Repellent and Insecticidal Activities of *Melia azedarach* L. against Cotton Leafworm, *Spodoptera littoralis* (Boisd.)

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A crude acetone extract and oil of ripe fruits from *Melia azedarach* L. were evaluated against the 2nd and 4th instar larvae of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Both oil and extract exhibited highly significant growth inhibition at all concentrations tested, while the oil of *M. azedarach* recorded higher insecticidal activity against both instars than the crude extract. GC-MS analysis of the oil revealed the presence of linoleic acid methyl ester, oleic acid methyl ester, and free oleic acid as the main components in addition to hexadecanol, palmitic acid, methyl esters of stearic acid and myristic acid. Fatty acids and their esters were not only the main constituents of essential oil from the ripe fruits of *M. azedarach*, but also mainly responsible for the insecticidal and growth inhibition activity against *S. littoralis*.

Key words: Melia azedarach, Fatty Acids, *Spodoptera littoralis*

Introduction

The cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is one of the pests that cause great damage to cotton plants and other crops (Bishara, 1954; Moussa *et al.*, 1960). The Egyptian cotton leafworm *S. littoralis* is considered as the major pest in a wide range of cultivation including cotton, corn, soybeans, peanuts, and vegetables. This pest is not only widely spread in Egypt but also in other Middle East countries in addition to temperate zones in Asia and Africa (Salama *et al.*, 1990).

Synthetic insecticides are important tools in pest control, although they have been used excessively with negative consequences such as toxicity towards farmers, consumers, and wild animals, interruption of natural control and pollination. The evolution of resistance pests has acquired to these products (Perry *et al.*, 1998). Botanical insecticides have been used in agriculture for at least two thousand years in Asia and the Middle East (Thacker, 2002). The interest in new botanical compounds for pest control is based on their bioefficiency, biodegradability, and physiological activity (Rodríguez, 1998; Isman, 1999).

The effectiveness of extracts from fruits and leaves of *Melia azedarach* L. has been previously demonstrated against insects (Carpinella *et al.*, 2002, 2003; Banchio *et al.*, 2003; Valladares *et al.*, 2003). The antifeedant effects of *M. azedarach* extracts are known for many insects (Juan *et al.*, 2000; Banchio *et al.*, 2003; Carpinella *et al.*, 2003; Nathan, 2006). Unfortunately, *M. azedarach* fruits are popularly believed to be toxic, but toxicity assays of the fruit extract carried out on mammals have not shown any adverse effects, when orally administered to rats (Carpinella *et al.*, 1999).

The present work aimed to evaluate the repellent and insecticidal effects of *M. azedarach* ripe fruit acetone extract and oil on the cotton leafworm *S. littoralis* in the laboratory and also to investigate the chemical composition of *M. azedarach* fruit oil.

Material and Methods

Plant material

Ripe fruits of the plant were collected from Menoufia, Egypt, in November 2008. The identification of the plant was kindly done by Dr. Adel Okeal, Director of El-Orman Garden, Giza, Egypt.

Preparation of crude extract

The ripe fruits of *M. azedarach* were crushed to fine particles and shade-dried at room temperature. Extraction was carried out according to the procedures of Warthen *et al.* (1984), with some modifications. In a 1000-mL flask, 200 g of crushed and dried fruits were stirred for 3 h in 800 mL of acetone. After leaving the acetone solution 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 mixed. The solvent was removed using a rotary evaporator, and a dark red residue was obtained (10 g/200 g plant). This crude extract was used to prepare a stock solution. Series of concentrations (1.25, 2.50, 5.00, 10.00, and 20.00 g/100 mL) of *M. azedarach* extract were carried out with acetone. One drop of emulsifier (Tween 20, Sigma Aldrich Chemical Company, St. Louis, MO, USA) was added to fruits extracts to ensure complete miscibility of the material in acetone.

Oil extraction

Ripe fruits (1200 g) of *M. azedarach* were crushed to fine particle size and shade-dried at room temperature. The dried fruits were extracted at room temperature three times with hexane. The solvent was removed under reduced pressure using a rotary evaporator to obtain 45 mL of oil. The oil underwent the toxicity assay on *S. littoralis*. Furthermore it was subjected to GC-MS for identification of its chemical constituents. Series of concentrations (1.25, 2.50, 5.00, 10.00, and 20.00 g/100 mL) of *M. azedarach* oil were carried out with acetone.

