

Vapor-Phase Toxicity of *Derris scandens* Benth.-Derived Constituents against Four Stored-Product Pests

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ABSTRACT: The vapor-phase toxicity of *Derris scandens* Benth.-derived constituents was evaluated against four stored-product pests (*Callosobruchus chinensis* L., *Sitophilus oryzae* L., *Rhyzopertha dominica* L., and *Tribolium castaneum* H.) using fumigation bioassays and compared to those of commonly used insecticides. The structures of all constituents were characterized by spectroscopic analyses [nuclear magnetic resonance (NMR) and mass spectrometry]. The sensitivity of the test insect to compounds varied with exposure time, concentration, and insect species. Over 100% mortality after 24 h was achieved with the compounds osajin (2), scandinone (5), sphaerobioside (8), and genistein (9) against all of the test insects, while laxifolin (3) and lupalbigenin (4) showed 100% mortality after 72 h against *T. castaneum* and *R. dominica*. Scandenone (1), scandenin A (6), and scandenin (7) were less effective. Among the insects, *C. chinensis*, *S. oryzae*, and *R. dominica* were more susceptible to the treatments, whereas *T. castaneum* was less susceptible. The results of fumigation tests indicated that compounds from *D. scandens* whole plant extract are potential candidates to control stored-product pests.

KEYWORDS: *Derris scandens*, insecticidal activity, stored pests, *Callosobruchus chinensis*, *Sitophilus oryzae*, *Rhyzopertha dominica*, *Tribolium castaneum*, vapor-phase toxicity

INTRODUCTION

The adzuki bean weevil, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae), rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae), lesser grain borer, *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae), and red flour beetle, *Tribolium castaneum* H. (Coleoptera: Tenebrionidae) are the most widespread and destructive insect pests of stored grains as well as stored products.^{1–5} Globally, about 10–30% of produced grains are lost every year due to insect damage.⁶ Control of these pests is primarily dependent upon repeated application of synthetic insecticides.⁷ The continuous use of synthetic insecticides for the eradication of insects has been effective but, nevertheless, has led to the development of pest resistance through the disruption of biological systems.^{8,9} Plants may provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals, are often active against a limited number of species, including specific target insects, are biodegradable to nontoxic products, and are potentially suitable for use in integrated pest management (IPM) programs.

As a part of our ongoing search for natural agrochemicals from Indian medicinal plants, we have thus far investigated the potential of species in the Fabaceae family. *Derris scandens* (Fabaceae), known by its common name gonj (Hindi), is widely distributed throughout India.¹⁰ One of the potential applications of the *Derris* species is the use to the control phytophagous pests. *D. scandens* root is an excellent insecticide, being harmful to chewing and sucking insects but not human beings. In a previous work, we reported the isolation of a new benzyl derivative along with several insecticidal constituents from *D. scandens*, which is

one of the dominant species of the south Indian forest.¹¹ These results have encouraged us to undertake the further studies of insecticidal activity against various stored-product insect species. Now, following the study of this plant, we have tested the isolates against the stored pest insects for their insecticidal activity. Herein, we report the insecticidal activity of isolates (1–9) from *D. scandens* against four major stored-product pest insect species viz. *C. chinensis* L. (Coleoptera: Bruchidae), *S. oryzae* L. (Coleoptera: Curculionidae), *R. dominica* F. (Coleoptera: Bostrichidae), and *T. castaneum* H. (Coleoptera: Tenebrionidae).

