Insecticidal Activity of *Melia azedarach L.* Triterpeniods against *Spodoptera littoralis* (Boisd.)

Melegi A. Abd El-Ghany[a,b], Mohamed Farag[b], Heba Yousef[b], Mohamed H. M. Ahmed[c], Samy S. El-badawey[b] and Adel A.-H. Abdel-Rahman[d]

[a] Central Lab., King Faisal University (KFU), Saudi Arabia, KSA
[b] Department of Pest Physiology, Plant Protection Research Institute, AgriculturalResearch Center, Dokki, Giza, Egypt
[c] Department of Chemistry, Faculty of Science, Benha University, Benha, Egypt.
[d] Department of Chemistry, Faculty of Science, Menoufia University, Shebin El- Koam, Egypt.
mhamedfarag_1@yahoo.com , hebayousef2004@yahoo.com , melegi_a@hotmail.com ,

Abstract: Ethyl acetate extract and the purified fraction extracted from ripe fruits of *Melia azedarach* were tested against the 2nd and 4th instar larvae of *Spodoptera littoralis*. The extract and the fraction showed high significant toxic activities and reduction in larval weight at all concentrations used. The LC_{50} values of the extract were 4.10 and 16.04 against the 2nd and 4th instars, respectively, while for the purified fraction were 1.19 and 2.01 against the same instars. The chemical constituents of the purified fraction were identified by LC-MS. Ten components were identified, four of these components were previously isolated and six compounds were identified for first time from fruits of *Melia. azedarach*.

[Melegi A. Abd El-Ghany, Mohamed Farag, Heba Yousef, Mohamed H. M. Ahme، Samy S. El-badawey and Adel A.-H. Abdel-Rahman. **Insecticidal Activity of *Melia azedarach L.* Triterpeniods against *Spodoptera littoralis* (Boisd.).** Journal of American Science 2012; 8(3): 661-667]. (ISSN: 1545-1003). http://www.americanscience.org. 89

Key words: *Melia azedarach, Spodoptera littoralis,* triterpenes

1. Introduction

The Meliaceae plant family is known to contain a variety of compounds that show insecticidal, antifeedant, growth regulating, and development modifying properties, **(D'Ambrosio and Guerriero, 2002; Nakatani *et al.*, 2004; Nathan and Kim, 2006).** One member of the Meliaceae, known as Chinaberry or Persian lilac tree (*M. azedarach*) is a deciduous tree that is native to northwestern India and has long recognized for its insecticidal properties **(Ascher *et al.*, 1995).** The insecticide activity of *M. azedarach* is due to biologically active triterpenoids with an antialimentary effect, i.e., they inhibit the feeding of phytophage insects producing death and malformations of subsequent generations **(Vergara *et al.*, 1997; Carpinella *et al.*, 2003).** Leaf and fruit extracts of *M. azedarach* have been evaluated on diverse pests with promising results **(Padrón *et al.*, 2003; Pérez-Pacheco *et al.*, 2004).**

The bioactivity of azadiractin (a tetranotriterpenoid) from *Azadirachta indica* has allowed to research natural insecticides in most similar types, including *melia* **(González-Gómez *et al.*, 2006).** Within the triterpenoids in the *M. azedarach* seeds, meliacarpine, similar to azadiractine, is also active in the regulation of insect growth **(Schmutterer, 2002).**

2. Material and methods

1- Plant material

Ripe fruits of the plant were collected from El-Menoufia, Egypt in November 2008.

2- 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.

3- Extraction and purification

Triterpenes were extracted and purified from *M. azedarach* according to the method of **Kleeberg (1997).**

3.1. Extraction

Ripe fruits (1200 g) were crushed to fine particle size and shade dried at room temperature. The dried seeds were extracted at room temperature three times with hexane, for 3 days each time **(Farag *et al.* 2011).** The solvent was filtered and the macerate was then extracted with ethyl acetate as the same with hexane in the first time. Ethyl acetate was removed from the extract under reduced pressure using a rotary evaporator (28 ± 2) °C to obtain 14 g of semi solid material.