Chemical analysis of oil

The prepared oil was subjected to GC-MS analysis using a Shimadzu GC-MS QP 5050A instrument (Duisburg, Germany); searched library, Wiley 229. LIB; column, DB5 (30 m, 0.53 mm ID, 1.5 μm film thickness); carrier gas, helium (flow rate, 1 mL/min); split ratio, 1:50; ionization mode: EL (70 eV); temperature program: 40 °C (static for 2 min), then gradually increased (at a rate of 2 °C/min) up to 250 °C (static for 7.5 min); detector temperature, 250 °C; injector temperature, 250 °C.

Strain of cotton leafworm S. littoralis

The *S. littoralis* strain was obtained from Faculty of Agriculture, Cairo University, Egypt and was reared in the laboratory of Physiology Department, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt, as described by El-Defrawi *et al.* (1964), under constant laboratory conditions of (25 ± 1) °C and (70 ± 5)% relative humidity.

Toxicity assay

The leaf-dipping technique, similar to that described by Tabashink *et al.* (1987), was used to determine the toxicity of the acetone extract and oil against the 2nd and 4th instar larvae using concentrations of 1.25, 2.50, 5.00, 10.00, and 20.00 g/100 mL of *M. azedarach* in acetone. Eight castor leaves were dipped for 5 s in each solution, and then the treated leaves were left for natural air-drying and were distributed in four jars (2 leaves/jar). Ten 2nd and 4th instar larvae were allowed to feed on treated leaves for 48 h, then larvae were fed on untreated leaves for 24 h. Four replicates of ten larvae were fed on acetone-treated leaves for 72 h to serve as control. Larval weight and mortality were recorded after 72 h. Mortality was calculated using the Abbott formula (Abbott, 1925) and subjected to probit analysis according to Finney (1971).

Repellency bioassay

Repellency was assessed according to the area preference method of Obeng Ofori *et al.* (1998), with some modifications. Samples of 0.30, 0.60, 1.25, and 2.50 g/100 mL of extract and oil solutions in acetone were applied to one half of filter paper discs with a pipette, and the solvent (acetone) on the other half served as control. After acetone was completely volatilized, each filter paper was placed in a culture dish with 9 cm diameter, and thirty larvae of *S. littoralis* were placed at the centre of the paper, covered with perforated lids lined with 4 mm wire mesh, and banned with rubber band. Three replications of each treatment were performed. After 24 h the number of larvae present on the treated (T) and the control (C) discs were counted. Percentage repellency (PR) values were computed using the formula: $PR = [(C - T)/(C + T)] \cdot 100$. PR data were analysed using Anal-

ysis of Variance after arcsine transforming them. Negative PR values were treated as zero.

Statistical analysis

The significances were calculated by ANOVA and Duncan's multiple range tests (ANOVA of arcsine square root transformed percentages). Differences between the treatments were determined by Tukey's multiple range test ($P < 0.05$) (Snedecor and Cochran, 1989).

Results and Discussion

Chemical analysis of oil constituents

GC-MS analysis showed that the ripe fruit oil is mainly composed of linoleic acid methyl ester (34.72%), oleic acid methyl ester (32.45%), and free oleic acid (15.16%) in addition to methyl esters of stearic acid (6.83%) and palmitic acid (6.77%), hexadecanol (3.07%), and myristic acid methyl ester (1.00%) (Table I). These results are supported by the findings of Carpinella *et al*. (2007), who reported that the fatty acids of ripe fruit oil of *M. azedarach* are mainly composed of linoleic and oleic acids, in addition to myristic, palmitic, palmitoleic, stearic, and linolenic acids.

Table I. Constituents pattern of *M. azedarach* oil.

Constituent	Retention time [min]	Relative content (%)[a]
Myristic acid methyl ester	16.274	1.00
Palmitic acid	16.663	6.77
Linoleic acid methyl ester	17.933	34.72
Oleic acid methyl ester	18.025	32.45
Stearic acid methyl ester	18.292	6.83
Oleic acid	18.383	15.16
Hexadecanol	18.617	3.07

[a] Mean values were measured by GC-MS.

