



Insecticidal Activity of *Melia toosendan* Extracts and Toosendanin Against Three Stored-product Insects

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Abstract—Repellency, toxicity, and fecundity-reducing effects of bark extracts of *Melia toosendan* on three stored-product beetles, the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), the rice weevil, *Sitophilus oryzae* (L.), and the red flour beetle, *Tribolium castaneum* (Herbst), were investigated in the laboratory. Wheat kernels treated with extracts containing 75 and 3% toosendanin at concentrations from 0.05 to 0.4% (375–3000 ppm toosendanin), and from 0.5 to 2.5% (150–750 ppm toosendanin), respectively, repelled beetles by 50–98%, compared with controls. The test materials were more toxic to *S. oryzae* than *C. ferrugineus* and were not toxic to *T. castaneum*. The LC₅₀ values for the extract containing 75% toosendanin for *S. oryzae* and *C. ferrugineus* after 6 weeks exposure were 675 and 1875 ppm toosendanin, respectively. Both extracts significantly reduced F₁ adults of all three insect species in most treatments. Pure (98%) toosendanin caused only 4% mortality of *C. ferrugineus* and *S. oryzae* at a concentration of 0.15% (1470 ppm toosendanin) after 2 weeks, but significantly reduced the F₁ adults compared with controls. This study indicates that a natural grain protectant based on *M. toosendan* extracts may be feasible.

Key words—*Melia toosendan*, toosendanin, repellency, toxicity, stored-product insects.

INTRODUCTION

The limonoid toosendanin has been used to kill gastrointestinal worms in humans in traditional Chinese medicine. It was first isolated from the bark of the trees *Melia toosendan* (Seid et Zucc) and *M. azedarach* L. (Meliaceae) in 1980 (Shu and Liang, 1980). Although its insecticidal activity has received considerable attention from Chinese scientists (Chiu, 1984, 1985, 1989; Chiu and Zhang, 1987; Liao and Chiu, 1986; Liao and Liu, 1986; Zhang and Chiu, 1983; Zhang *et al.*, 1992), the potential of this botanical chemical as a stored-product protectant has been neglected. Production of a botanical insecticide (Toosendanin, 0.5 EC) based on bark extracts (containing 3% toosendanin as the active ingredient) has recently begun in the People's Republic of China (Zhang *et al.*, 1992); making this natural product available for insect control on a commercial scale. Around the world, residual chemical insecticides and fumigation are currently the methods of choice for the control of stored-product insects (White, 1995). Their widespread use has led to some serious problems, including development of insect strains resistant to insecticides (Champ and

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Dyte, 1976; Zettler and Cuperus, 1990; White, 1995), toxic residues on stored grain, and health hazards to grain handlers. Thus, new approaches are being sought. Insecticides derived from higher plants, such as toosendanin, are desirable because they can be safe for the environment, users, and for consumers (Finney, 1990). The purpose of the present study was to investigate the insecticidal action of toosendanin and toosendanin-based extracts against three stored-product beetles.

MATERIALS AND METHODS

Insects and test materials

Three stored-product beetles, the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), the rice weevil, *Sitophilus oryzae* (L.), and the red flour beetle, *Tribolium castaneum* (Herbst), were used in this study. *C. ferrugineus* were reared on Canada Western hard red spring wheat (cv. Columbus) at 16% moisture content mixed with 10% cracked wheat (wt:wt). *S. oryzae* and *T. castaneum* were reared on the same medium with 14% moisture content. All species were collected from western Canada and kept in laboratory culture [30 ± 1 C, $70 \pm 5\%$ relative humidity (r.h.)] for over 3 years.

Toosendanin (98% purity) was isolated from the bark of *M. toosendan* (Shu and Liang, 1986). Dry powder of *M. toosendan* bark was extracted either by 70% ethanol or by water. The ethanol extract was further processed with chloroform to obtain refined extract containing 75% toosendanin. The crude water extract contained 3% toosendanin. The toosendanin concentration was measured by high performance liquid chromatography.