3.2. Purification

Ethyl acetate extract (5 g) was dissolved in 50 mL ethyl acetate, and then 400 mL petroleum ether was added with occasional stirring during which, the triterpenes containing active substances precipitates and is totally sediment to the bottom after 30 min. The liquid layer was decanted and the precipitate was whirled up in 20 mL petroleum ether. The sediment was dried for 12 h at 30 ^{0}C yielding 3 g of yellow material. Toxicity assay was carried out to examine the effect of the obtained ethyl acetate extract and purified fraction on 2nd and 4th instar larvae of *S. littorals*. Furthermore the purified fraction was subjected to LC-MS for identification of its chemical constituents.

4- LC-MS analysis

LC-MS (electrospray ionization) was carried out using Thermo Finnigan Electron Corporation (LCQ Advantage Max) ion trap. Infusion experiments were performed using a syringe pump (Hamilton syringe, 500 µl) directly connected to the electrospray ionization (ESI) at a flow rate 10 µl/min. The spectrometer was run in positive mode.

The technique used was Total Ion Mapping Experiment, where you get product ion scans for each parent ion, so you can determine which parent ions lost a particular fragment to yield a particular product ion.

5. Toxicity assay

Leaf-dipping technique similar to that described by **Tabashink *et al.* (1987),** was used to determine the toxicity of the extract and the fraction against the 2nd and 4th instar larvae using concentrations of 1.25, 2.5, 5, 10 and 20 g/100 mL of the extract and 0.3 0.6, 1.25, 2.5 and 5 g/100 mL of the purified fraction of in acetone. Eight castor leaves were dipped for 5 s in each concentration, were left for natural air-dryness and were distributed in four jars (2 leaves/jars). Ten 2nd and 4th instar larvae were allowed to feed for 48 h on treated leaves, then, larvae were fed for 24 h on untreated leaves. Four replicates of 10 larvae each 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).**

Statistical analysis

The differences 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 the purified fraction

LC-MS of the purified fraction from EtOAc extract led to isolation of 15 compounds, four of them were previously isolated from the fruits of *M. azedarach* and were identified as:

Meliartenin (1)

The molecular formula $C_{28}H_{36}O_{10}$, deduced from the molecular ion peak at m/z 535 in MS-MS (positive mode), was compatible with meliartenin m/z 535 - 18 = 517 indicated the elimination of one molecule of water, m/z 517 - (68) = 449, which indicated the elimination of furyl ring, m/z (418 - 60) + $2H^+$ = 360, indicated the elimination of AcOH molecule. The molecular ion peak and the fragmentation pattern were compatible with the chemical structure of meliartenin. This compound was isolated and identified from fruits of Argentine *M. azedarach*, **(Carpinella *et al*., 2002, 2003).**

Toosendanin (2)

The molecular formula $C_{30}H_{38}O_{11}$, deduced from the molecular ion peak at m/z 572 in MS-MS (positive mode), was compatible with toosendanin. m/z 572 - H^+ = 571, m/z 571 - AcOH = 511, m/z 511 – AcOH = 451, m/z (451 - $3H_2O$) + H^+ = 398, m/z 398 - O = 382, m/z (382 – 67 [furyl]) + H^+ = 316. The molecular ion peak and the fragmentation pattern were in agreement with the chemical structure of toosendanin.

Toosendanin was isolated and identified from leaves of *M. azedarach* **(Zhang *et al*., 1988)** and from fruits **(Wang *et al*., 1994).**

Nimbolinin B (3)

The molecular formula $C_{35}H_{46}O_{10}$, deduced from the molecular ion peak at m/z 626 in MS-MS (positive mode), was compatible with nimbolinin B. m/z 626 - 18 = 608 indicated the elimination of water molecule, m/z 626 - CH_3COO^- [59] = 567, m/z 567 - furyl [67] = 500, m/z (500 - tiglate [99]) + $2H^+$ = 403, m/z 626 - 118 [$2CH_3COO^-$] = 508.