Toxicity test

Data presented in Table II revealed that both extract and oil showed significant toxic effects on larvae of *S. littoralis*. The oil was slightly more effective than the crude acetone extract against 2nd and 4th instar larvae at all concentrations tested except for 10.0 g/100 mL, where the toxicity of the crude extract was higher than that of the oil (57.5% and 42.5%, respectively) against 4th instar larvae. This finding is in agreement with results obtained by Carpinella *et al*. (2007) who stated that the oil of *M. azedarach* was slightly more effective than the fruit ethanol extract at the same test concentrations, although no significant differences were observed at lower concentrations. Schmidt *et al*. (1997) indicated that the percentage of mortality increased with application of higher concentrations of *Melia* extract in *S. littoralis* and *Agrotis ipsilon*. The insecticidal effect of the *M. azedarach* extract against *S. littoralis* larvae was very high when used at high concentrations; percentage mortality was 16% at 10 ppm and 100% at 50 ppm. This suggested that the extract acted as a stomach poison (Salam and Ahmed, 1997). In no-choice tests, adults of *Xanthogaleruca luteola* fed on leaves treated with 2.5 or 10% *M. azedarach* extract showed a dramatic increase in mortality rates (Defago *et al*., 2006).

Table II. Toxic effect of the crude acetone extract and oil from ripe fruits of *M. azedarach* against 2nd and 4th instar larvae of *S. littoralis*.

Treatment	Corrected mortality (%)			
	Ripe fruit extract		Ripe fruit oil	
	2nd instar	4th instar	2nd instar	4th instar
Control	0.00	0.00	0.00	0.00
1.25 g/100 mL	30.0[e]	5.00[e]	32.50[e]	10.00[e]
2.50 g/100 mL	37.5[d]	15.00[d]	42.50[d]	30.00[d]
5.00 g/100 mL	42.5[c]	27.50[c]	67.50[c]	32.50[c]
10.00 g/100 mL	70.0[b]	57.50[b]	77.50[b]	42.50[b]
20.00 g/100 mL	85.0[a]	65.00[a]	100.0[a]	77.50[a]
LC_{50} [g/100 mL]	4.25	10.11	2.73	8.70
F value	4194.20***	2486.90***	6426.04***	6030.69***
LSD	1.138	1.665	1.067	1.005

Values in a column followed by the same letters are not significantly different.
*** Highly significant effect.

Table III. Effect of the acetone extract and oil of *M. azedarach* on mean larval weight (M.W.) and weight reduction (W.R.) of *S. littoralis*.

Treatment	Ripe fruit extract				Ripe fruit oil			
	2nd instar		4th instar		2nd instar		4th instar	
	W.R. (%)	M.W. [mg]	W.R. (%)	M.W. [mg]	W.R. (%)	M.W. [mg]	W.R. (%)	M.W. [mg]
Control	11.19		42.18		12.4		42.18	
1.25 g/100 mL	48.26^{e}	5.79^{a}	73.33^{e}	11.25^{a}	51.11^{e}	6.06^{a}	76.53^{e}	9.90^{a}
2.50 g/100 mL	61.75^{d}	4.28^{b}	82.62^{d}	7.34^{b}	56.93^{d}	5.34^{b}	84.92^{d}	6.36^{b}
5.00 g/100 mL	66.58^{c}	3.74^{c}	87.43^{c}	5.39^{c}	67.90^{c}	3.98^{c}	88.03^{c}	5.05^{c}
10.00 g/100 mL	71.40^{b}	3.20^{d}	89.68^{b}	4.35^{d}	79.84^{b}	2.50^{d}	90.99^{b}	3.80^{d}
20.00 g/100 mL	87.04^{a}	1.45^{e}	92.98^{a}	2.96^{e}	90.08^{a}	1.23^{e}	93.62^{a}	2.69^{e}
F value	381.38***		4651.48***		2744.89***		2653.89***	
LSD	0.246		0.1475		0.1183		0.1712	

Values in a column followed by the same letters are not significantly different.
*** Highly significant effect.
Weight reduction (%) = [(Control – M.W.) / Control] · 100.

