Antifeedant Activity of Fruit and Seed Extracts of *Melia azedarach* and *Azadirachta indica* on Larvae of *Sesamia nonagrioides*

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Methanolic extracts of seeds and fruits of the chinaberry tree, *Melia azedarach* L. (Meliaceae), showed strong antifeedant activity against 2nd instar larvae of *Sesamia nonagrioides* Lefèbvre (Lepidoptera: Noctuidae), a very serious pest of maize (*Zea mays* L.) in Mediterranean countries. Extracts were applied in an artificial diet at concentrations of 1000 and 2000 ppm. The parameters used to evaluate the activity were larval growth rates; quantity of food ingested; phagodepression/phagostimulation index; quantity of frass produced; quantity of material ingested; duration of larval development; and cumulative mortality. Seed extract showed high bioactivity at both doses, while fruit extract proved to be less active, and only at the higher dose used (2000 ppm) did it display a slight antifeedant activity. The activity of the *M. azedarach* seed extract at the higher dose (2000 ppm) was comparable to that of pure azadirachtin applied at a dose of 1.25 ppm, or to 'Mubel', a commercial extract of *Azadirachta indica* A. Juss. (Meliaceae), applied at a dose of 75 ppm.
KEY WORDS: *Melia azedarach*; *Azadirachta indica*; Meliaceae; *Sesamia nonagrioides*; Lepidoptera; Noctuidae; antifeedant activity; phagodepression; azadirachtin; commercial extract of Meliaceae.

INTRODUCTION

Multiple strategies of plant defence have resulted from plant–insect coevolution. One of them is the production of allelochemical compounds that affect the growth and survival of phytophagous insects (9). These substances can also interfere with the communication mechanisms of insects at the interspecific level (28). Substances that can influence behavioral aspects, such as compounds with antifeedant or oviposition deterrent activity (12), or insect-growth regulators (21), are included in this group.

Some species of Meliaceae are characterized by containing compounds that possess a high level of bioactivity against insects and acari. Among them, the Indian neem tree, *Azadirachta indica* A. Juss., stands out. Specific seed extracts with known effectiveness in pest control have been marketed from this tree (18,25). In these extracts, azadirachtin, a tetranortriterpenoid compound of the limonoid family, exhibits the highest biological activity against a large number of insect pests (2,18,25), although other bioactive compounds are also present (14).

The chinaberry tree, *Melia azedarach* L., is another tree of the Meliaceae family which is native to the Arabian Peninsula (Asia Minor), but is now naturalized in many tropical

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and subtropical regions of Asia and Africa, as well as in large temperate areas of America and southern Europe (20). It is also quite widespread in Spain, and in Lleida province it is present in many towns as an ornamental tree in gardens, parks, streets and avenues.

The effects of compounds, products and extracts obtainable from *M. azedarach* on insects have been reviewed by Ascher *et al.* (4). Antifeedant effects of *M. azedarach* extracts are known for many insects (3,23,25). For Lepidoptera, feeding deterrence has been documented for several species (6,7,8,19,24). In addition, treatments in the field did not seem to inhibit activities of beneficial parasitoids and the predatory carabids (6).

Preliminary studies carried out in our laboratory showed that aqueous, methanolic or acetonic extracts of fruits and seeds of this tree exhibited an interesting antifeedant and insect growth-regulating (IGR) activity for several lepidopteran, coleopteran, and heteropteran pests. The aim of this paper was to evaluate the biological activity of methanolic extracts of seeds and fruits on larvae of the corn borer *Sesamia nonagrioides* Lefèbvre (Lepidoptera: Noctuidae), an important maize pest in the Mediterranean basin. The antifeedant activity of extracts from *M. azedarach* was compared with 'Mubel', a commercial neem extract that is reportedly active against many Lepidoptera, and with pure azadirachtin, the most bioactive compound in neem extracts.

MATERIALS AND METHODS

Insects *Sesamia nonagrioides* was reared on an artificial diet (10). Pupae were sexed and reared individually in an environmental chamber (16L:8D, 25±2°C) at 80% r.h. The emerged adults were used for reproduction. All the biological tests were carried out using 2nd instar larvae.

