

## Antifeedant and Insecticide Properties of a Limonoid from *Melia azedarach* (Meliaceae) with Potential Use for Pest Management

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In the course of screening for novel naturally occurring insecticides from plants, the activity of the fruit extract of the Argentinian *Melia azedarach* L. (Meliaceae) and its recently described limonoid meliartenin were investigated. The antifeedant activity of the fruit extract was tested on a variety of herbivore and granivorous insects through choice tests. Sixteen of 17 species belonging to three orders consume significantly less food when treated with the extract. The bioactivity of the isolated active compound meliartenin and its interchangeable isomer 12-hydroxiamoorastatin (**1**) was further studied. In choice tests, compound **1** inhibited feeding of *Epilachna paenulata* Germ. (Coleoptera, Coccinellidae) larvae, with an ED<sub>50</sub> value of 0.80 μg/cm<sup>2</sup>, comparable to that of azadirachtin (**2**) and lower than that of toosendanin (**3**) (0.72 and 3.69 μg/cm<sup>2</sup>, respectively), both compounds used for comparison purposes. In no-choice tests, *E. paenulata* larvae reared on food treated with **1** or **2** ate less, gained less weight, and suffered greater mortality rates than control larvae. The activity of compound **1** was comparable to that of **2**, with LD<sub>50</sub> values of 0.76 and 1.24 μg/cm<sup>2</sup>, respectively, at 96 h. Shorter LT<sub>50</sub> values were recorded for **1** at 4 and 1 μg/cm<sup>2</sup> in comparison with **2**. Thus, *M. azedarach* fruit extract and its active principle have interesting potential for use in pest control programs.

**KEYWORDS:** *Melia azedarach*; Paraiso; insect antifeedant; limonoid; meliartenin

### INTRODUCTION

Approximately one-third of the global food production is destroyed annually by field and storage pests (*1*). Despite expensive and often environmentally hazardous control measures, insects remain the chief pests of crops and stored products (*2*). Synthetic pesticides are currently the most effective means of pest control, but the appearance of insect resistance and other negative side effects has prompted a search for new alternatives. Wild plants may derive adequate protection against insect herbivores from an “umbrella” of chemical compounds, which may be exploited to protect susceptible crop plants (*3*) and represent a basis for effective and environmentally safe botanical pesticides.

Chemicals isolated from species belonging to the Meliaceae family, among them *Melia azedarach* L. and *Azadirachta indica* A. Juss, have in recently received particular attention from applied entomologists because of their excellent properties as

insect control agents (*3*). This effect could be attributed to the presence of limonoids (*4–8*) with insect antifeedant activity (*9–12*), as well as a high potential for toxic interference with the basic biochemical and physiological functions of insect herbivores (*13*).

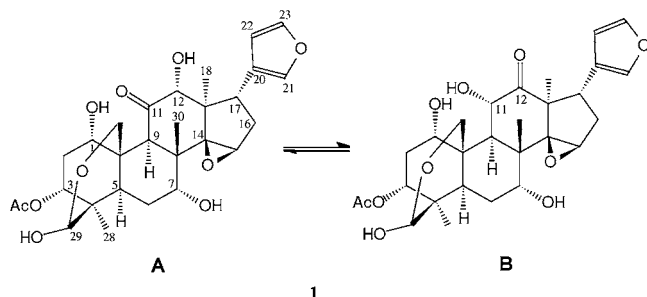
Although native to India and China, *M. azedarach* is currently found in Africa, Australia, and the Americas (*4, 12*). This tree is widespread in Argentina, being used for timber and ornamental purposes. Extracts of Argentinian *M. azedarach* trees have shown strong insect repellent effects (*14, 15*). Compounds from some chemotypes of *M. azedarach* have been reported to be toxic to mammals (*16, 17*), but studies on fruit extract from trees growing in Argentina revealed no such toxicity (*18*).