Repellency

Test materials were dissolved in methanol for stock solutions, then diluted with distilled water to provide the required concentrations of 0.05, 0.1, 0.2, 0.3 and 0.4% (wt:wt) for the extract containing 75% toosendanin (375–3000 ppm toosendanin); and 0.5, 1, 1.5, 2 and 2.5% (wt:wt) for the extract containing 3% toosendanin (150–750 ppm toosendanin). Distilled water was used as a control. Triton X-100 served as an emulsifier at a concentration of 0.1%. 800 g of wheat kernels were sprayed with 16 ml of aqueous solution using a small atomizer, placed in a glass jar and rotated on a roller for 30 min to obtain uniform distribution. The initial moisture content of wheat was 13%, and the final moisture content was 15%. Using standard food preference chambers (Loschiavo, 1952), a multiple choice bioassay was conducted to test for repellency. The brass chamber, divided into six equal sections by brass partitions, was cylindrical (6 cm high, 30 cm dia), with a raised arena (2 cm high, 10 cm dia) in the centre of the chamber. Six 200 g portions of treated wheat kernels with different concentrations of *M. toosendan* extract were randomly placed into each section. Two hundred unsexed adult beetles (1–2 weeks old) were introduced into the centre of an arena, and the chamber was kept at 30 ± 1 C and $70 \pm 5\%$ r.h. for 24 h. At the end of 24 h, the contents of each section were vacuumed and the number of insects in each section was determined by sifting the grain. The entire experiment was repeated four times.

Toxicity and fecundity reduction

Wheat kernels used in the repellency tests were held at -35°C for at least 3 h to kill any eggs laid during the multiple choice tests. For pure toosendanin (98%), only one concentration (0.15%, wt:wt) (1470 ppm toosendanin) was prepared, and its toxicity and fecundity-reducing effect were tested against *C. ferrugineus* and *S. oryzae*. Two sets of 5 glass vials (7 cm high, 2.7 cm dia) with 20 g of wheat per vial were prepared for each treatment. Ten unsexed adult beetles (1–2 weeks old) were introduced into each vial for the first set of vials (5 replicates). After 2 weeks at 30°C and 70% r.h., adult beetles were removed and placed in the second set of vials with wheat treated at the same concentration. Mortality of adult beetles was determined after 2, 4, and 6 weeks (except for pure toosendanin, which was tested for 2 weeks only). The first set of vials was held in the incubator for an additional 5 weeks before counting F_1 adults. The fecundity-reducing effect, if it occurred, was reflected in the reduction of F_1 adults.

Statistical analyses

The χ -squared test was used to test for differences between extract concentrations for a given species and between species for a given extract (SigmaStat, 1994). When correlations were found

Table 1. Number of *F*₁ adults developing in wheat kernels treated with extracts of *M. toosendanii* after 6 weeks