Methanolic extracts of fruit and seeds of *M. azedarach* Fruits of *M. azedarach* were picked when ripe from trees on the Lleida University campus in February 1997. Some of the fruit was used to obtain the methanolic extract and the remainder for the recovery of the seeds. Extracts of fruits and seeds were obtained according to the following methodology: First, the plant sample was crushed to fine particle size and dried in an oven at 35–40°C for 20 h. Extraction was carried out according to the procedure of Warthen *et al.* (27). In a 1000 ml flask, 50 g of crushed and dried plant material and 500 ml of methanol were stirred for 3 h. After leaving the methanolic solution to rest overnight, it was filtered through Whatman no. 40 filter paper. The solid filtration residue was extracted again following an identical procedure, and the two filtrates were combined. The solvent was removed by vacuum evaporation in a rotary evaporator (20 Torr, 30°C), and an oily and very viscous dark-red residue was obtained. The yields of the extractive processes were 15% for the fruit extract and 11% for the seed extract.

Bioassays of antifeedant activity Larvae were reared for 24 days on an artificial diet containing one of the test compounds dissolved in acetone:water (2 ml acetone:750 ml water, to obtain 1000 g diet). The diet was cut into small pieces and put in small cylindrical PVC cages (4 × 7 cm diam) in which one or two larvae were placed. Diet pieces were identical cylinders (1 cm diam × 1 cm height). Diet was changed every 2 days, when surviving insects, remaining diet and frass produced were weighed, and mortality was recorded. After 24 days, surviving larvae were fed untreated diet until the pupal stage. For every treatment approximately 15–25 larvae of *S. nonagrioides* were used.

Test materials consisted of: seed extract of *M. azedarach* at 1000 and 2000 ppm,

fruit extract (including seeds) of *M. azedarach* at 1000 and 2000 ppm, azadirachtin (95%, Sigma-Aldrich Co., St. Louis, MO, USA) at 0.25 and 1.5 ppm, and a commercial neem extract, 'Mubel' (Fertimet SL, Castellon, Spain), containing 2% w/v of azadirachtin at 12.5 and 75 ppm, a blank control consisting of untreated diet, and diet treated with acetone (2 ml per 1000 g diet) as a control. The parameters used to assess the antifeedant activity of the treatments were:

- Larval wet weights (mg) 5, 10, 15, 24 and 30 days after the onset of the experiment.
- Cumulative quantity (mg) of diet ingested per larva after 5, 15 and 24 days.
- Index of phagodepression/stimulation (P). This index is defined as:

$$P_{i(p/s)} = \frac{\Delta F_T}{\Delta F_C}$$

  where ΔF is the quantity of fresh diet ingested by the larvae in the treatments (T) and in the acetone-free control (C).
- Quantity of test material (g) ingested by larva (I_q), estimated in the following way:

$$(I_q)_i = C_i \Delta F$$

  where C_i = μg of product i ingested per g of diet, and ΔF = quantity of fresh diet ingested (g). The products are *M. azedarach* fruit and seed extract, neem extract or azadirachtin.
- Cumulative quantity of frass (mg) produced per larva by days 5, 15 and 24.
- Length of the larval period (days) from 2nd instar to pupation.
- Mortality at the end of the treatment period (24 days) and at pupation.

Statistical analysis All statistical analyses were performed on SAS V.6.12 (22). Data satisfied the assumptions of the general linear model and were not transformed. Statistical significance of data was assessed by analysis of variance (ANOVA). When ANOVA indicated there were significant effects ($P<0.05$), the LSD test was used to compare means.

RESULTS AND DISCUSSION

Larval weight gain Larvae from the blank control and acetone control treatments did not differ significantly ($P>0.05$) in weight (Table 1). However, the mean weight of larvae from the acetone control after 30 days was 33% lower (176 *vs* 265 mg). This could indicate a slight effect of the solvent, also confirmed by the 0% mortality in the blank control *vs* 7–13% mortality in the acetone control (Table 5). From observation of the data in Table 1, three levels of activity could be observed. The first type was that of the controls and that of fruit extract at low dose, which show a progressive and continued weight increase. There was little activity at 1000 ppm of fruit extract. Although at 5, 10 and 15 days significant differences from the controls were detected in the larval weight, these differences disappeared in the further course of the experiment, and at the end of the treatment no differences from the controls were observed.