The molecular ion peak and the fragmentation pattern were in agreement with the chemical structure of nimbolinin B. This compound was previously isolated and identified from the root bark and fruits of *M. azedarach* **(Ruo- Chun *et al*., 1996; Singh *et al*., 1998; Ayyad *et al*., 2008).**

Nimbolinin A (4)

The molecular formula $C_{37}H_{44}O_{10}$, deduced from the molecular ion peak at m/z 650 in MS-MS (positive mode), was compatible with nimbolinin A. m/z 650 - H_2O [18] = 632, m/z 650 - $PhCOO^-$ [121] = 529, m/z 529 – AcOH [60] = 469, m/z 650 - O2 [32]

= 618, m/z 618 - furan [68] = 550. The molecular ion peak and the fragmentation pattern were in agreement with the chemical structure of nimbolinin A. Extract of fruits of *M. azedarach* contained this compound **(Ayyad *et al*., 2008).**

There are six compounds which, isolated for first time from ripe fruits of *M. azedarach*, and were identified as:

Azedarachin C (5)

The molecular formula $C_{32}H_{42}O_{10}$, deduced from the molecular ion peak at m/z 585 in MS-MS (positive mode), was compatible with azedarachin C. m/z 585 - H_2O [18] = 567, m/z 567 – isobutyric [88] + 2H = 481, m/z 585 - $2CH_4$ [32] = 553, m/z 553 – furyl [67] = 486, m/z 585 - $(CH3)_2$-CH_2 [44] = 541, m/z 585 - furyl [67] + $3H^+$ = 521, m/z 521 - CH_3COO^- [59] = 462.

Trichilin K (6)

The molecular formula $C_{32}H_{42}O_{11}$, deduced from the molecular ion peak at m/z 600 in MS-MS (positive mode), was compatible with compound.

m/z 600 - H_2O = 582, m/z 600 - $3CH_3$ [45] = 555, m/z 600- furyl [67] – AcOH [60] = 473, m/z 600 - $(CH_3)_2CHCOOH$ [88] - AcOH [60] - 2H = 450. The molecular ion peak and the fragmentation pattern were consistent with the chemical structure of Trichilin K.

Nimbolidin C (**7**)

The molecular formula $C_{37}H_{50}O_{12}$, deduced from the molecular ion peak at m/z 686 in MS-MS (positive mode), was compatible with nimbolidin C. m/z 686 - H_2O [18] = 668, m/z 686 - AcOH [60] = 626, m/z 686 - 2AcOH [120] = 566, m/z 686 - CH_3COOMe [74] - isobutyric acid [88] = 524, m/z 686 - isobutyrate [87] - CH_2COOMe [73] - $C_{12}H_{13}O_3$ [205] = 321, m/z 321 – 16 [O] - CH4 [16] = 289. The molecular ion peak and the fragmentation pattern were in agreement with the chemical structure of nimbolidin C.

Trichilin H (8)

The molecular formula $C_{36}H_{46}O_{14}$, deduced from the molecular ion peak at m/z 702 in MS-MS (positive mode), was compatible with compound.

m/z 702 - 1 = 701, m/z 701 - AcOH [60] = 641, m/z (701 - $(CH_3)_2$-CHCOOH [88]) - H =612, m/z (701 - 2ACOH [120]) - 2H = 579, m/z 701 - $(CH3)_2$-CHCOO[87] = 614 - CH_3COO[59] = (555 - furyl [67]) + H = 489. The molecular ion peak and the fragmentation pattern were consistent with the chemical structure of Trichilin H.

