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Feeding Deterrents from *Dictamnus dasycarpus* Turcz Against Two Stored-Product Insects

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The screening for insecticidal principles from several Chinese medicinal herbs showed that the root bark of *Dictamnus dasycarpus* possessed significant feeding deterrence against two stored-product insects (*Tribolium castaneum* and *Sitophilus zeamais*). From the methanol extract, two feeding deterrents were isolated by bioassay-guided fractionation. The compounds were identified as fraxinellone and dictamnine from their spectroscopic data. Fraxinellone was demonstrated to possess feeding deterrent activity against adults and larvae of *T. castaneum* as well as *S. zeamais* adults with EC_{50} values of 36.4, 29.1, and 71.2 ppm, respectively. Dictamnine was shown to have feeding deterrent activity against adults and larvae of *T. castaneum* as well as *S. zeamais* adults with EC_{50} values of 57.6, 47.9, and 91.7 ppm, respectively.

KEYWORDS: Feeding deterrents; *Dictamnus dasycarpus*; *Tribolium castaneum*; *Sitophilus zeamais;* fraxinellone; dictamnine

INTRODUCTION

Botanical pesticides have the advantage of providing novel modes of action against insects that can reduce the risk of cross-resistance as well as offering new leads for design of target-specific molecules. During the screening program for new agrochemicals from Chinese medicinal herbs, *Dictamnus dasycarpus* Turcz (Family Rutaceae) was found to possess significant feeding deterrence activity against two stored-product insects (*Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch.). The root bark of this plant has been used for treatment of jaundice, cough, rheumatism, and other diseases (*1*). Aqueous extracts of *D. dasycarpus* have been used to control the aphids on vegetables (*2*).

The known chemical constituents of *D. dasycarpus* include furoquinoline alkoids, limonoids, reduced limonoids, flavonoids, sesquiterpenes, sesquiterpene glycosides, and steroids (*3*−*10*). Dictamnine and γ-fagarine were found to be mutagenic compounds (*11*). Woo et al. (*12*) showed that fraxinellone possesses antifertility activity. Fraxinellone and dictamnine were shown to have antiplatelet aggregation and vascular relaxing activities as well as being effective vasorelaxants (*13, 14*). Miyazawa et al. (*15*) showed that isofraxinellone also had antimutagenic activity. Six compounds isolated from *D. dasycarpus* were found to be active against the plant pathogenic fungus *Cladosporium cucumerinum* L. (*16*). In this paper, we report the isolation and identification of two feeding deterrents contained in *D. dasycarpus* against two stored-product insects, *T. castaneum* and *S. zeamais*.

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MATERIALS AND METHODS

Extraction of Plant Material. *D. dasycarpus* (10 kg, root bark material), purchased from a local Chinese herbs shop, were ground to a powder and extracted with methanol and chloroform at room temperature over a period of three weeks. The extracts were concentrated using a vacuum rotary evaporator to afford a syrupy gum (940 g). This syrup was partitioned between methanol−water and *n*-hexane (3 × 6000 mL). The *n*-hexane extracts were evaporated off to give a residue (98 g). The aqueous layer was re-partitioned with chloroform (3 × 6000 mL) to provide a residue (61 g) after evaporation of the chloroform. Further partitioning with ethyl acetate (3 × 6000 mL) gave a residue (45 g) after evaporation of the solvent.

Apparatus. Melting points were measured on a Buchi 535. ^{1}H and ^{13}C NMR spectra were recorded on Bruker ACF300 [300 MHz (^{1}H) and 75 MHz (^{13}C)] and AMX500 [500 MHz (^{1}H) and 125 MHz (^{13}C)] instruments using $CDCl_3$ as solvent with TMS as internal standard. EIMS were determined on a Micromass VG7035 mass spectrometer at 70 eV (probe). Infrared spectra were recorded in a Bio-Rad FTIR spectrophotometer.

Chromatography. From the feeding deterrence bioassays with the two species of stored-product insects, the hexane soluble fraction was found to be active. Chromatography of this fraction through a silica gel (Merck 9385, 1000 g) column using a *n*-hexane−ethyl acetate gradient provided 20 fractions. Fractions 14 and 15 were determined to be the most active. Fraxinellone (3.8 g) was recrystallized from fraction 14. Fraction 15 was further chromatographed on silica gel TLC using *n*-hexane/acetone (20:1) to provide a further 5 fractions. The most active fraction was further separated by silica gel TLC using *n*-hexane/acetone (20:1) to provide the bioactive compound which was recrystallized and determined to be dictamnine.