Extract (%)	Toosendanin (ppm)	Extract containing 3% toosendanin						Extract containing 75% toosendanin						98% toosendanin			
		Concentration		Number of offspring		Concentration		Number of offspring		Concentration		Number of offspring		Concentration		Number of offspring	
		<i>C. ferrugineus</i>	<i>S. oryzae</i>	<i>T. castaneum</i>	Extract (%)	Toosendanin (ppm)	<i>C. ferrugineus</i>	<i>S. oryzae</i>	<i>T. castaneum</i>	Extract (%)	Toosendanin (ppm)	<i>C. ferrugineus</i>	<i>S. oryzae</i>	<i>T. castaneum</i>	Extract (%)	Toosendanin (ppm)	<i>C. ferrugineus</i>
0	0	51.6a*	131.8a	62.4a	0	90.2a	272.8a	86.0a	0	0	100.6a	304.2a	0	0	100.6a	304.2a	
0.5	150	42.8ab	76.2ab	54.0ab	0.05	85.8a	205.8b	79.0ab	0.15	1470	52.8b	218.2b					
1.0	300	34.8bc	65.0b	44.8b	0.1	77.8ab	154.6bc	74.6abc									
1.5	450	33.4bc	58.8b	48.2b	0.2	64.4b	140.0c	67.6bc									
2.0	600	27.4bc	31.2b	42.4b	0.3	31.4c	110.0cd	60.0cd									
2.5	750	23.0c	30.6b	39.8b	0.4	30.0c	60.6d	45.6d									
<i>Regression parameters</i> †																	
Intercept		49.1	111.8	58.9		92.8	237.1	85.1									
Slope		-10.9	-37	-8.25		168.9	-456.2	-93.2									
R ² -value		0.96	0.86	0.85		0.95	0.89	0.98									
P-value		0.0005	0.0076	0.0088		0.001	0.0042	0.0001									
Extract EC ₅₀ (%) ‡		2.11	1.24	3.04		0.28	0.22	0.45									
(95% C.I.)		(1.78-2.44)	(0.80-1.68)	(1.91-4.18)		(0.22-0.33)	(0.15-0.28)	(0.39-0.50)									
Toosendanin EC ₅₀ (ppm) ‡		633	372	912		2100	1650	3375									
(95% C.I.)		(534-732)	(240-504)	(573-1254)		(1650-2475)	(1125-2100)	(2925-3750)									

*Means followed by the same letters within columns for each test material indicate no significant difference ($P > 0.05$) in the LSD test.

†Regression was performed between extract concentration and number of *F*₁ adults.

‡EC₅₀ represents effective concentration resulting in 50% reduction of *F*₁ adults relative to controls.

to be significant, linear regression was conducted to define dose–response relationships (Table 1). A logarithmic transformation [$\text{Log}_{10}(x + 0.1)$, where x = concentration] was performed before regression analysis. The EC_{50} values (effective concentrations of *M. toosendan* extracts required to reduce F_1 adults by 50% relative to controls) (Table 1) were determined by regression-prediction analysis (Anonymous, 1991). Analysis of variance (ANOVA) was performed for F_1 data (Table 1) and the least significant difference (LSD) test was used to compare means. Probit analysis (Finney, 1971) was applied to determine the lethal concentration of *M. toosendan* extract containing 75% toosendanin causing 50% mortality (LC_{50}) for *C. ferrugineus* and *S. oryzae*. Abbott's formula (Abbott, 1925) was used to correct for control mortality (less than 6%).

RESULTS AND DISCUSSION

Repellency

Extracts containing 75 and 3% toosendanin significantly repelled the three insect species (Fig. 1, $\chi^2 > 50$, $\text{df} = 5$, $P < 0.0001$). For each extract, all insect species responded to the repellency significantly different ($\chi^2 > 50$, $\text{df} = 5$, $P < 0.0001$). With the extracts containing 3% toosendanin, *S. oryzae* was the most repelled, followed by *T. castaneum*, with *C. ferrugineus* being the least