12-O-Acetyl trichilin B (9)

The molecular formula $C_{37}H_{48}O_{14}$, deduced from the molecular ion peak at m/z 716 in MS-MS (positive mode), was compatible with 12-O-Acetyl trichilin B.

m/z 716 – AcOH (60) = 656, m/z 716 - CH_3COO^- (59) – Furyl (67) + $2H^+$=592, m/z 716 - $2AcO^-$ [118] = 598 - OH^- [17] = 581, m/z 716 - $2CH_3COOH$ (120) = 596 - C_4H_9COOH (102) = 594 - $2H^+$ = 592, m/z 716 - $C_5H_9O_2$ [101] = 615 - $3CH_3COO^-$ [177] + $2H^+$ = 440, m/z 440 - $2H_2O$ [36] = 404, m/z 716 – furyl [67] = 649 - CH_3COO^- [59] = 590 - CH_3 [15] = 575. This compound was isolated from the root bark of *M. azedarach* by **Nakatani *et al*. (1994)**, but this is first time isolated and identified from fruits of *M. azedarach*.

The molecular ion peak and the fragmentation pattern were in agreement with the chemical structure of azedarachin C, which was isolated from the root bark of *M. azedarach* by **Huang *et al.* (1995)**, but this is first time isolated from fruits of *M. azedarach*.

1-Cinnamoyl-3-Methacryl-11-Methoxy-Meliacarpinin (10)

The molecular formula $C_{40}H_{46}O_{14}$, deduced from the molecular ion peak at m/z 748 in MS-MS (positive mode), was compatible with compound.

m/z 748 - H_2O = 730, m/z 748 - AcOH [60] = 688, m/z 748 - cinnamic [148] –H_2O [18] = 626, m/z 748 - cinnamate [147] - methacrylate [85] - CH_3 [15]=501, m/z 748 - cinnamic [148] - H_2O [18] = 582, and m/z 748 - [195] = 553 indicated the elimination of a part of the main skeleton of meliacarpinins [$C_{10}H_{11}O_4$]. The molecular ion peak and the fragmentation pattern were consistent with the chemical structure of 1-Cinnamoyl-3-Methaacryl-11-Methoxy-Meliacarpinin.

Unfortunately, we couldn't identify the rest of compounds (5 compounds) by this method, but the MS2 of them were (456, 676, 696, 722 and 763). Preparative HPLC is recommended for more isolation and structure elucidation of new compounds from ripe fruits of *M. azedarach*, which will be our ingoing work.

Toxicity tests

Data represented in (Tables I, II) revealed that EtOAc extract and the purified fraction of *M. azedarach* fruits showed significant highly toxic effects on larvae of *S. littoralis*. The purified fraction was more effective than the extract against 2nd and 4th instar larvae. Our results were in agreement with **Schmidt *et al.* (1997)**, who mentioned that methanol extract of *M. azedarach* fruits showed toxic and antifeedant activity against larvae of *S. littoralis* and *Agrotis ipsilon*. The percentage of mortality increased

with application of higher concentrations of *Melia* extract in both species. Insecticidal effect of *M. azedarach* against other insects were recorded by **Wen and Schmutterer (1991) and Breuer and Devkota (1991).**

In this study, the highest toxicity rates were recorded for EtOAc extract of *M. azedarach* (82.5%) and (57.5%) mortality with 2nd and 4th instar, respectively at the highest concentration of 20 g/100 mL, while, the purified fraction caused 82.5% and 67.5% with 2nd and 4th instars, respectively at the highest concentration of 5 g/100 mL. The insecticidal activity of *M. azedarach* extracts against *S. littoralis* was recognized by several reports. **Salam and Ahmed (1997)** reported that the insecticidal effect of methanol extract of *M. azedarach* was very high when used at high concentrations against *S. littoralis*. **Carpinella *et al.* (2003)** stated that *M. azedarach* fruit extract and its active principle have interesting potential for use in pest control programs.

The results in (Table I, II) showed that ($LC_{50,}$) of extract was 4.10 and 16.04 g/100 mL with 2nd and 4th instar larvae respectively, while, the fraction showed ($LC_{50,}$) 1.19 g/100 mL with 2nd instar and 2.01 g/100 mL with 4th instar.

Table I. Toxic effect and LC_{50} of EtOAc extract from ripe fruits of *M. azedarach* against 2nd and 4th instar larvae of *S. littoralis*.