MATERIALS AND METHODS

General Procedures. All solvents were distilled before use and were removed by rotary evaporation at temperatures up to 40 °C. ¹H and ¹³C nuclear magnetic resonance (NMR) spectra were measured on a Bruker 300 MHz spectrometer using tetramethylsilane as an internal standard. Mass spectra were recorded on VG Auto Spec-M [fast atom bombardment mass spectrometry (FABMS)], and the infrared spectra were recorded on a Thermo Nicolet Nexus 670 Fourier transform infrared (FTIR) spectrometer. Melting points were measured on a Fischer Scientific melting point apparatus and are uncorrected. Column chromatography was carried out using 60–120 mesh silica gel (Merck). Thin-layer chromatography (TLC) was performed using precoated silica gel 60 F₂₅₄ TLC plates, visualized with a UV lamp, and then dipped in 5% H₂SO₄ solution followed by

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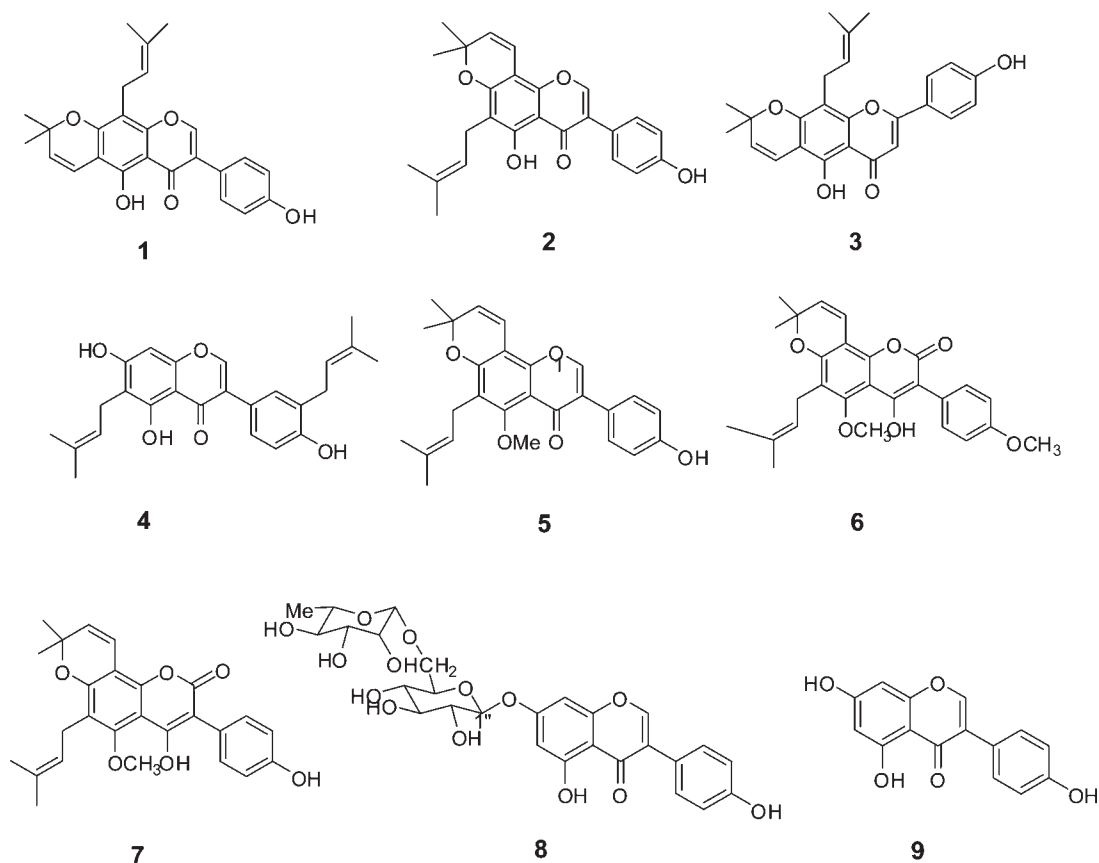


Figure 1. Compounds isolated from *D. scandens* Benth.

heating. The optical rotations were measured on a Jasco Dip 360 digital polarimeter.

Test Insects. Test insects were obtained from laboratory cultures of the Entomology Division, Directorate of Maize Research, Rajendra-Nagar, Hyderabad, India. Green gram (*Phaseolus mungo* L.) (Fabaceae) for *C. chinensis* and wheat (*Triticum aestivum* L.) for *S. oryzae*, *R. dominica*, and *T. castaneum* are the respective rearing medium for test insects. Insect rearing was maintained in the laboratory of the Indian Institute of Chemical Technology (IICT), Hyderabad, India. The cultures were maintained at $28 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ relative humidity.