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Table 1. Nutritional and Feeding Deterrence Indices of Adults and Larvae of *T. castaneum* (T. C.) and *S. zeamais* (S. Z.) Adults to Fraxinellone[a]

insect	life stage	conc. (ppm)	RGR (mean ± SD) (μg/mg/d)	RCR (mean ± SD) (μg/mg/d)	ECI (mean ± SD) (%)	mort. (%)	FDI (%)	EC_{50} (ppm)
S. Z.	adult	0	15.11 ± 1.20a	137.16 ± 4.70a	10.99 ± 1.29a	0		
		3	15.22 ± 0.79a	133.85 ± 7.83a	11.41 ± 1.62a	0	2.92	71.2
		10	13.29 ± 1.21a	120.27 ± 8.30ab	11.25 ± 1.82a	0	15.68	
		30	10.06 ± 0.91b	113.45 ± 6.57b	9.57 ± 1.71a	2	30.76	
		100	7.05 ± 0.89c	79.29 ± 7.60c	9.66 ± 1.18a	0	54.09	
		300	4.26 ± 0.78d	35.29 ± 5.12d	6.56 ± 1.34b	4	77.77	
T. C.	adult	0	30.58 ± 1.57a	146.20 ± 5.04a	20.94 ± 1.73a	0		
		3	30.45 ± 2.16a	140.68 ± 6.29a	21.68 ± 2.53a	0	8.46	36.4
		10	23.64 ± 2.36b	120.94 ± 10.31b	21.98 ± 2.50a	0	18.64	
		30	19.84 ± 3.05b	83.91 ± 10.27c	19.10 ± 1.75a	0	40.07	
		100	9.41 ± 2.65c	60.43 ± 10.80d	17.57 ± 2.01a	0	69.28	
		300	1.48 ± 0.96d	7.39 ± 6.26e	10.07 ± 1.65b	4	95.05	
T. C.	larvae	0	53.77 ± 3.14a	257.40 ± 13.65a	20.92 ± 1.35a	0		
		3	52.62 ± 3.49a	233.06 ± 15.73a	22.62 ± 1.48a	0	11.65	29.1
		10	41.30 ± 4.79b	198.13 ± 16.11ab	20.90 ± 2.84a	0	24.45	
		30	23.22 ± 5.98c	163.61 ± 10.45b	17.81 ± 7.3a	0	50.33	
		100	11.71 ± 6.06d	127.99 ± 13.00c	13.65 ± 7.11b	0	72.76	
		300	–8.44 ± 5.32e	30.12 ± 7.65d	-	12	95.68	

[a] Within each stage of species, means in the same column followed by same letters do not differ significantly ($P > 0.05$) in ANOVA test.

Feeding Deterrence Bioassay. *T. castaneum* and *S. zeamais* were obtained from laboratory cultures maintained for the last 14 years in the dark in incubators at 30 ± 1 °C and 70−80% relative humidity. *T. castaneum* was reared on wheat flour mixed with yeast (10:1, w:w), and *S. zeamais* was reared on whole wheat at 12−13% moisture content. Adults of the two species used in all the experiments were about 2 weeks old, and the larvae of *T. castaneum* were 14 days old. A flour disk bioassay was used to direct the isolation of active compounds from *D. dasycarpus* according to the method of Xie et al. (*17*) with some modifications. Wheat flour (1.0 g) was ultrasonically stirred in 5 mL of distilled water, and 50 μL of hexane containing a fraction was added. Pure compounds were first dissolved in 500 μL of ethanol and two drops of Tween-20 (approximately 50 μg) were added to the wheat flour suspension. Aliquots of 200 μL of this stirred suspension were placed on the bottom of a polystyrene Petri dish to form disks. The pipet was fitted with a disposable tip that had an opening enlarged to about 2-mm internal diameter by cutting about 1 cm from the bottom of the tip with a razor blade. The same amounts of ethanol and Tween-20 were applied to produce the control flour disks. The disks were left in the fume hood overnight to air-dry. The disks were then transferred to an incubator to equilibrate at 30 ± 1 °C and 70−80% RH for 48 h. Each flour disk weighed between 36 and 39 mg. The moisture content of the disk was determined to be 13.5 ± 0.1% using the Kett's Grain moisture tester (model PB-1D2, Japan). The disks were placed in glass vials (diameter 2.5 cm, height 5.5 cm) for weighing. Ten group-weighed, unsexed insects were then added to each vial prior to further weighing. All the insects were starved for 24 h before use. The experimental setup was left in the incubator for 3 days. Glass vials containing treated flour disks but without insects were prepared to determine any decrease in weight that might have occurred due to evaporation of solvents. The following calculations were made for the study of nutritional indices and feeding deterrent index (*18*, *19*):