In the present paper, we first describe the antifeedant activity exhibited by the fruit extract of *M. azedarach* on a variety of herbivore and granivorous insects, to assess the potential use of the extracts in pest management programs. After important antifeedant effects of the extract had been verified, its most active principle obtained via a bioassay-guided isolation process (*19*) was further studied in comparison to azadirachtin (**2**) and toosendanin (**3**). Effects on feeding behavior, development, and

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**Figure 1.** Chemical structures of compound **1**, 12-hydroxyamoorastatin (**A**), and meliartenin (**B**).

mortality of *Epilachna paenulata* Germ. (Coleoptera, Coccinellidae) were analyzed in that framework.

## MATERIALS AND METHODS

**Plant Material.** Ripe fruits of *M. azedarach* were collected at Córdoba, Argentina, in October 1999. A voucher specimen has been deposited at the Botanical Museum of Córdoba (CORD 229, Córdoba, Argentina).

**General Experimental Procedures.** Azadirachtin was purchased from Sigma Chemical Co., Inc. (St. Louis, MO); toosendanin (**3**) was a gift from Dr. M. B. Isman (Department of Plant Science, University of British Columbia, Vancouver, BC, Canada).  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were obtained at Serveis Científico-Tècnics-University of Barcelona with a Bruker AC 500 spectrometer operated at 500 MHz for  $^1\text{H}$  and at 125 MHz for the  $^{13}\text{C}$  nucleus in  $\text{CD}_3\text{CN}$  (Aldrich Chemical Co., Inc.), using tetramethylsilane as an internal standard. UV spectra were recorded in  $\text{CH}_3\text{CN}$  on a Shimadzu UV-260 spectrophotometer, and optical rotation angles were recorded using a JASCO DIP-370 spectropolarimeter (JASCO Co., Tokyo, Japan). HPLC was performed on a Phenomenex Prodigy  $5\mu$  ODS (10 mm i.d.  $\times$  250 mm) reversed-phase column, and UV detection was at 210 nm. MS spectra were measured with a Finnigan 3300-f100 instrument. Silica gel grade 70–230 mesh, 60 Å, for column chromatography were purchased from Sigma Chemical Co., Inc. All solvents were purchased from Merck (Darmstadt, Germany).

**Extraction and Isolation.** Crushed ripe fruits (200 g) of *M. azedarach* were extracted with a Soxhlet apparatus, first using hexane to defat the crude raw material and then using ethanol. After an exhaustive evaporation of the alcohol, the complete viscous extract (66 g), dissolved in the necessary amount of distilled water to reach the desire dosages, was ready for testing antifeedant activity.

For the isolation of the anti-insect principle (**19**), the air-dried kernels of ripe fruits (290 g) were extracted with ethanol after defatting with hexane to yield, after evaporation under vacuum, 10.5 g of extract [antifeedant index (AI) on *E. paenulata* = 95.3%, 5% extract]. The resulting extract was partitioned between  $\text{MeOH}/\text{H}_2\text{O}$  and  $\text{CH}_2\text{Cl}_2$ . The  $\text{CH}_2\text{Cl}_2$ -soluble extract (4.5 g) was twice vacuum liquid-chromatographed, and those fractions with antifeedant activity >90% were then separated in successive radial chromatography; the resulting limonoid fraction (AI = 93%, 400 ppm, corresponding to  $8\ \mu\text{g}/\text{cm}^2$ ) was separated by means of HPLC to yield compound **1** (17 mg) (**Figure 1**).  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and other physical data were further determined in order to confirm the chemical structure.

**Compound 1:**  $\text{C}_{28}\text{H}_{36}\text{O}_{10}$ ; mp 243–244 °C from  $\text{Me}_2\text{CO}$ ;  $[\alpha]_{\text{D}}^{24}$   $-43.7^\circ$  ( $c$  0.4,  $\text{CH}_3\text{CN}$ ); UV  $\lambda_{\text{max}}$  ( $\text{CH}_3\text{CN}$ ) nm ( $\epsilon$ ) 204 (6032); EIMS,  $m/z$  532 ( $\text{M}^+$ ), 514 ( $\text{M}^+ - \text{H}_2\text{O}$ ), 496 ( $\text{M}^+ - 2\text{H}_2\text{O}$ ), 478 ( $\text{M}^+ - 3\text{H}_2\text{O}$ ), 472 ( $\text{M}^+ - \text{AcO}$ ), 454 ( $\text{M}^+ - \text{H}_2\text{O} - \text{AcO}$ ), 408, 311, 239, 163, 94.