Plant Material. The whole plant of *D. scandens* was collected from the Tirumala Forest, Tirupati, Andhra Pradesh, India, in August 2005. It was authenticated by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, India. A voucher specimen was deposited in the herbarium of the Botany Department, Sri Venkateswara University, Tirupati, India.

Extraction and Isolation of the Compounds. The whole plant of *D. scandens* (5 kg) was shade-dried, powdered, and extracted with chloroform in a Soxhlet apparatus for 72 h at 50°C . The resulting chloroform extract was evaporated to dryness under reduced pressure, affording a syrupy residue (21 g). Then, this chloroform extract was subjected to column chromatography on a silica gel column (60–120 mesh, 150×15 cm) and eluted with a stepwise gradient of hexane/EtOAc (98:2, 95:5, 92:8, 90:10, and 88:12 by volume) to give seven fractions (F_1 , F_2 , F_3 , F_4 , F_5 , F_6 , and F_7). Fraction F_2 was chromatographed on silica gel (60–120 mesh) column using hexane/EtOAc (99:1 and 98:2, by volume) to give compound 1 (0.2 g) and compound 2 (0.08 g). Fraction F_4 was concentrated under reduced pressure to give compound 3 as a pale yellow solid (0.12 g). Fraction F_5 was extracted with hexane to obtain compound 5 (5.8 g) as a residue. The filtrate was concentrated to give compound 4 (4.6 g) as a pale yellow solid. Fraction

F_6 was concentrated to give a dark brown residue (2.5 g), which was chromatographed on a 100–200 mesh silica gel column with an isocratic elution of the solvent system of hexane/chloroform/acetone (80:15:5, by volume) to give two subfractions A and B, which were further fractionated on a silica gel column with isocratic elution of chloroform/carbon tetrachloride/acetone (55:40:5, by volume) to give compound 6 (0.26 g) and compound 7 (1.2 g). Fraction F_7 was chromatographed on a silica gel column (60–120 mesh, 50×5 cm) and eluted with a stepwise gradient of chloroform/methanol (90:10 and 85:15, by volume) to give compounds 8 (2.8 g) and 9 (3.9 g).

All of these compounds were identified as scandenone (1),¹² osajin (2),¹² laxifolin (3),¹³ lupalbigenin (4),¹⁴ scandinone (5),¹⁴ scandenin A (6),¹⁴ scandenin (7),¹⁴ sphaerobioside (8),¹⁵ and genistein (9),¹⁶ from ^1H and ^{13}C NMR data comparison to those reported in the literature (Figure 1).

Bioassay. The insecticidal properties of isolated compounds from *D. scandens* were evaluated against adults of four stored-product insects by the fumigation assay.¹⁷ In this method, 20 adults of 3–8-day-old *C. chinensis*, *S. oryzae*, *R. dominica*, and *T. castaneum* were separately placed on the bottom of a polyvinyl chloride (PVC) container (100 mL) and then covered with a lid. Appropriate concentrations (0.1–1.0 $\mu\text{g}/\text{mL}$) of each tested material in acetone were applied to filter papers (Whatman No. 1; 2×3 cm diameter). After drying in a fume hood for 5 min, each treated paper was attached to the inner side of a lid with a small amount of solid glue and the container was covered with the lid. Control filter papers received 50 μL of acetone. The tests were carried out at $28 \pm 2^\circ\text{C}$ temperature and $65 \pm 5\%$ relative humidity. Mortality was ensured by probing the insect body with a slender paintbrush. Dead insects were counted every 24 h for a total period of 72 h post-treatment. There were five replicates per treatment, and the tests were repeated 3 times on different dates each time, to avoid any day-to-day variation.