$$\text{relative growth rate (RGR)} = \frac{(A - B)}{(3B)}$$

$$\text{relative consumption rate (RCR)} = \frac{D}{(3B)}$$

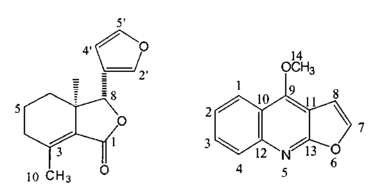
$$\text{efficiency of conversion of ingested food (ECI) (\%)} = \left(\frac{\text{RGR}}{\text{RCR}}\right) \times 100$$

$$\text{feeding deterrence index (FDI) (\%)} = \frac{(C - T) \times 100}{C}$$

where A = weight of live insects on the third day/number of live insects on the third day, B = initial weight of insects/10, D = biomass ingested/number of the insects on the third day, C = the consumption of control disks, and T = the consumption of treated disks. The EC_{50} (the concentration needed to inhibit insect feeding by 50% relative to controls) was determined by linear regression (*20*).

Data for Isolated Bioactive Compounds. Fraxinellone, colorless needles, mp 114−116 °C (mp 114−115 °C (*15*)). $[\alpha]_D^{31} = -42.1°$ (*c*. 2.2 Chloroform). EIMS *m/z*: 232 [M^+]. (27): $C_{14}H_{16}O_3$, 136 (90%), 103 (90%), 93 (95%), 39 (66%). IR(ν_{max} cm^{-1}): 1750 cm^{-1} (γ-lactone), 1674 cm^{-1}, 3123 cm^{-1}, 1600 cm^{-1}, 1502 cm^{-1}, 1030 cm^{-1}, 875 cm^{-1}, 802 cm^{-1} (furan). UV λ_{max} nm (log ε): 230 (3.52). ^{1}HNMR: δ 0.83 (3H, s, H-11), 1.42 & 1.78 (4H, m, H-5 & H-6), 2.10 (3H, d, J = 0.62, H-10), 2.22 (2H, m, H-4), 4.85 (1H, s, H-8), 6.32 (1H, dd, J = 1.5, 0.5, H-4′), 7.41 (1H, dd, J = 1.5, 1.69, H-5′), 7.44 (1H,dd, J = 0.5, 1.70, H-2′). The ^{13}C NMR data were in agreement with the reported data (*21*).

Dictamnine, colorless needles, mp 130−132 °C. EIMS *m/z*: 199 [M^+]. (100): $C_{12}H_9NO_2$, 156 (30%), 120 (28%), 101 (22%), 76 (22%), 50 (12%), 39 (6%). UV λ_{max} nm (log ε): 308 (3.86). ^{13}C NMR: δ 58.85 (C-14), 103.28 (C-10), 104.62 (C-8), 118.56 (C-11), 122.25 (C-1), 123.60 (C-2), 127.51 (C-4), 129.52 (C-3), 143.37 (C-7), 145.38 (C-12), 163.64 (C-13). The ^{1}H NMR spectrum was in agreement with the known data (*13*).



Fraxinellone Dictamnine

RESULTS AND DISCUSSION

The nutritional and feeding deterrence indices of the two stored-product insects exposed to fraxinellone and dictamnine are shown in **Tables 1** and **2**. Fraxinellone significantly ($P < 0.05$) reduced both the growth rate (RGR) and food consumption (RCR) of both the adults and larvae of *T. castaneum* at concentrations of 10 ppm and above in a concentration-dependent manner. The feeding deterrence index (FDI) at 10−300 ppm ranged from 18.6% to 95.1% for the adults and from 24.5% to 95.7% for the larvae. EC_{50} values of adults and larvae of *T. castaneum* were calculated to be 36.4 and 29.1 ppm, respectively. Fraxinellone also significantly ($P < 0.05$) reduced the growth rate and food consumption of *S. zeamais* at concentra-