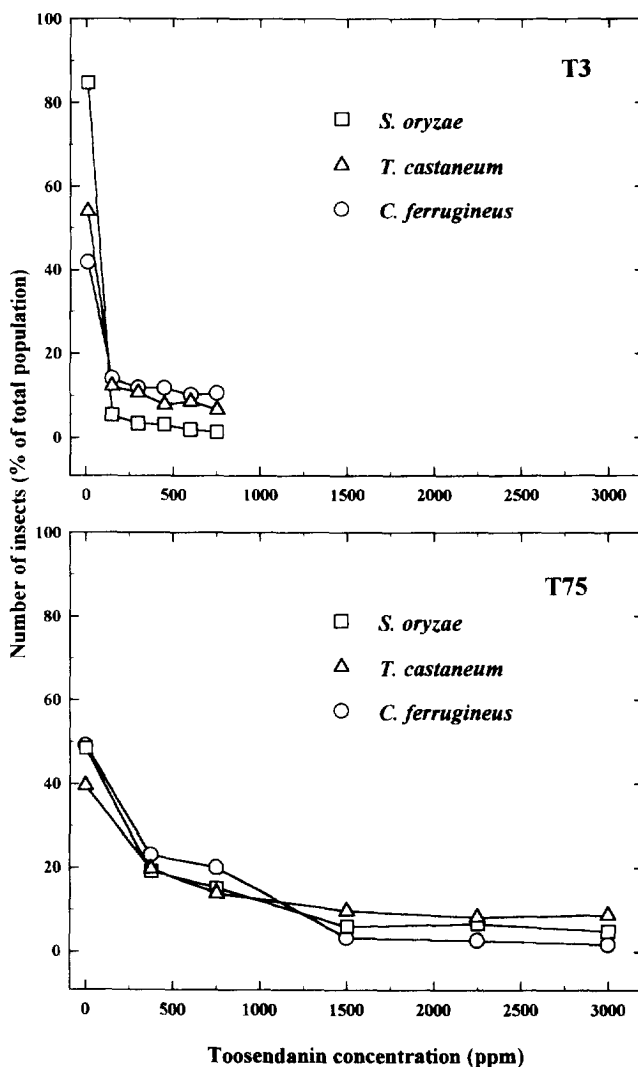


Fig. 1. Number of adult *C. ferrugineus*, *S. oryzae*, and *T. castaneum* in wheat treated with various concentrations of the bark extracts containing 75% toosendanin (T75) or 3% toosendanin (T3) under multiple choice conditions.

repelled. Although there were statistical differences with the extracts containing 75% toosendanin, the patterns were less clear and the differences were smaller than the extract containing 3% toosendanin.

Azadirachtin and neem are well known as repellents against various stored-grain insects, including the species we tested in this study (Jilani *et al.*, 1988; Jilani and Saxena, 1990; Xie *et al.*, 1995). Compared with the previous work with azadirachtin and neem extracts (Xie *et al.*, 1995), *M. toosendan* extracts showed a similar repellency pattern to the three beetles under identical test procedures. Furthermore, the present study indicated that insects could be significantly repelled from grain treated with *M. toosendan* extracts at a toosendanin equivalent of ≤ 300 ppm. However, *M. toosendan* extract was far less repellent than azadirachtin and azadirachtin-rich neem extracts, which repelled the three beetles at levels as low as 10 ppm (Xie *et al.*, 1995).