Table 1. Insecticidal Activity of Isolated Compounds from *D. scandens* against *C. chinensis* by the Fumigation Method

compounds	toxicity (%) ^a (1.0 µg/mL)			LC ₅₀ (95% CL) ^b (µg/mL)	χ ² (df)	p level
	24 h	48 h	72 h			
1	27.9 ± 5.1 c	29.1 ± 3.6 e	31.4 ± 2.8 f	>1.0	1.78 (5)	0.774
2	100 ± 0.0 b			0.29 (0.06–0.38)	35.05 (5)	0
3	88.8 ± 6.8 a	93.2 ± 3.4 d	96.4 ± 3.7 d	0.39 (0.26–0.55)	14.62 (5)	0.005
4	100 ± 0.0 b			0.23 (0.10–0.36)	23.08 (5)	0.001
5	100 ± 0.0 b			0.31 (0.14–0.53)	34.43 (5)	0
6	79.0 ± 5.7 e	81.4 ± 2.5 a	83.0 ± 4.1 a	0.43 (0.39–0.47)	3.38 (5)	0.495
7	49.5 ± 6.6 d	56.0 ± 2.8 c	58.2 ± 3.3 e	0.90 (0.81–1.02)	3.12 (5)	0.528
8	100 ± 0.0 b			0.21 (0.16–0.35)	23.93 (5)	0.001
9	100 ± 0.0 b			0.17 (0.12–1.00)	126.6 (5)	0

^a Values are the mean ± standard deviation (SD). Each column followed by the same letter is not significantly different from another (one-way ANOVA; Tukey test at <0.05). ^b Confidence level, after 24 h of treatment.

Table 2. Toxicity of Isolated Compounds from *D. scandens* against *S. oryzae* by the Fumigation Method

compounds	toxicity (%) ^a (1.0 µg/mL)			LC ₅₀ (95% CL) ^b (µg/mL)	χ ² (df)	p level
	24 h	48 h	72 h			
1	50.1 ± 7.3 b	60.0 ± 3.5 d	63.4 ± 3.8 c	0.84 (0.76–0.94)	2.30 (5)	0.680
2	100 ± 0.0 a			0.28 (0.09–0.46)	17.72 (5)	0
3	100 ± 0.0 a			0.20 (0.08–0.33)	22.80 (5)	0
4	100 ± 0.0 a			0.26 (0.10–0.46)	35.21 (5)	0
5	100 ± 0.0 a			0.24 (0.15–0.34)	11.61 (5)	0.020
6	69.4 ± 5.2 d	72.0 ± 4.8 e	75.6 ± 4.6 a	0.76 (0.69–0.84)	3.32 (5)	0.505
7	82.0 ± 8.9 e	83.6 ± 4.4 a	85.4 ± 5.4 b	0.45 (0.31–0.66)	15.64 (5)	0.003
8	100 ± 0.0 a			0.18 (0.13–0.29)	9.55 (5)	0.048
9	100 ± 0.0 a			0.31 (0.12–0.55)	34.63 (5)	0

^a Values are the mean ± SD. Each column followed by the same letter is not significantly different from another (one-way ANOVA; Tukey test at <0.05). ^b Confidence level, after 24 h of treatment.

Percentage mortality was calculated using the corrected formula by Abbott¹⁸ for natural mortality in untreated controls.

Data Analysis. Mortality counts were corrected for control mortality as suggested by Abbott,¹⁸ wherever it was considered necessary. Statistical analysis of the toxicity data was performed using probit analysis to determine the LC₅₀.¹⁹ All experimental data were subjected to a one-way analysis of variation (ANOVA) to determine differences between samples, using the statistical software Sigmasat, version 3.5. Means were separated using Tukey's honestly significant difference (HSD) test at the 5% level.