**12-Hydroxyamoorastatin (A):**  $t_{\text{R}}$  = 32.8 min (by HPLC);  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ )  $\delta$  0.75 (3H, s, 28-Me), 1.01 (3H, s, 30-Me), 1.07 (3H, s, 18-Me), 1.60 (1H, dt,  $J$  = 14.3, 3.9 Hz, H-6 $\alpha$ ), 1.72 (1H, dt,  $J$  = 16.0, 1.4 Hz, H-2 $\beta$ ), 1.85 (1H, dd,  $J$  = 14.1, 2.2 Hz, H-6 $\beta$ ), 1.93 (1H, m, overlapping H-16 $\beta$  and  $\text{CH}_3\text{CN}$ ), 1.99 (3H, s,  $\text{COCH}_3$ ), 2.21 (1H, m, H-16 $\alpha$ ), 2.66 (1H, dd,  $J$  = 13.6, 5.1 Hz, H-5), 2.70 (1H, dt,  $J$  = 16.1, 4.8 Hz, H-2 $\alpha$ ), 2.84 (1H, dd,  $J$  = 11.0, 6.4 Hz, H-17), 3.52 (1H, dd,  $J$  = 5.5, 3.3 Hz, H-7), 3.73 (1H, br s, H-15), 3.91 (1H, d,  $J$  = 2.0 Hz, H-12), 4.16 (1H, d,  $J$  = 12.6 Hz, H-19b), 4.17 (1H, d,  $J$  = 12.5 Hz, H-19a), 4.24 (1H, br t,  $J$  = 4.0 Hz, H-1), 4.47 (1H, s, H-9), 4.70 (1H,

dd,  $J$  = 5.1, 0.9 Hz, H-3 *endo*), 4.78 (1H, d,  $J$  = 4.3 Hz, H-29), 5.11 (1H, dd,  $J$  = 4.8, 1.3 Hz, H-3 *exo*), 6.50 (1H, dd,  $J$  = 0.5, 1.85 Hz, H-22), 7.26 (1H, q,  $J$  = 1.2 Hz, H-21), 7.34 (1H, t,  $J$  = 1.7 Hz, H-23);  $^{13}\text{C}$  NMR  $\delta$  14.2 (C-18), 19.3 (C-28), 21.1 (C-3'), 22.6 (C-30), 25.3 (C-6), 28.5 (C-5), 36.3 (C-2), 39.5 (C-17), 40.1 (C-4), 42.0 (C-10), 42.7 (C-8), 46.6 (C-13), 48.5 (C-9), 58.8 (C-15), 64.4 (C-19), 66.1 (C-14), 70.0 (C-7), 70.1 (C-1), 73.3 (C-3), 79.3 (C-12), 96.3 (C-29), 113.6 (C-22), 124.9 (C-20), 141.3 (C-21), 142.5 (C-23), 170.7 (C-2'), 214.3 (C-11).