Table 2. Nutritional and Feeding Deterrence Indices of Adults and Larvae of *T. castaneum* (T. C.) and *S. zeamais* (S. Z.) Adults to Dictamnine[a]

insect	life stage	conc. (ppm)	RGR (mean ± SD) (μg/mg/d)	RCR (mean ± SD) (μg/mg/d)	ECI (mean ± SD) (%)	mort. (%)	FDI (%)	EC_{50} (ppm)
S. Z.	adult	0	15.11 ± 1.20a	137.16 ± 4.70a	10.99 ± 1.29a	0		
		3	15.71 ± 1.26a	126.78 ± 8.04a	9.10 ± 2.37a	0	9.69	91.7
		10	14.32 ± 1.09ab	110.95 ± 9.25b	10.42 ± 1.13a	0	17.87	
		30	12.08 ± 1.08b	86.75 ± 12.97c	9.78 ± 2.74a	0	30.99	
		100	9.49 ± 1.06b	52.65 ± 7.65c	9.73 ± 1.90a	0	51.77	
		300	3.21 ± 1.34c	35.13 ± 6.12d	6.81 ± 1.04b	0	68.04	
T. C.	adult	0	30.58 ± 1.57a	146.20 ± 5.04a	20.94 ± 1.73a	0		
		3	29.56 ± 2.03a	132.89 ± 7.45a	21.25 ± 1.54a	0	7.31	57.6
		10	27.30 ± 1.67a	129.71 ± 4.93a	19.91 ± 2.05a	0	17.06	
		30	20.27 ± 1.44b	101.27 ± 9.27b	18.35 ± 2.15a	0	32.38	
		100	13.49 ± 2.05c	59.80 ± 3.43c	17.88 ± 2.23ab	0	59.58	
		300	6.45 ± 1.64d	22.21 ± 5.72d	13.81 ± 2.51b	2	81.24	
T. C.	larvae	0	53.77 ± 3.14a	257.40 ± 13.65a	20.92 ± 1.35a	0		
		3	52.88 ± 2.38a	254.20 ± 6.19a	19.80 ± 2.87a	0	4.59	47.9
		10	47.56 ± 3.74a	213.17 ± 10.31b	18.07 ± 1.63a	0	21.77	
		30	31.75 ± 3.08b	168.23 ± 8.15b	20.53 ± 1.74a	0	38.52	
		100	19.14 ± 4.54c	123.74 ± 9.65c	17.43 ± 1.20a	0	64.42	
		300	7.08 ± 2.54d	84.83 ± 8.07d	13.20 ± 1.45b	4	82.21	

[a] Within each stage of species, means in the same column followed by same letters do not differ significantly ($P > 0.05$) in ANOVA.

tions of 30 ppm and above. The feeding deterrence index at 30−300 ppm ranged from 30.8% to 77.8% for the *S. zeamais* adults, and the EC_{50} value of *S. zeamais* was calculated to be 71.2 ppm. However, food utilization (ECI values) was not significantly ($P > 0.05$) different from that of the controls except at the higher concentrations in all insect groups studied (**Table 1**).

Dictamnine significantly ($P < 0.05$) reduced the RGR and RCR of both the adults and larvae of *T. castaneum* as well as *S. zeamais* adults at concentrations of 30 ppm and above in a concentration-dependent manner. The feeding deterrence index at 30−300 ppm ranged from 30.9% to 68.1% for the *S. zeamais* adults, from 32.4% to 81.2% for *T. castaneum* adults, and from 38.5 to 82.2% for *T. castaneum* larvae. EC_{50} values of adults and larvae of *T. castaneum* as well as *S. zeamais* adults were calculated to be 57.6, 47.9, and 91.7 ppm, respectively. However, food utilization was not significantly ($P > 0.05$) different between the controls and the treated insects except at 300 ppm for all groups of insects.

Comparing the feeding deterrent activity of the two compounds, fraxinellone was more active than dictamnine to adults of the two species of insects and larvae of *T. castaneum*. In the case of *T. castaneum*, both adults and larvae showed similar feeding deterrence caused by both the compounds.

It is possible that the reduction in growth rate of both adults and larvae of *T. castaneum* as well as *S. zeamais* adults was mainly due to the behavioral (starvation, feeding deterrent) action rather than post-ingestive toxicity (*22*, *23*) because their food utilization was not affected, except at the higher concentrations (**Tables 1** and **2**).

When compared with the commercial feeding deterrent, azadirachtin, fraxinellone and dictamnine were less active against *T. castaneum* and *S. zeamais* (for azadirachtin, EC_{50} values of the adults and larvae of *T. castaneum* as well as *S. zeamais* adults were 3, 1, and 57 ppm respectively, Liu et al. unpublished data). They possess feeding deterrent activity against the two species of insects similar to that of another commercial feeding deterrent, toosendanin (EC_{50} values of the adults and larvae of *T. castaneum* as well as *S. zeamais* adults were 66, 48, and 100 ppm respectively, Liu et al. unpublished data). Aqueous extracts of *D. dasycarpus* have been consumed for treatment of jaundice, cough, rheumatism, and other diseases (*1*). However, there are no toxicity data available regarding human consumption of the two compounds.

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