Treatment	Corrected mortality (%)	
	EtOAc extract	
	2nd instar	4th instar
Control	0.00	0.00
1.25 [g/100 mL]	22.50e	20.00e
2.50 [g/100 mL]	40.00d	22.50d
5.00 [g/100 mL]	57.50c	30.00c
10.00 [g/100 mL]	67.50b	40.00b
20.00 [g/100 mL]	82.50a	57.50a
LC_{50} [g/100 mL]	4.10	16.04
F	4115.63 ***	1165.63 ***
LSD	1.150	1.41

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

Table II. Toxic effect and LC_{50} of purified fraction from ripe fruits of *M. azedarach* against 2nd and 4th instar larvae of *S. littoralis*.

Treatment	Corrected mortality (%)	
	purified fraction	
	2nd instar	4th instar
Control	0.00	0.00
0.30 [g/100 mL]	27.50e	25.00e
0.60 [g/100 mL]	35.50d	30.00d
1.25 [g/100 mL]	40.00c	35.0c
2.50 [g/100 mL]	67.50b	55.000b
5.00 [g/100 mL]	82.50a	67.50a
LC_{50} [g/100 mL]	1.19	2.01
F	8164.50***	3642.44***
LSD	0.81	0.94

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

As shown in (Tables III, IV) the percentage of reduction in larval body weight was positively correlated with concentrations of EtOAc extract and purified fraction from ripe fruits of *M. azedarach*, the same observation was recorded with both 2nd and 4th instar larvae of *S. littoralis*. Approximately, the highest decrease of larval body weight recorded at concentration 20 g/100 mL with 2nd and 4th instar for extract, and concentration of 5 g/100 mL for the fraction with the same two instars. The percentage of reduction in larval body weight of extract against the two instars increase gradually at all tested concentrations; this is obvious in (Tables III, IV).

The percentages of larval body weight reduction for the ethyl acetate extract were, 73.37, 75.07, 80.64, 82.57 and 95.5% with 2nd instar, while, 79.9, 86.43, 90.99, 91.09 and 94.07% with 4th instar at concentrations of 1.25, 2.5, 5, 10 and 20 g/100 ml respectively. In case of the purified fraction, also, the percentage of reduction in larval body weight increase as follow 66.73, 68.93, 72.44, 77.1, 87.57% and 56, 72.91, 74.95. 75.36 82.04% respectively, with the 2nd and 4th instar larvae at concentrations of

0.3, 0.6, 1.25, 2.5 and 5 g/100 mL. The mentioned results are in agreement with the results of **Schmidt *et al.* (1997)** showed that, the conversion of digested food was lowered gradually by using higher concentrations of *Melia* extract against *S. littoralis* and *Agrotis ipsilon* and antifeedant activity was observed in larvae of the both insects. **Ahmed *et al.* (1978)** reported that *M. azedarach*, *Aegle marmelos*, *C. splendens* and *C. inerme* afforded a significant degree of determent with some instars of *S. littoralis*. A 1% methanol extract of seed kernels of *M. azedarach* caused 80% inhibition of feeding in 1st and 2nd instar larvae of the noctuid *Mythimna separata* **(Chiu, 1987).** Other report showed that fruits of *M. azedarach* caused growth inhibition and larval mortality above 80% against *Spodoptera frugiperda* **(Hernandez and Vendramim, 1997). 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% concentration significantly deterred feeding by adults of the elm leaf beetle, *Xanthogaleruca luteola*. **Akhtar *et al.* (2008)** found that most of the extracts and botanicals (*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 inhibitory to growth 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*.