**Meliartenin (B):**  $t_{\text{R}}$  = 41.7 min (by HPLC);  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ )  $\delta$  0.82 (3H, s, 28-Me), 1.04 (3H, s, 30-Me), 1.06 (3H, s, 18-Me), 1.70 (1H, dt,  $J$  = 16.0, 1.4 Hz, H-2 $\beta$ ), 2.01 (3H, s,  $\text{COCH}_3$ ), 2.57 (1H, dd,  $J$  = 13.6, 4.1 Hz, H-5), 2.72 (1H, dt,  $J$  = 16.1, 4.8 Hz, H-2 $\alpha$ ), 2.85 (1H, dd,  $J$  = 11.2, 6.0 Hz, H-17), 3.48 (1H, dd,  $J$  = 5.9, 3.6 Hz, H-7), 3.72 (1H, br s, H-15), 3.97 (1H, dd,  $J$  = 11.6, 1.0 Hz, H-11), 4.12 (1H, d,  $J$  = 11.9 Hz, H-19b), 4.13 (1H, d,  $J$  = 11.6 Hz, H-19a), 4.44 (1H, d,  $J$  = 12.0 Hz, H-9), 4.29 (1H, br t,  $J$  = 4.4 Hz, H-1), 4.66 (1H, d,  $J$  = 3.2 Hz, H-29), 6.51 (1H, dd,  $J$  = 0.6, 2.0 Hz, H-22);  $^{13}\text{C}$  NMR  $\delta$  14.2 (C-18), 18.5 (C-28), 21.1 (C-3'), 22.8 (C-30), 27.4 (C-6), 25.9 (C-5), 36.7 (C-2), 39.4 (C-17), 40.3 (C-4), 41.9 (C-10), 42.8 (C-8), 46.6 (C-13), 48.3 (C-9), 66.0 (C-14), 58.9 (C-15), 73.2 (C-3), 70.4 (C-1), 79.3 (C-11), 96.0 (C-29), 124.9 (C-20), 142.5 (C-23), 170.6 (C-2'), 214.2 (C-12).

**Insects.** The experiments with complete fruit extract were conducted using field populations of insects. Tests were made with those species from which at least 20 individuals in the same life cycle stage were collected. Leaves from the same crop where the insects were collected were used as substrate for the assays (**Table 1**); rice wafers were used in tests with granivorous insects.

*E. paenulata* larvae were obtained from a laboratory colony, reared on a natural diet of *Cucurbita maxima* leaves and maintained in a growth chamber at  $24 \pm 1$  °C and 70–75% relative humidity, with a photoperiod of 16/8 h light cycle, and periodically renewed with field specimens. This insect represents the local equivalent of *E. varivestis*, a species showing high tolerance to feeding inhibitors and therefore considered to be most suitable for studies of biological activities of phytochemical compounds (20).

**Insects Bioassays. Feeding Choice Assays.** To scan the antifeedant activity of the complete extract, conventional choice tests were used for herbivore insects (14) and the technique explained by Taludker and Howse (21) for granivorous species. For the root-feeding larvae of *Diabrotica speciosa*, five individuals were given a choice of five treated and five untreated seedling corn grains in a Petri dish; the numbers of larvae feeding on each type of corn were compared. The substrate and number of replicates used in each test are indicated in **Table 1**. The relative amounts (recorded in percentage from 0 to 100) of the treated and untreated substrate area eaten in each feeding choice test were estimated visually by dividing the food area in imaginary quarters. The measurements were always done by the same operator. Data were then compared by using the Wilcoxon signed paired rank test,  $\alpha$  = 0.05. The antifeedant index (AI %) was calculated as  $(1 - T/C) \times 100$  (22), where  $T$  and  $C$  represent the consumption of treated and untreated foods, respectively.

The antifeedant experiments of kernel crude extract and chromatography fractions (dissolved in EtOH,  $\text{Me}_2\text{CO}$ , or  $\text{Et}_2\text{O}$  depending on the polarity) were carried out on *E. paenulata* by a modified leaf-disk choice test (23, 24). Two cotyledon leaves from a *C. maxima* seedling were placed in a Petri dish, and a glass disk with two 1  $\text{cm}^2$  diameter holes was placed on top. A third-instar *E. paenulata* was placed equidistant from both a treated and an untreated (solvent control) leaf disk and allowed to feed for 24 h. The AI % was then calculated.

The antifeedant dose–response of **1** was also tested on *E. paenulata* larvae as described above. Different dosages of **2**, the most active principle from *Azadirachta indica*, and **3**, isolated from *Melia toosendan*, were used for comparison of antifeedant activity.

Pure compounds **1–3** were dissolved in  $\text{Me}_2\text{CO}$ . After calculation of the AI, the relative potency ( $\text{ED}_{50}$  values, the effective dosage for 50% feeding reduction) for each compound was determined by Probit analysis.