A second group of treatments showed moderate activity: the treatments at the lower dose of azadirachtin (0.25 ppm), the lower dose of Mubel (12.5 ppm), the lower dose of seed extract (1000 ppm), and the higher dose of fruit extract (2000 ppm). The larvae fed

TABLE 1. Weight gain of *Sesamia nonagrioides* larvae exposed to extracts of *Azadirachta indica* and *Melia azedarach* during and after the treatment period

Treatment	ppm	Weight per larva (mean±SE, mg) during the treatment, after:			Weight per larva (mean±SE, mg) after treatment	
		5 days	10 days	15 days	24 days	30 days
Blank control	0	12±1 d	55±4 e	146±12 d	244±22 cd	265±17 d
Acetone control	0	13±2 d	60±7 e	155±14 d	209±16 c	176±9 cd
Azadirachtin	0.25	8±1 c	20±2 c	38±4 b	110±12 b	203±15 d
	1.50	8±1 c	12±2 abc	17±3 ab	42±8 a	94±17 bc
Mubel	12.5	8±1 c	21±2 c	35±3 b	98±8 b	201±8 d
	75	5±1 b	6±1 a	11±4 ab	22±1 a	20±0 a
M. azedarach seed extract	1000	8±1 c	19±3 bc	37±4 b	103±9 b	208±10 d
	2000	2±1 a	4±1 a	6±1 a	21±3 a	69±11 ab
M. azedarach fruit extract	1000	7±1 c	40±5 d	109±11 c	254±19 d	203±30 d
	2000	3±1 ab	11±1 ab	27±3 ab	119±9 b	204±13 d

Within columns, means followed by a common letter do differ significantly (LSD test, $P<0.05$).

TABLE 2. Effect of exposure to extracts of *Azadirachta indica* and *Melia azedarach* on ingestion of fresh diet by *Sesamia nonagrioides* larvae during the 24 days of the bioassay

Treatment	ppm	Cumulative ingestion of fresh diet per larva (mean±SE, mg)		
		5 days	15 days	24 days
Blank control	0	73±15 a	1249±86 d	3160±151 e
Acetone control	0	62±12 a	1284±118 d	2873±200 e
Azadirachtin	0.25	78±15 a	443±67 b	1018±130 abcd
	1.50	69±14 a	302±71 ab	740±164 abc
Mubel	12.5	68±9 a	391±40 b	943±97 abd
	75	83±18 a	155±11 ab	278±31 a
M. azedarach seed extract	1000	59±12 a	357±45 b	882±123 abc
	2000	22±14 a	148±40 a	308±79 ab
M. azedarach fruit extract	1000	33±10 a	931±135 c	3116±262 e
	2000	35±4 a	273±34 ab	1403±115 d

Within columns, means followed by a common letter do not differ significantly (LSD test, $P<0.05$).

with diet containing these fractions showed appreciably lower weights than the controls after 24 days (Table 1). However, after the diet was replaced with the untreated one the survivors recovered, showing a growth curve parallel to that of the controls (Table 1), and almost all of them pupated at a similar weight to the controls, although in a considerably longer development time (Table 5).

Finally, a third group of more active treatments consisted of the higher doses of Mubel (75 ppm), azadirachtin (1.5 ppm), and seed extract (2000 ppm). In these treatments the larvae always showed significantly different weights from the controls, even in the post-treatment period (Table 1). The different activity of the fruit and seed extracts indicates that bioactive compounds are concentrated mostly in the seeds, as reported previously (25). Using the more active fraction, Mubel at 75 ppm, the larvae could not recover when the diet was replaced and all of them died before pupation (Table 5). It should be noted that 75 ppm of Mubel corresponds to 1.5 ppm of azadirachtin according to the product label (2% w/v in azadirachtin). Thus, it seems that other compounds in that commercial extract heighten the activity of azadirachtin on the insect mortality, showing a synergistic effect.

This antifeedant effect of Meliaceae extracts on insect growth is well documented, mainly in Lepidoptera. Thus, the effects of neem extracts were previously reported on *S. nonagrioides* (17), and on *Ostrinia nubilalis* Hübner (16), another corn borer. Also, the

TABLE 3. Index of phagodepression/phagostimulation and quantity of material ingested by *Sesamia nonagrioides* larvae exposed to extracts of *Azadirachta indica* and *Melia azedarach*

Treatment	ppm	Phagodepression/ phagostimulation index ($P_{i(d/s)}$) (mean±SE)	Quantity of material ingested (I_q) (mean±SE, μg)
Blank control	0	–	–
Acetone control	0	0.91±0.06 e	–
Azadirachtin	0.25	0.34±0.04 abcd	0.09±0.01 a
	1.50	0.23±0.05 abc	0.34±0.07 b
Mubel	12.5	0.30±0.03 abc	0.07±0.01 a
	75	0.09±0.01 a	0.14±0.01 ab
M. azedarach seed extract	1000	0.28±0.04 abc	0.28±0.04 ab
	2000	0.10±0.02 ab	0.20±0.05 ab
M. azedarach fruit extract	1000	0.98±0.08 e	0.96±0.08 c
	2000	0.44±0.04 d	0.88±0.07 c

Within columns, means followed by a common letter do not differ significantly (LSD test, $P<0.05$).