Table III. Effect of EtOAc extract from ripe fruits of *M. azedarach* on mean larval weight (M.W.) and weight reduction (W.R.) of *S. littoralis*

Treatment	Mean larval weight (mg) and weight reduction (%)			
	EtOAc extract			
	2nd instar		4th instar	
	M.W. [mg]	W.R. (%)	M.W. [mg]	W.R. (%)
Control	11.19	0.00	42.18	0.00
1.25 [g/100 mL]	2.98e	73.37e	8.47e	79.90e
2.50 [g/100 mL]	2.17d	75.07c	5.76d	86.43d
5.00 [g/100 mL]	2.79c	80.64d	3.80c	90.99c
10.00 [g/100 mL]	1.95b	82.57b	3.758b	91.09b
20.00 [g/100 mL]	0.50a	95.50a	2.50a	94.07a
F	1170.97 ***		443.22 ***	
LSD	0.81		0.82	

Values in a column followed by the same letters are not significantly different.
*** means highly significant effect Weight Reduction (%) = [(Control - M.W.) / Control].100

Table IV. Effect of purified fraction from ripe fruits of *M. azedarach* on mean larval weight (M.W.) and weight reduction (W.R.) of *S. littoralis*

Treatment	Mean larval weight (mg) and weight reduction (%)			
	purified fraction			
	2nd instar		4th instar	
	M.W. [mg]	W.R. (%)	M.W. [mg]	W.R. (%)
Control	15.93	0.00	49.10	0.00
0.30 [g/100 mL]	5.30e	66.73e	21.60e	56.00e
0.60 [g/100 mL]	4.95d	68.93d	13.30d	72.91d
1.25 [g/100 mL]	4.39c	72.44c	12.30c	74.95c
2.50 [g/100 mL]	3.65b	77.10b	12.10b	75.36b
5.00 [g/100 mL]	1.98a	87.57b	8.82a	82.04a
F	513.79 ***		1032.17 ***	
LSD	1.15		0.94	

Values in a column followed by the same letters are not significantly different.
*** means highly significant effect
Weight Reduction (%) = [(Control - M.W.) / Control].100

The insecticidal and larval weight reduction activity of the EtOAc extract and the purified fraction are due to the presence of the active triterpenes as major constitutes. The isolated compounds showed toxic effect against different insects. These results were in agreement with several pervious investigations. **Carpinella *et al.* (2002)** reported that meliartenin is a limonoid existed as a mixture of two interchangeable isomers, at 4 micro g/cm2 and 1 micro g/cm2, the isomeric mixture was as active as azadirachtin in strongly inhibiting the larval feeding of *Epilachna paenulata* (Coleoptera: Coccinellidae) and the polyphagous pest, *Spodoptera eridania* (Lepidoptera: Noctuidae), respectively. Toosendanin a triterpene isolated from the fruits of *M. azedarach* possessed strong antifeedant activities against the

pierid rapae and showed considerable toxicity to the insect on consumption **(Wang *et al.*, 1994)**. Six fractions from the fruits of *M. azedarach* tested for their toxic properties against *Helicoverpa armigera*. There was a sharp increase in mortality at the higher concentrations for all fractions. Nimbolinin B and ohchinolide-A, tetranortriterpenoids, determined the antifeeding and toxic properties of the different fractions **(Singh *et al.*, 1998).** Nimbolinin B and nimbolidin B exhibited antifeedant properties against larvae of *S. eridania* **(RuoChun *et al.*, 1996).**

In conclusion, fruits of *M. azedarach* are still a source for new compounds. triterpenes were proven to be the major constitutes of EtOAc extract from *M. azedarach* fruits and also mainly responsible for the results of insecticidal activity and growth inhibition against *S. littoralis* larvae**.**

Corresponding author
Mohamed Farag[b]
[b] Department of Pest Physiology, Plant Protection Research Institute, AgriculturalResearch Center, Dokki, Giza, Egypt

References

Abbott W. S. A. (1925), Method for computing the effectiveness of an insecticide. *J. Econ. Entomol.* **18**, 265.

Ahmed S. M., Shalaby F. F. and El-Khishin M. K. (1978), Antifeedant properties of some plant extracts for the larvae of *Spodoptera littoralis* (Boisd.), Plant Protection Department, Faculty of Agricultural Sciences, Moshtohor, Zagazig University, Egypt. Proceedings of the 4th Conference of Pest Control, 1Part I. Cairo, Egypt, pp. 336-345.

Akhtar Y., Yeoung Y. R. and Isman M. B. (2008), Comparative bioactivity of selected extracts from Meliaceae and some commercial botanical insecticides against two noctuid caterpillars, *Trichoplusia ni* and *Pseudaletia unipuncta*. Phytochem. Rev. **7**, 77–88.