**No-Choice Feeding Assays.** These tests were carried out to analyze possible effects of the pure compound **1** in comparison with **2** and **3** on insect development and survival while we further studied its

**Table 1.** Feeding Choice Tests with *M. azedarach* Fruit Extract and Different Insect Species (Results at 24 h)

species	stage <sup>a</sup>	substrate (leaves)	n	AI <sup>b</sup> (%)	dosage ( $\mu\text{g}/\text{cm}^2$ )	
Order Coleoptera						
family Curculionidae	<i>Sitophylus oryzae</i> (Linné)	A	rice wafer	8 × 10	51.8 <sup>c</sup>	2000
	<i>Pantomorus leucoloma</i> Boheman	A	soy	20	81.0 <sup>c</sup>	400
	<i>Priocoryphus bosqui</i> (Hustache)	A	soy	20	95.0 <sup>c</sup>	2000
family Tenebrionidae	<i>Tribolium confusum</i> Duval	A	rice wafer	8 × 10	88.4 <sup>c</sup>	2000
family Coccinellidae	<i>Epilachna paenulata</i> (Germ)	A	squash	20	90.0 <sup>c</sup>	400
family Chrysomelidae	<i>Diabrotica speciosa</i> (Germar)	L III		20	88.0 <sup>c</sup>	2000
		L III	corn grain	16 × 5	65.7 <sup>c</sup>	2000
	<i>Chrysodina</i> sp.	A	alfalfa	17	100 <sup>c</sup>	400
		A	alfalfa	17	100 <sup>c</sup>	2000
	<i>Epitrix argentiensis</i> Bryan	A	eggplant	20	97 <sup>c</sup>	2000
	<i>Eumolpinae</i> sp.	A	eggplant	20	100 <sup>c</sup>	1000
	<i>Plagioneda erythroptera</i> (Blanchard)	A	willow	20	91.0 <sup>c</sup>	400
	<i>Xanthogalleruca luteola</i> (Müller)	A	elm	20	100 <sup>c</sup>	2000
L			13	86.4 <sup>c</sup>	400	
Order Orthoptera						
family Romaleidae	<i>Cromachris miles</i> (Drury)	N	duraznillo negro	30	72.0 <sup>c</sup>	2000
Order Lepidoptera						
family Arctiidae	<i>Spilosoma virginica</i> (Fabricius)	L IV	quinoa	20	96.0 <sup>c</sup>	400
family Noctuidae	<i>Anticarsia gemmatalis</i> (Hubner)	L V				
		L III	soy	20	86.0 <sup>c</sup>	2000
	<i>Rachiplusia nu</i> Guenée	L IV				
		L IV L V	alfalfa	26	22.0	2000
		L IV	alfalfa	20	60.0 <sup>c</sup>	2000
family Pieridae	<i>Colias lesbias</i> (Fabricius)	L V				
		L V	soy	20	76.0 <sup>c</sup>	400

<sup>a</sup> A, adult; L, larva; N, nymph. <sup>b</sup> AI (%): antifeedant index =  $(1 - \text{treatment consumption}/\text{control consumption}) \times 100$ . <sup>c</sup> Consumption significantly lower on extract-treated food,  $p < 0.05$ , Wilcoxon signed paired rank test.

antifeedant activity. Four *E. paenulata* larvae (third instar) were placed in a Petri dish and fed *C. maxima* leaves (renewed every 48 h) on which known quantities of either the pure compounds or solvent were applied with a Hamilton syringe. Six replicates were used for each treatment. A similar set of larvae were not fed at all and acted as starved controls. Leaf consumption, body weight, and mortality were recorded every 24 h. Mortality data were used for lethal dosage (LD<sub>50</sub>) calculation through Probit analysis. The time required for 50% mortality (LT<sub>50</sub>) was estimated through linear regression.

Data shown in the figures are presented as means  $\pm$  standard errors. Mortality rates at each date, as well as body weights and average daily consumption, were compared among treatments by analysis of variance and Tukey's honestly significant difference test, with  $\alpha = 0.05$ .