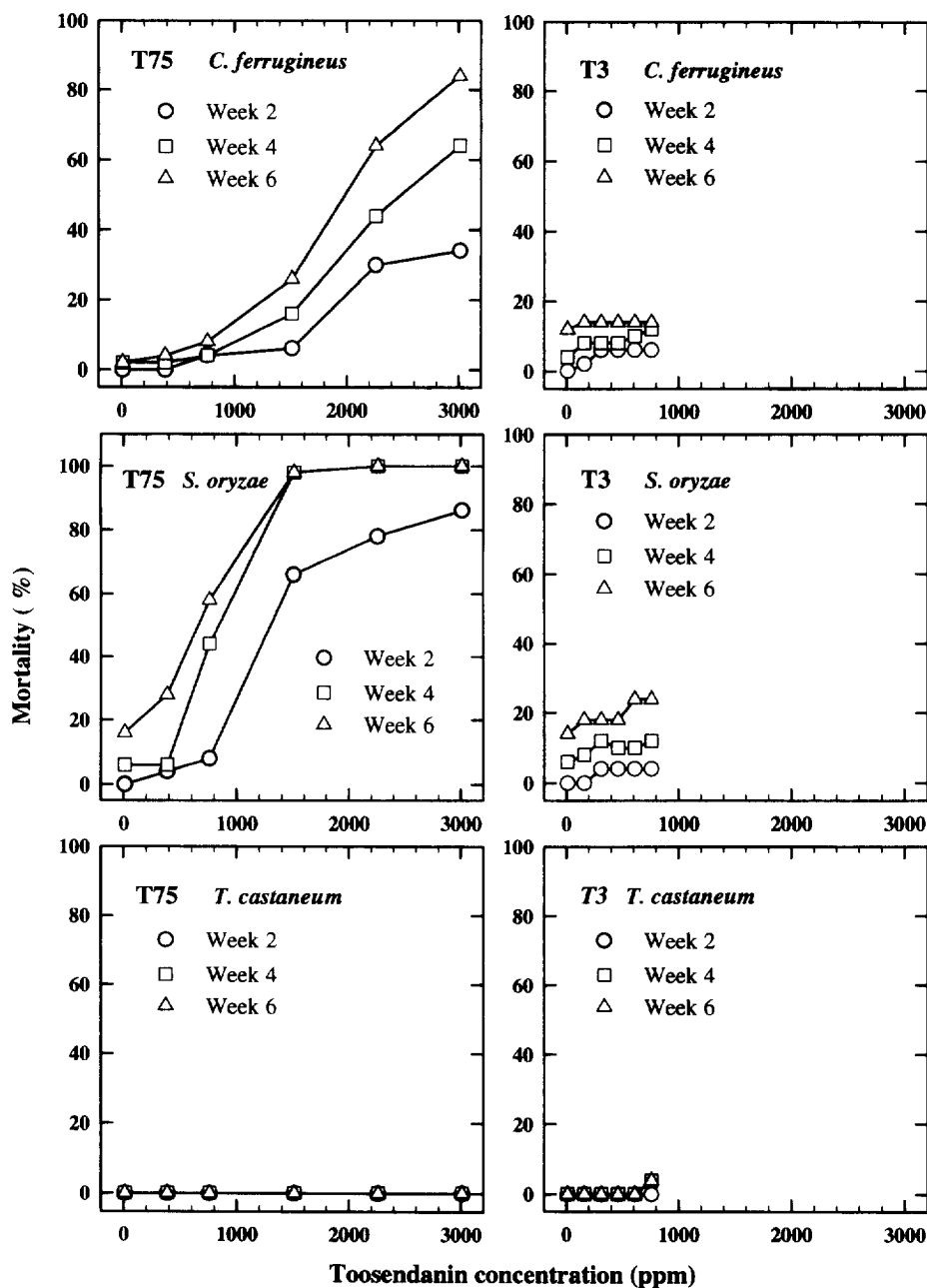


Fig. 2. Mortality of adult *C. ferrugineus*, *S. oryzae*, and *T. castaneum* held on wheat grains treated with various concentrations of the bark extracts containing 75% toosendanin (T75) or 3% toosendanin (T3). Standard errors for all mean values are less than 10%.

Toxicity

The toxic action of *M. toosendan* extracts varied with test materials and insect species. With the extract containing 75% toosendanin, *S. oryzae* was the most susceptible, followed by *C. ferrugineus*, whereas for *T. castaneum* no mortality was observed at the highest concentration tested (3000 ppm toosendanin) (Fig. 2). At a concentration of 1500 ppm toosendanin, it could provide excellent control (98% mortality) for *S. oryzae* and moderate control for *C. ferrugineus* (26% mortality) within 6 weeks. The LC₅₀ values (95% confidence interval) for *S. oryzae* and *C. ferrugineus* after 6 weeks were 675 ppm (525–825 ppm) toosendanin ($n = 250$; slope = 4.90 ± 0.56 ; $\chi^2 = 3.14$; $P < 0.01$) and 1875 ppm (1575–2250 ppm) toosendanin ($n = 250$; slope = 3.88 ± 0.49 ; $\chi^2 = 7.89$; $P < 0.01$), respectively. With the extract containing 3% toosendanin, less than 25% mortality for the three species studied was observed at the highest concentration tested (750 ppm toosendanin) after 6 weeks (Fig. 2). With pure (98%) toosendanin at a concentration of 1470 ppm toosendanin, only 4% mortality for *S. oryzae* and *C. ferrugineus* was observed after 2 weeks.