Ascher K. R. S., Schmutterer H., Zebitz C. P. W. and Naqvi S. N. H. (1995), The Persian lilac or Chinaberry tree: *Melia azedarach* L., in: H. Schmutterer (Ed.), The Neem Tree: Source of Unique Natural Products for Integrated Pest Management Medicine Industry and Other Purposes, VCH, Weinheim, Germany, pp. 605-642.

Ayyad N. S., Hoye T. H., Sreerama L., Dawidar M. A., Alarif M. W. and Abdel-Mogib M. (2008), New nimbolinin and meliacarpin derivatives from *Melia azedarach.* Rev. Latinoamer. Quim. **36**, 7-15.

Breuer M. and Devkota B. (1991), Control of Thauemtopoea pityocampa (Den. & Schiff.) by extracts of *Melia azedarach* L. (Meliaceae). J. Appl. Entomol. **110**, 128-135.

Carpinella C., Ferrayoli C., Valladares G., Defago M. and Palacios S. (2002), Potent limonoid insect antifeedant from *Melia azedarach*. Biosci. Biotech. and Biochem. **66**, 1731-1736.

Carpinella C., Defago´ M., Valladares G. and Palacios S. (2003), Antifeedant and insecticide properties of a limonoid from *Melia azedarach* (Meliaceae) with potential use for pest management. J. Agric. Food Chem. **51**, 369-74.

Chiu S. F. (1987), Experiments on the practical application of chinaberry, *Melia azedarach*, and other naturally occurring insecticides in China. Natural pesticides from the neem tree *Azadirachta indica* A. Juss and other tropical plants Proceedings of the 3rd International Neem Conference, Nairobi, Kenya, 10 – 15 July, 1986, pp. 661-668.

D'Ambrosio M. and Guerriero A. (2002), Degraded limonoids from *Melia azedarach* and biogenetic implications, Phytochemistry **60**, 419–424.

Defago, M., Valladares, G., Banchio, E., Carpinella, C. and Palacios, S. (2006), Insecticide and antifeedant activity of different plant parts of *Melia azedarach* on *Xanthogaleruca luteola.* Fitoterapia **77**, 500-505.

El-Defrawi M. E., Toppozada A., Mansour N. and Zeid M. (1964), Toxicological studies on Egyptian cotton leafwrom *Prodenia litura* (F.). I: Suceptiblity of different larval instars to insecti-cides. J. Econ. Entomo. **57**, 591-593.

Farag M., Ahmed M. H. M., Yousef H., and Abdel-Rahman A. A.-H. (2011), Repellent and insecticidal activities of Melia azedarach *L. against cotton leafworm* Spodoptera littoralis *(Boisd.). Z. Naturforsch. 66C, 129-135.*

Finney D. J. (1971), Probity analysis statistical treatment of the sigmoid response curve. Cambridge University Press, p. 256.

González-Gómez R., Otero-Colina G., Villanueva-Jiménez J. A., Pérez-Amaro J. A. Soto-Hernández R. M. (2006), Toxicidad y repelencia de *Azadirachta indica* contra *Varroa destructor* (Acari: Varroidae). Agrocie. **40**, 741-751.

Hernandez C. R. and Vendramim J. D. (1997), Bioactivity evaluation of aqueous extracts of Meliaceae to *Spodoptera frugiperda* (J.E. Smith). Revist. de Agric. Piracica. **72**, 305-318.

Huang R. C., Okamura H., Iwagawa T., Tadera K. and Nakatani M. (1995), Azedarachin C, a limonoid antifeedant from *Melia azedarach.* Phytochemistry **38**, 593-594.

Kleeberg H. (1997), Method for the reproduction of storage stable azadirachtin from seed kernels of the neem tree. United States patent, 5,695,763.

Nathan S. S. and Kim S. (2006), Effects of *Melia azedarach* L. extract on the teak defoliator *Hyblaea puera* Cramer (Lepidoptera: Hyblaeidae). Crop Protect. **25**, 287-291.