## RESULTS AND DISCUSSION

The usefulness of antifeedant activity studies limited to one test species has been seriously questioned (25). Following this criticism, the present study included a variety of species as seen in **Table 1**, which shows that fruit extract from *M. azedarach* inhibited the feeding activity of several pest species belonging to three different orders. With the only exception of *Rachiplusia nu* Guenée, all of the species tested ate significantly less food when this was treated with *M. azedarach* fruit extract (Wilcoxon,  $p < 0.05$ ) at 400, 1000, and 2000  $\mu\text{g}/\text{cm}^2$  (corresponding to ca. 5.5, 13.7, and 27.6  $\mu\text{g}/\text{cm}^2$  of compound **1**). For most of the species, AI values indicated a high (>75%) inhibitory activity, or at least a moderate one (50–75%). Coleoptera species appeared to be particularly sensitive to this extract, showing in many cases antifeedant index values as high as 90–100%, in contrast with the strongest response showed by Lepidoptera species in experiments with azadirachtin (24).

Through a chromatographic fractionation of the extract, led by bioassays on *E. paenulata*, compound **1** (see **Figure 1**) was isolated as the most active principle from kernels of Argentinian *M. azedarach* (19). The characterization of this compound, existing as a mixture of two interchangeable isomers, has been described previously (19).

**Table 2.** Effective Dosage (ED<sub>50</sub>) of the Test Compounds 1–3 against *E. paenulata* Larvae in Choice Test

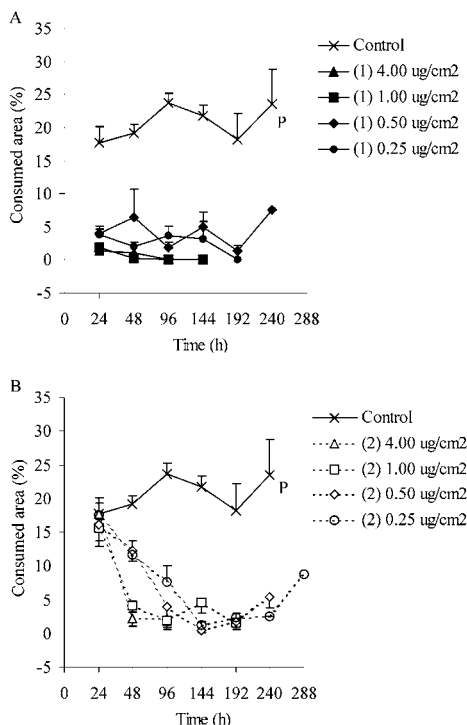
compd	ED <sub>50</sub> <sup>a</sup> ( $\mu\text{g}/\text{cm}^2$ ) values and 95% confidence limits (lower, upper)
1	0.80 (0.02, 25.53)
2	0.72 (0.02, 18.22)
3	3.69 (0.03, 387.01)

<sup>a</sup> ED<sub>50</sub> is the dosage required to give an antifeedant index of 50%.

When we compared the relative potencies (ED<sub>50</sub>) of compounds **1–3** (**Table 2**), compound **1** exhibited the same level of activity as **2** and a nearly 5 times greater activity than **3**. Other compounds have been isolated from *M. azedarach* fruit extract (26–28) and from *M. toosendan* (29, 30), exhibiting an antifeedant effect comparable to or lower than that of compound **1**.

In the no-choice experiments and after 24 h (**Figure 2**), larvae exposed to leaves treated with compound **1** had eaten at least 4 times less than those either confronted with **2** at the same dosages or receiving untreated leaves ( $F = 21.83$ ;  $\text{df} = 8$ ;  $p < 0.001$ ). Moreover, compound **2** did not differ significantly from the control at this time but showed an abrupt reduction in food consumption 24 h later. After 6 days of exposure to treated food, larvae receiving either **1** or **2** showed nearly null consumption values, significantly lower than those of control larvae ( $F = 9.95$ ;  $\text{df} = 6$ ;  $p < 0.001$ ).

