repellency of 46.95 and 45.00%, respectively, for a 4-month period. Both are in the scale of Class III, which is considered as the standard for a promising repellent (Laudani et al. 1955;

Rate of application	at ind	0	% repel period a		Overall	Repellency	
$(\mu g/cm^2)$	1 wk	2 wk	1 mo	2 mo	4 mo		(class)
			С.	cassia e	extract		
600	54.50	60.75	40.75	44.75	34.00	46.95 ± 3.98 a	III
400	47.50	55.50	32.00	42.50	47.50	45.00 ± 3.19 a	III
200	28.75	52.00	36.25	32.75	32.25	36.40 ± 3.56 ab	II
100	28.50	40.25	26.50	20.25	20.75	27.25 ± 3.26 bc	II
			(C. cassic	ı oil		
600	48.00	24.25	19.50	18.50	17.25	25.50 ± 4.03 bc	Π
400	38.25	18.75	22.75	17.50	10.50	$21.55\pm4.15~{ m c}$	II
200	37.00	9.75	4.75	13.50	16.50	$16.30\pm3.91~ m cd$	Ι
100	6.75	5.50	8.50	13.75	12.25	9.35 ± 2.36 d	I

Table 4. Average repellency of C. cassia acetone extract and C. cassia oil toTribolium confusum adults from treated paper method.

*Average of data from four replicates, 10 insects per replicate.

[†] Average data of 1 wk, 2 wk, 1 mo, 2 mo, and 4 mo.

[‡] Values followed by the same letter within a column are not significantly different at the 0.05 level according to Duncan's multiple range test.

McDonald et al. 1970). But the oil, at 600 and 400 μ g/cm², produced average repellency of only 22.50 and 21.55%, an equivalent of Class II in the scale. The repellency decreased as the application rate of the material decreased. The repellency of the extract deteriorated very slowly during the 4-month exposure period, but at a faster rate for the oil. This could be because comparatively more volatile components are present in the oil than in the extract.

From this study, the Chinese cinnamon extract and its oil showed low to moderate contact toxicity to rice weevils and cigarette beetles. Both the extract and oil on wheat showed strong repellency to rice weevils, and the extract showed a longer effect against confused flour beetles than the oil when applied on paper. These results suggest that Chinese cinnamon is a good candidate to consider for development as a short term protectant for grain in storage against the above insects.

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