The highest toxicity rates were recorded for oil of *M. azedarach*, 100% and 77.5% mortality with 2nd and 4th instars, respectively, at the highest concentration of 20 g/100 mL, while the crude extract caused 85% and 65% mortality with the two instars at the same concentration. The toxicity rate was positively correlated with the concentration of both crude extract and oil of *M. azedarach*. These results of the effectiveness of *M. azedarach* insecticidal extract and oil coincide with those obtained by other authors (Chiu, 1987; Wei *et al*., 1989; Kheirallah *et al*., 1994; Hashem *et al*., 1998; Hamed, 2000; El-Khayat, 2000).

The results showed that the LC_{50} values of the oil were lower than those of the extract with 2nd and 4th instar larvae, 2.73 and 8.70 g/100 mL for the oil, 4.25 and 10.11 g/100 mL for the extract, respectively.

In fact, evaluation of the insecticidal activity of each constituent in both oil and acetone extract of *M. azedarach* could not easily be accomplished, but some of these constituents such as fatty acids and their methyl esters were reported to have growth inhibition and toxic effects against insects. The presence of fatty acids and fatty acid methyl esters with high concentration in the oil of *M. azedarach* may be the reason for growth inhibition of *M. azedarach* oil against *S. littoralis*. This conclusion was confirmed by several reports in the literature.

The potency of botanical fatty acids was reported by Abdallah *et al.* (2009) against *Aphis craccivora*, and Messina and Renwick (1983) and Abdallah *et al*. (1986) against weevil species. Tare and Sharma (1991) compared the larvicidal properties of different fatty acids constituents against *Aedes aegypti* and found that oleic acid was the most effective one. Deshpande *et al.* (1974) reported oleic acid as insecticidal component of *Nigella sativa* (Ranunculaceae), which was found to be toxic to the pulse beetle, *Callosobruchus chinensis*. Barakat *et al*. (2004) reported that the ethanol and hexane crude extracts of *Cassia fistula* (L.) reduced pupation, egg production, and hatchability, and increased percent sterility; the dominant constituents were fatty acids, linoleic acid, hexadecanoic acid, and octadecanoic acid, and their alkyl esters. Another study carried out by Farlane and Henneberry (1965) indicated that the growth of cricket, *Gryllodes sigillatus* (Walk.), was inhibited by fatty acids and their methyl esters; the effective fatty acids were lauric, myristic, stearic, and behenic acids. Similar results were reported by Andrews and Miskus (1972) and Juárez and Napolitano (2000).

As shown in Table III the reduction in larval body weight was positively correlated with the crude acetone extract and oil concentrations of *M. azedarach*; the same observation was recorded with both 2nd and 4th instar larvae of *S. littoralis*.

Generally the highest decrease in larval body weight was recorded at a concentration of 20 g/100 mL of the oil and extract with the 4th instar larvae. The percentages of larval weight reduction of the acetone extract and oil against *S. littoralis* 2nd and 4th instar larvae increased gradu-

Table IV. Repellency of the acetone extract and oil of *M. azedarach* against *S. littoralis* larvae at different concentrations.

Treatment	Repellency (%)			
	Ripe fruit extract		Ripe fruit oil	
	2nd instar	4th instar	2nd instar	4th instar
0.30 g/100 mL	51.11[d]	55.55[d]	30.00[d]	33.30[d]
0.60 g/100 mL	61.11[c]	65.55[c]	38.88[c]	54.66[c]
1.25 g/100 mL	71.11[b]	75.55[b]	65.55[b]	88.66[b]
2.50 g/100 mL	78.86[a]	81.11[a]	78.88[a]	91.30[a]
F value	141801.46***	38622.88***	4677.10***	253059.44***
LSD	0.105	0.1872	1.096	0.1812

Values in a column followed by the same letters are not significantly different.
*** Highly significant effect.

ally with the increasing concentrations. Larval weight reduction percentages of the acetone extract were 48.26, 61.75, 66.58, 71.4, 87.04% and 73.33, 82.62, 87.43, 89.68, 92.98% with 2nd and 4th instars, respectively, at 1.25, 2.50, 5.00, 10.00, 20.00 g/100 mL, while the percentages for oil were 51.11, 56.93, 67.90, 79.84, 90.08% and 76.53, 84.92, 88.03, 90.99, 93.62% with 2nd and 4th instars, respectively, and at the same concentrations.