Earlier studies with *M. toosendan* extracts suggest that the limonoid toosendanin acts as an insect antifeedant, growth inhibitor and stomach poison (Zhang and Chiu, 1983; Chiu, 1985, 1989; Chiu and Zhang, 1987; Chen *et al.*, 1995). Our study indicated that *M. toosendan* extracts were toxic to *S. oryzae* and *C. ferrugineus*, although the mode of action is unknown. Since the antifeedant action of toosendanin has been well demonstrated, the observed mortality in this study was likely a consequence of both toxic and antifeedant (starvation) effects. Specific bioassays are needed to clarify the antifeedant effect on stored-product insects.

The toxicity of toosendanin was far less than that of azadirachtin, the active compound in neem extracts. Under an identical test procedure, azadirachtin-rich neem extracts (containing 7, 23 and 48% azadirachtin) had a LC₅₀ of 9 ppm azadirachtin for *C. ferrugineus* (Xie *et al.*, 1995). In contrast, the LC₅₀ for the extract containing 75% toosendanin was 3300 ppm toosendanin for *C. ferrugineus* in the present study.

The extract containing 75% toosendanin was more effective than pure toosendanin at the same level of toosendanin. This strongly suggests that some constituents in the extract other than toosendanin are also toxic to insects. Similar results were obtained for the growth inhibitory effect on the variegated cutworm, *Peridroma saucia* Hübner (Chen *et al.*, 1995). The isolation and identification of these other active compounds from the bark extract are currently under investigation.

Fecundity reduction

The extracts containing 3 and 75% toosendanin significantly reduced numbers of F₁ adults of all three insect species at concentrations of ≥ 300 and ≥ 1500 ppm toosendanin, respectively (LSD, $P < 0.05$) (Table 1). All these reductions were dose-dependent (Table 1). At a concentration of 1470 ppm pure (98%) toosendanin also significantly reduced the F₁ adults of *C. ferrugineus* or *S. oryzae* compared with controls (LSD, $P < 0.05$) (Table 1).

S. oryzae is the most sensitive to the fecundity-reducing effect, followed by *C. ferrugineus*, with *T. castaneum* being the least sensitive (significantly less sensitive compared with the other two species based on the failure of the 95% confidence interval to overlap). The differences in F₁ progeny could be explained by a number of factors. With the extract containing 75% toosendanin, the adult mortality during the first 2 weeks could account for the major portion of the differences in F₁ progeny (Fig. 2), as adult mortality at the highest concentration after 2 weeks was observed as 86, 34, and 0%, for *S. oryzae*, *C. ferrugineus* and *T. castaneum*, respectively. However, with the extract containing 3% toosendanin and for pure toosendanin, the reduction of F₁ progeny was probably a consequence of ovicidal and larvicidal action, or inhibition of oviposition. The adult mortality during the first 2 weeks could not explain the differences in F₁ progeny as the three species suffered similar mortality when they were exposed to the treated wheat for 2 weeks, yet the EC₅₀ values were significantly different between three species (Table 1).

The present study indicates that extracts from *M. toosendan* bark have repellent, toxic, and fecundity-reducing effects on stored-product insects. Plant natural products with good insecticidal activity are more likely to be used as leads for synthesis of new insecticides rather than as protectants *per se* (Benner, 1993). However, this may not be the case for extracts of *M. toosendan* because of two factors: (1) the structural complexity of toosendanin makes it difficult to be

synthesized; and (2) the natural resource (*M. toosendan*) is locally abundant. Although the effects of application of the *M. toosendan* extracts on baking, malting, noodle production and other food processes would have to be tested before they could be used in commercial grain stores, the data from this study indicated that these extracts could be used as a stored grain protectant. Finally, more in depth toxicological studies would be required to prove that *M. toosendan* extracts have no adverse effects on mammals.

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