TABLE 4. Cumulative amount of frass produced by *Sesamia nonagrioides* larvae fed on artificial diet containing extracts from *Azadirachta indica* and *Melia azedarach* during the 24 days of the bioassay

Treatment	ppm	Weight of cumulative frass per larva (mean±SE, mg)		
		5 days	15 days	24 days
Blank control	0	22±4 de	859±87 de	2503±156 d
Acetone control	0	24±4 e	940±98 e	2448±174 d
Azadirachtin	0.25	19±3 cde	219±43 abc	682±102 b
	1.50	14±2 bc	144±42 abc	479±132 ab
Mubel	12.5	12±3 bc	192±27 abc	602±78 b
	75	10±2 ab	70±25 abc	169±11 ab
M. azedarach seed extract	1000	12±2 bc	167±26 abc	555±100 b
	2000	2±1 a	40±10 ab	141±37 a
M. azedarach fruit extract	1000	16±2 bcd	713±102 d	2656±220 d
	2000	2±1 a	199±25 abc	1178±109 c

Within columns, means followed by a common letter do not differ significantly (LSD test, $P<0.05$).

efficacy of azadirachtin has been proved in several noctuid species (5,11,13). The effects of *M. azedarach* extracts are less documented. They have been reported in *Agrotis ipsilon* Hufnagel (1,24), *Thaumetopoea pityocampa* (Den. & Schiff.) (6), *Plutella xylostella* L. (7,8), *Spodoptera littoralis* Boisduval (24), and *S. exigua* Hübner (19). The results on the antifeedant activity in the present study can not discriminate whether they stem from altered behavior based on a gustatory effect (chemoreception) or from the growth-disrupting and toxic effects (18).

Larval consumption rates Table 2 shows the cumulative amount (mg wet weight) of diet ingested at different periods of the assay. After 5 days, no differences were observed between any treatments ($P>0.05$). At 15 days all the treatments showed significantly lower values of ingested diet than of either of the controls. Finally, at the end of the treatment period, all the treatments were significantly different from the controls, except the fruit extract at 1000 ppm. The higher rates of Mubel and seed extract showed the lowest quantity of ingested food.

Index of phagodepression/phagostimulation According to the definition of this index, values higher than 1 indicate phagostimulation, and those less than 1 – phagodepression.

TABLE 5. Development time from 2nd instar larvae to pupation and percent mortality for *Sesamia nonagrioides* larvae exposed to extracts of *Azadirachta indica* and *Melia azedarach* in an artificial diet

Treatment	ppm	Development time (days, mean±SE)	Mortality (%)[z]	
			At the end of treatment	At pupation
Blank control	0	28.9±1.3 ab	0 (25)	0 (25)
Acetone control	0	26.7±0.6 a	7 (25)	13 (25)
Azadirachtin	0.25	40.0±1.2 c	0 (23)	0 (23)
	1.50	53.8±2.9 d	20 (25)	20 (25)
Mubel	12.5	39.5±0.9 c	7 (15)	13 (15)
	75	–	87 (20)	100 (20)
M. azedarach seed extract	1000	39.7±0.8 c	0 (18)	0 (18)
	2000	52.7±1.1 d	20 (20)	20 (20)
M. azedarach fruit extract	1000	31.5±0.8 b	27 (22)	27 (22)
	2000	42.4±1.8 c	13 (15)	20 (15)

Means followed by a common letter do not differ significantly (LSD test, $P<0.05$).
[z] In parentheses, number of insects tested.

In our case none of the treatments was a phagostimulant (Table 3). The lowest values of this index, corresponding to a greater level of deterrency, were around 0.1–0.2 for the higher doses of azadirachtin, Mubel and seed extract. Values for the lower doses of these treatments, which showed a lower antifeedant activity, were around 0.3–0.4. Evidence for the low bioactivity of the fruit extract applied at the low dose is confirmed again from the index value of near 1, which did not differ from that for the acetone control (P_i=0.91).