The above mentioned results are in agreement with the results of Schmidt *et al*. (1997), who stated that the larval weight of *S. littoralis* and *Agrotis ipsilon* significantly reduced until pupation in 25 ppm and higher extract contents of *Melia* extract. Ahmed *et al.* (1978) stated that the acetone extract of *M. azedarach* afforded a significant degree of determent with some instars of *S. littoralis*. Defago *et al*. (2006) reported that treatment of elm leaves with extracts obtained from unripe fruits and green or senescent leaves of *M. azedarach* at 1–10% content significantly deterred feeding of the adult elm leafbeetle, *Xanthogaleruca luteola*. Jianzhang *et al*. (1983) found that the seed oil of *M. azedarach* had various adverse effects on several important rice pests. The seed oil had marked antifeedant and some systemic activity against *Scirpophaga incertulas* (Wlk.) (*Tryporyza incertulas*), *Sogatella furcifera* (Horv.), and *Nilaparvata lugens* (Stal). Chiu *et al*. (1983) reported that petroleum ether extracts of the seed kernels of *Melia toosendan* and *M. azedarach* had strong antifeedant effects on nymphs of the rice pest *Nilaparvata lugens* (Stal). Akhtar *et al*. (2008) reported that most of the extracts of *Azadirachta indica*, *A. excels* (sentang), *Melia volkensii*, *M. azedarach*, and *Trichilia americana* proved to be strong growth inhibitors, contact toxins, and significant feeding deterrents to two lepidopteran species. All botanicals tested were more growth inhibitory and toxic (through feeding) to *Trichoplusia ni* than to *Pseudaletia unipuncta*, except for *M. azedarach*, which was more toxic to *P. unipuncta* than to *T. ni*.

Repellency bioassay

The repellency rates of the oil and extract against *S. littoralis* are shown in Table IV. Generally, repellency was increased with the increase of concentration. On the other hand higher repellency rates were recorded in 4th instar than in 2nd larvae at all concentrations tested.

The highest repellency (91.30%) of oil was recorded at the highest concentration with the 4th instar larvae, also the highest repellency rate (81.11%) of extract was recorded with the same larvae and at the same concentration. The fruits of *M. azedarach* showed excellent repellency effects against many insects as reported by Panji (1964) who stated that 5% ethanolic extract of *M. azedarach* repelled adults of *Aulacophora foveicollis* L. Tandon and Sirohi (2009) stated that 5% ethanolic *M. azedarach* extract repelled minimum 30% beetles of *Raphidopalpa foveicollis* Lucas (1 h) and maximum 65% beetles (48 h) whereas 10% extract repelled maximum 76% beetles in 48 h. *M. azedarach* oil at 2% produced 95.13% (95% CI = 90.74–99.52) protection for 7 h and 20 min, while the 5% oil gave 96.20% (95% CI = 86.98–105.41) protection for 8 h and 20 min against *Phlebotomus orientalis* (vector of visceral leishmaniasis) (Kebede *et al*., 2010).

Khan and Siddiqui (1994) recorded good repellency of *M. azedarach* (Bakain) seeds and leaves

against *Tribolium castenteum*. Another report described the repellent properties of *M. azedarach* against the red pumpkin beetle (*Aulacophora foveicollis* Lucas) attacking musk melon (*Cucumis melo* L.) crop (Khan and Wasim, 2001).

The data obtained in the present work were confirmed by many previous reports and can lead to the conclusion that the fruit acetone extract and oil from *M. azedarach* can be considered as effective natural insecticides of plant origin for control of the cotton leafworm, *S. littoralis*. Fatty acid methyl esters were proven to be the major constituents of the oil from the fruits of *M. azedarach* and also may be mainly responsible for insecticidal and repellent activities against *S. littoralis*.

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