Nakatani M., Abdelgaleil S. A. M., Saad M. M. G. Huang R. C, Iwagawa N., and Doe T. (2004), Phragmalin limonoids from *Chukrasia tabularis*, Phytochemistry **65**, 2833–2841.

Nakatani M., Huang R. C., Okamura H., Naoki H. and Iwagawa T. (1994), Limonoid antifeedants from Chinese *Melia azedarach*. Phytochemistry, **36**, 39-41.

Padrón B., Oranday A., Rivas C. and Verde M. (2003), Identificacin de compuestos de *Melia azedarach*, *Syzgium aromaticum* y *Cinnamomum zeylanicum* con efecto inhibitorio sobre bacterias y hongos. Ciencia UANL.Verlag, Berlin, Germany. **6**, 333-338.

Pérez-Pacheco R., Rodríguez C., Lara-Reyna J., Montes R. and Ramírez G. (2004), Toxicidad de aceites, esencias y extractos vegetales en larvas del mosquito *Culex quinquefasciatus* (Say.) (Diptera: Culicidae). Acta Zool. Mex. Nueva Serie **20**, 141-152.

RuoChun H., Tadera K., Yagi F., Minami Y., Okamura H., Iwagawa T. and Nakatani M. (1996), Limonoids from *Melia azedarach*. Phytochemistry, **43**, 581-583.

Salam A. L. and Ahmed A. I. (1997). Evaluation of using the extract of chinaberry fruits, *Melia azedarach* L. in the control of the cotton leafworm, *Spodoptera littoralis* in Egypt. International conference on pests in agriculture, 6 - 8 January 1997, at le Corum, Montpellier, France **3**, 1159-1162.

Schmidt G. H., Ahmed A. A. I. and Breuer M. (1997), Effect of *Melia azedarach* extract on larval development and reproduction parameters of *Spodoptera littoralis* (Boisd.) and *Agrotis ipsilon* (Hufn.) (Lep. Noctuidae) Anzeiger fur Schadlingskunde, Pflanzenschutz, Umweltschutz **70**, 4-12.

Schmutterer H. (2002), The neem tree. Neem Found, Mumbai, India. p. 892.

Snedecor G. W. and Cochran W. G. (1989), Statistical Methods, 8th ed. Iowa State University Press, Ames, Iowa.

Singh R. P., Dilawari V. K., Kochhar D. K. and Dhaliwal G. S. (1998), Toxic effects of enriched fractions of *Melia azedarach* L. against the larvae of *Helicoverpa armigera* (Hubner). Proceedings of International Conference on Ecological Agriculture, Ecological agriculture and sustainable development, 15 - 17 November 1997, Chandigarh, India, **2**, pp. 262-266.

Tabashink B. E., Cushing N. L. and Johnson M. W. (1987), Diamond back moth (Lepidoptera: Plutellidae) resistance to insecticides in Hawaii: Intra-island variation and cross resistance. J. Econ. Entomol. **80**, 1091-1099.

Vergara R., Escobar C. and Galeano P. (1997), Potencial insecticida de extractos de *Melia azedarach* L. (Meliaceae). Actividad biológica y efectos. Rev. Fac. Nac. Agron. Medellín **50**, 186.

Wang W. L., Wang Y. and Chiu S. F. (1994), The toxic chemical factors in the fruits of *Melia azedarach* and their bio-activities toward Pieris rapae. Acta Entomolo. Sinic. **37**, 20-24.

Wen J. H. and Schmutterer H. (1991), Effects of extracts from fruit and leaves of *Melia azedarach* L. on Locusta *migratoria migratorioides* (R. & F.). Anzeiger fur Schadlingskunde, Pflanzenschutz, Umweltschutz **64**, 128-133.

Zhang X., Znang M. L. and Chiu S. F. (1988), The relationship between the bioactivity and the content of toosendanin in chinaberry. J. South China Agri. Univ. **9**, 21-30.

3/2/2012