The different activities of the compounds at the initial stage of this experiment (24 and 48 h) could be linked to differences in how their antifeedant activity is attained. There is evidence that **2** may act on insect gut musculature, reducing motility and consequently suppressing feeding (31). Moreover, a reduction of feeding has been observed with administration of **2** via topical application or injection (32). These findings support the hypothesis of a secondary antifeedant effect, where reduction of food intake follows some initial consumption (32). Our



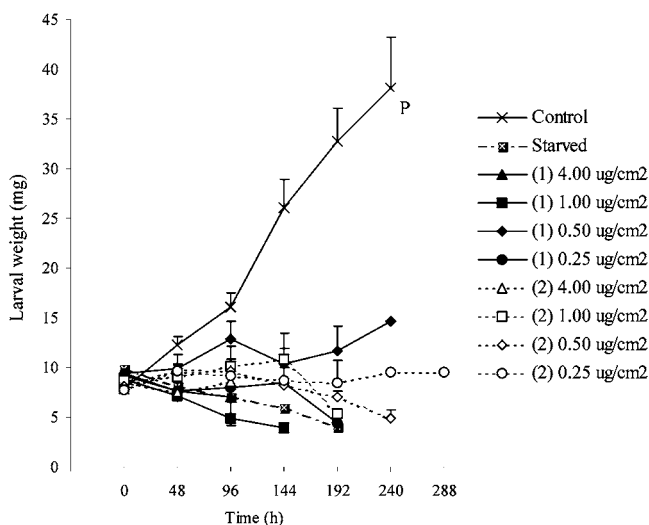
**Figure 2.** Average area consumed by each *E. paenulata* larva on leaves treated with **1** (A) and **2** (B) in no-choice tests. P indicates pupation time. See text for additional experimental details.

observations from no-choice tests, with larvae initially eating as much on leaves treated with **2** as untreated leaves and showing an inhibitory effect only after 48 h, agree with what would be expected from a secondary antifeedant. It must be noted that **2** can also behave as a primary antifeedant compound (24) and acted as such in our choice tests. The different results between no-choice and choice tests could be associated with differences in the protocol of both types of experiments, which may involve a change in the insect's perception of the food (33).

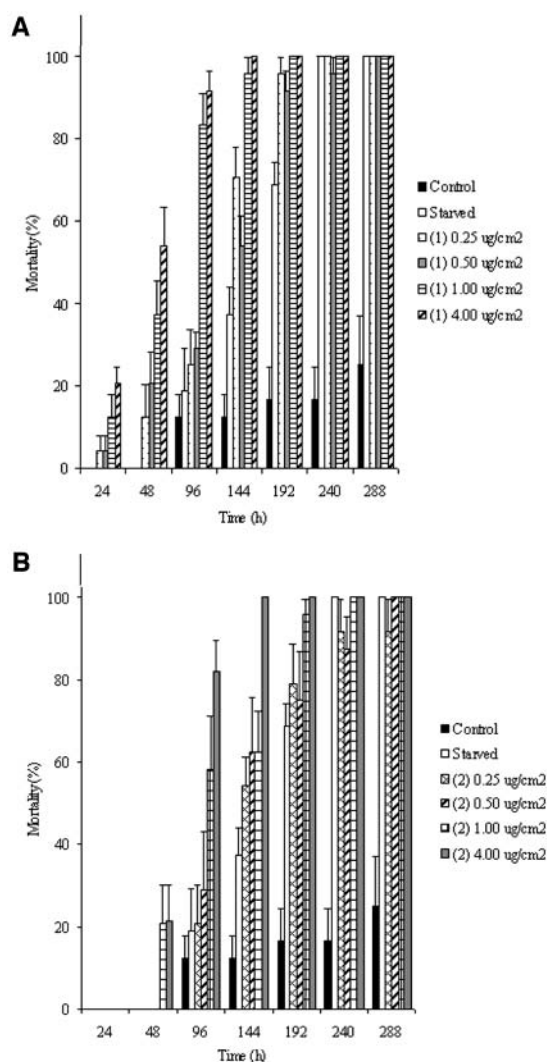
Instead, compound **1** elicited an immediate rejection for treated food, acting as could be expected from a primary antifeedant, probably via the gustatory pathway regulated by sensory organs of the mouthparts (32).