RESULTS AND DISCUSSION

All of the compounds (1–9) isolated from *D. scandens* were tested on adults of *C. chinensis*, *S. oryzae*, *R. dominica*, and *T. castaneum* for their insecticidal activity. As shown (see Tables 1–4), the test insects showed high susceptibility to all compounds, except for the compounds 1 and 7. The compounds 2, 3, 4, 5, 8, and 9 were highly effective against all of the test insects, showing 100% mortality after 72 h of treatment (Table 1–4). In contrast, *T. castaneum* was slightly tolerant compared to the other insect species studied. In all cases, a strong difference in insect mortality was observed as the concentration increased. For *C. chinensis*, all of the test compounds except 1 and 7 showed significant ($P = 540.22$; $df = 8$; $p < 0.001$) toxicity at a concentration of 1.0 µg/mL after 24 h of treatment (Table 1). The test compounds 2, 4, 5, 8, and 9 had toxicity adequate to kill 50% of *C. chinensis*

at concentrations between 0.17 and 0.31 µg/mL. In these treatments, frantic movements and immediate knockdown of *C. chinensis* occurred soon after their release into the test containers. In the case of *S. oryzae*, a high mortality (100%) of the test insects occurred at 1.0 µg/mL concentration after 24 h of exposure to the compounds (P = 246.01; $df = 5$; $p < 0.001$) (Table 2). The compounds 2, 5, 8, and 9 caused 100% toxicity after 24 h against *R. dominica*, whereas compounds 4 and 3 showed 100% or >90% toxicity only after 72 h of treatment at 1.0 µg/mL concentration, respectively (Table 3). However, *T. castaneum* exhibited less susceptibility to the treatments in comparison to the other insects. At a concentration of 1.0 µg/mL, only the compounds 2, 5, 8, and 9 caused 100% mortality in these insects after 24 h of exposure (Table 4). It is concluded that *C. chinensis* and *S. oryzae* were more responsive to the *D. scandens* derivatives than *R. dominica* and *T. castaneum* in vapor-phase toxicity trials and the test compounds were effective to these pest insects.

Stored grains treated separately with compounds from *D. scandens* were highly toxic in vapor form to all of the insects at a 1.0 µg/mL concentration. In light of our results, they can be considered as promising insecticidal agents in stored-product management. The major advantage of the plant compounds tested in the present study is their high toxicity, which is a desirable characteristic for insecticidal preparations for the control of stored-product pests.^{20–23} This was confirmed in the present study. Notably, the sensitivity of species differs in the

Table 3. Insecticidal Activity of Isolated Compounds from *D. scandens* against *R. dominica* by the Fumigation Method

compounds	toxicity (%) ^a (1.0 µg/mL)			LC ₅₀ (95% CL) ^b (µg/mL)	χ ² (df)	p level
	24 h	48 h	72 h			
1	15.8 ± 4.6 d	18.6 ± 4.0 d	21.8 ± 2.3 a	>1.0	5.37 (5)	0.250
2	100 ± 0.0 c			0.32 (0.11–0.57)	44.41 (5)	0
3	89.3 ± 5.9 ba	90.8 ± 2.8 e	92.0 ± 1.9 f	0.34 (0.21–0.48)	14.22 (5)	0.006
4	93.4 ± 4.3 b	94.6 ± 2.9 c	100 ± 0.0 e	0.29 (0.09–0.48)	45.61 (5)	0
5	100 ± 0.0 ca			0.33 (0.22–0.47)	12.11 (5)	0.016
6	60.6 ± 4.0 e	64.2 ± 3.9 a	67.8 ± 5.4 g	0.82 (0.75–0.91)	1.01 (5)	0.908
7	72.3 ± 6.5 f	75.8 ± 2.7 b	78.2 ± 3.0 b	0.74 (0.61–0.94)	5.84 (5)	0.210
8	100 ± 0.0 c			0.27 (0.11–0.45)	27.4 (5)	0
9	100 ± 0.0 c			0.28 (0.08–0.51)	50.49 (5)	0

^a Values are the mean ± SD. Each column followed by the same letter is not significantly different from another (one-way ANOVA; Tukey test at <0.05).

^b Confidence level, after 24 h of treatment.