According to our results, a good correlation was found between this index and the other parameters defined to evaluate the biological activity of the treatment. The feeding deterrence index can be used routinely to determine the antifeedant activity of a sample.

Quantity of material ingested The values of the index I_q for the different treatments are shown in Table 3. There were no significant differences between doses for the treatments with plant extracts (fruit or seed) ($P>0.05$). However, for azadirachtin these differences showed up clearly, and the larvae ingested a higher amount of product at the higher dose. Mubel would be an intermediate case, showing a similar trend, although the differences were not significant.

Treatments with fruit extracts allowed the ingestion of a higher quantity of material than with seed extracts. This difference could be explained by the different chemical composition and nutritional quality of the substrate for the larva, or by the absence of antifeedants in the fruit extracts.

Similar results were obtained when methanolic extract of *M. azedarach* fruits was used for laboratory treatments of two lepidopteran pests in Egypt (*S. littoralis* and *A. ipsilon*) (24). These experiments, carried out with several concentrations of the toxic substance in the diet, showed that insect food consumption, weight gain, and conversion of injected food into body matter underwent considerable decreases with increasing amounts of extract.

Frass production Table 4 shows the weight (mg) of frass produced by the larvae at the end of the treatment. All the treatments, except the fruit extract at low dose, showed mean values lower than the controls. The higher doses of seed extract, Mubel and azadirachtin

were the most active treatments.

As could be expected, a high correlation can be observed among the weight increase, the quantity of diet ingested, and the quantity of frass produced during the whole assay period. This shows the basically antifeedant character of the products tested. This effect is the most important and the most widely reported one in most of the papers on the application of neem extracts in insect diets (25).

Development time of the insects The data shown in Table 5 on the development time from the beginning of the assay up to the pupal stage agree with what has been stated above. The values of 27–29 days obtained for the controls, are considerably lower than the values of 53–54 days found for the most active fractions. Treatments of moderate activity showed intermediate values of 39–40 days. In all cases except the fruit extract at 1000 ppm, significant differences from the controls were found.

In the literature, many other references documenting neem or azadirachtin effects extending the duration of larval instars can be found for insects of different orders (25). Similar effects have also been described for *M. azedarach* extracts (7,24).

Cumulative mortality At the doses used, only Mubel at 75 ppm resulted in 100% mortality (Table 5). This mortality occurred progressively throughout the entire assay. The remaining treatments caused low mortality (6–27%), which occurred generally on the first days of the assay. Most of the insects that reached the pupal stage eclosed as apparently normal adults, in contrast to the results of Simmonds *et al.* (26), who found higher mortalities in various noctuid insects when pupae fail to eclose as adults.

Mortality of the larvae in some cases was the result of molting failure. Observations using a binocular lens revealed the inability to detach the exuvia, and sometimes it was noted that the larvae died from starvation, with a very low weight and after having acquired a blackish coloring.

Mortality is also reported in many Lepidoptera. *P. xylostella* larvae usually die from failure to molt completely when they feed on a diet into which extracts of *M. azedarach* have been incorporated (7). In *T. pityocampa* such extracts caused 100% mortality when sprayed on twigs of *Pinus mugo* (6).

Lepidoptera seem to be the group most sensitive to the growth-regulating effects caused by Meliaceae extracts. Thorough studies of the effects on the hormonal system of the application of azadirachtin showed a dose-dependent effect. This effect can produce a reduction in the rate of embryonic development, alteration of metamorphosis, occurrence of supernumerary larval stages, inability to molt, and even death of the insect (15).

From the results obtained in the current experiment, the following conclusions can be drawn: The methanolic extracts of fruit (2000 ppm) and seed (1000 ppm) of *M. azedarach* showed a strong antifeedant bioactivity to larvae of *S. nonagrioides*. Larvae treated with fruit extract at 2000 ppm, and seed extract at 1000 ppm showed a behavior very similar to that of the ones treated with azadirachtin at 0.25 ppm, and with Mubel at 12.5 ppm. At higher concentrations, an analogous behavior is shown by treatments of seed extract at 2000 ppm, 1.5 ppm azadirachtin, and 75 ppm Mubel. These treatments showed the greatest antifeedant activity. The fruit extract at 1000 ppm showed hardly any bioactivity.

A good correlation was found among all the indices used to evaluate the bioactivity of these extracts. The evolution of the indices of weight gain of the larvae, the cumulative ingestion of fresh diet, and the cumulative frass production of the larvae confirmed that the activity shown by the different active components of the extracts is essentially antifeedant.

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