Analysis of larval weight data (Figure 3) showed that whereas control larvae steadily increased their body weight, treated larvae either remained stationary or lost weight. From 96 h onward, significant differences in body weight ( $F = 3.26$ ;  $df = 9$ ;  $p = 0.005$ ) were observed between the control and higher dosages of compound **1**, whereas after 6 days (144 h), all treated larvae were less than half the weight of those receiving untreated food ( $F = 8.87$ ;  $df = 6$ ;  $p < 0.001$ ). Reduced weight gain compared to control was also observed after 72 h on *Spodoptera litura* larvae treated with meliatoxin A<sub>2</sub> and meliatoxin B<sub>1</sub>, both compounds isolated from *M. azedarach*, at 480 and 600  $\mu\text{g}/\text{cm}^2$ , respectively (26).

Larval mortality rates (Figure 4) were also affected by food being treated with the studied compounds. Significant differences were observed 24 h after the start of the experiment ( $F = 5.86$ ;  $df = 9$ ;  $p < 0.001$ ), when treatments with compound **1** suffered up to 20% mortality (increasing with extract dosage), whereas all larvae in any other treatment were still alive. The observation that larvae fed with compound **1** were dying more quickly than the starved insects could suggest a toxic activity, although further studies are needed to confirm this hypothesis. An important increase in larval mortality was also observed with



**Figure 3.** Average body weight of each *E. paenulata* larva confronted with leaves treated with **1** and **2** in no-choice tests. P indicates pupation time. See text for additional experimental details.



**Figure 4.** Mortality of *E. paenulata* larvae confronted with leaves treated with **1** (A) and **2** (B) in no-choice tests. See text for additional experimental details.

the highest dosage of compound **2**, but only after 4 days (96 h) of treatment ( $F = 7.29$ ;  $df = 9$ ;  $p < 0.001$ ). At lower dosages of both compounds, mortality rates did not differ from those of

**Table 3.** Lethal Time (LT<sub>50</sub>) of Compounds **1** and **2** and Starvation Control for Larvae of *E. paenulata* in No-Choice Tests

dosage (μg/cm <sup>2</sup> )	LT <sub>50</sub> <sup>a</sup> (h)		starved larvae
	<b>1</b>	<b>2</b>	
4.00	25.69	86.98	
1.00	64.18	115.06	
0.50	130.75	146.04	
0.25	127.49	151.91	
			155.59

<sup>a</sup> LT<sub>50</sub> is the time required to obtain 50% mortality.

starved larvae. However, after 6 days of receiving food treated with any dosage of compound **1** or **2**, larval mortality had significantly increased in comparison with control larvae ( $F = 13.91$ ;  $df = 9$ ;  $p < 0.001$ ), and there were practically no survivors after 12 days. This is probably related to the strong feeding inhibiting effect of the compounds, mortality being due to the reduced food consumption previously mentioned (see **Figure 2**).

From these mortality data, LD<sub>50</sub> was calculated at 96 h, at which time both compounds exhibited mortality values below as well as above 50%, thus allowing the Probit calculation. The results indicate a better performance for **1** (0.76 μg/cm<sup>2</sup>; 95% confidence interval = 0.28–2.09) than for **2** (1.24 μg/cm<sup>2</sup>; 95% confidence interval = 0.34–4.47), which might be associated with the early manifestation of effects from the first compound.

Lethal times (**Table 3**) were also shorter for compound **1**, particularly at higher dosages (4 and 1 μg/cm<sup>2</sup>), which presented values ~2–3 times lower than those exhibited by compound **2**. This could again be linked to the difference in toxicity noticed in the first hours of the experiment.

From the results presented here, the activity shown by compound **1** was comparable to the commercial limonoid azadirachtin, the most potent antifeedant compound currently known. Feeding inhibition and toxicity appeared even slightly earlier for compound **1**. These findings suggest that compound **1** could be a promising alternative for secure pest control in crops.

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