Table 4. Insecticidal Activity of Isolated Compounds from *D. scandens* against *T. castaneum* by the Fumigation Method

compounds	toxicity (%) ^a (1.0 µg/mL)			LC ₅₀ (95% CL) ^b (µg/mL)	χ ² (df)	p level
	24 h	48 h	72 h			
1	30.7 ± 5.6 b	32.8 ± 2.4 e	37.2 ± 2.8 g	>1.0	122.4 (5)	0
2	100 ± 0.0 e			0.26 (0.13–0.41)	27.29 (5)	0
3	92.3 ± 5.4 a	94.6 ± 1.3 a	100 ± 0.0 a	0.34 (0.27–0.40)	4.05 (5)	0.398
4	85.4 ± 3.9 d	88.4 ± 3.1 c	91.0 ± 2.6 d	0.38 (0.32–0.45)	4.71 (5)	0.318
5	100 ± 0.0 e			0.20 (0.16–0.28)	10.42 (5)	0.033
6	58.2 ± 6.1 c	60.8 ± 3.2 d	63.8 ± 4.6 e	0.82 (0.74–0.94)	1.23 (5)	0.993
7	24.7 ± 1.0 b	29.6 ± 1.9 b	32.2 ± 1.7 c	>1.0	11.98 (5)	0.017
8	100 ± 0.0 e			0.20 (0.08–0.31)	17.68 (5)	0.001
9	100 ± 0.0 e			0.18 (0.10–0.26)	9.95 (5)	0.041

^a Values are the mean ± SD. Each column followed by the same letter is not significantly different from another (one-way ANOVA; Tukey test at <0.05).

^b Confidence level, after 24 h of treatment.

tests. In fumigation bioassays, the volatile compound penetrates via the respiratory system; this leads to insect death by direct inhalation of the toxic compound in the insect body. Many plant extracts are known for their ovicidal, repellent, antifeedant, and insecticidal properties against various insect species.^{24,25} Some plant-derived materials are highly effective against insecticide-resistant strains of insect pests.^{24,26}

The insecticidal activity varied with insect species, concentrations of the test compounds, and exposure time. The results showed higher mortality rates in *C. chinensis* and *S. oryzae* than in *R. dominica* and *T. castaneum*. Moreover, our results indicated that higher concentrations of the test compounds for a relatively short period of time are much more effective than lower concentrations for a longer period. Studies have not been reported previously concerning the activity of *D. scandens* as an insecticidal agent on stored pests. The results of this study indicate that stored commodities can be protected satisfactorily against four major stored pests using compounds 2, 3, 4, 5, 8, and 9 from *D. scandens* at a concentration of 1.0 µg/mL. Surprisingly, even an application of these compounds at lower concentrations (0.20–0.40 µg/mL) can cause a high level of mortality in insect species and provides a good level of prevention against grain infestation. Hence, compounds 2, 3, 4, 5, 8, and 9 are proven to be superior to the other compounds 1, 6, and 7. In our results, *T. castaneum* was slightly resistant to the compounds 1, 3, 4, 6, and 7 tested. Only compounds 2, 5, 8, and 9 applied even at the

lowest concentration (0.2 µg/mL) showed highest efficacy against *T. castaneum* within 24 h of exposure. This indicates that *T. castaneum* is unable to detoxify the inhaled toxic compounds in its body, which led to its death.

The plant-derived insect-control agents could be incorporated into an IPM strategy, because these agents are selective to pests, have no or little harmful action against nontarget organisms and the environment, and demonstrate a differential mode of action against pests.^{24,25,27} On the basis of the results of this study, it can be concluded that chloroform extract *D. scandens* could have great potential for the control of stored pests. From this active extract, nine known compounds were isolated and shown to be potent insecticides for the control of four major stored pests in stored commodities. For the practical use of these plant extracts and their active ingredients as novel grain protectants, further research is required as far as safety issues for human health are concerned. Other areas requiring attention are development of cost-effective formulations with improved efficacy, insecticidal potency, and stability against the stored-product pests.

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