The Journal of Animal & Plant Sciences, 25(2): 2015, Page: 519-527
ISSN: 1018-7081

PESTICIDAL ACTIVITY OF AN ISOLATED LIMONOID FROM *MELIA AZEDARACH* L. FRUITS

Ahmed S. Abdel-Aty[1] and Ahmed Abdel-Megeed[2,3]

[1]Department of Pesticide Chemistry and Technology, Faculty of Agriculture, 21545-El-Shatby, Alexandria University; Alexandria, Egypt.
[2] Department of Plant Protection, Faculty of Agriculture, (Saba Basha), Alexandria University, Egypt.
[3] Department of Botany and Microbiology, College of Science, King Saud University, P. O. Box 2455 – Riyadh 11451, Saudi Arabia
Corresponding author E-mail: sabry2000@yahoo.com; aamahmoud@ksu.edu.sa

ABSTRACT

Melia azedarach is one of a famous botanical pesticides source. Extraction of its fruits with aqueous acetone followed by liquid partitioning with n-butanol, ethanol and methanol lead to a heterocyclic compound (limonoid). Through IR, UV, ^{1}H NMR, ^{13}C NMR, C-H COSY and MS spectroscopic measurements, the isolated compound was identified as 7-(3'-furyl)-benzo[3,4-c]-7H-oxol-2-one. The isolated compound exhibited moderate lethal effects on *Spodoptera littoralis* 4th larval instar with antifeeding activity increasingly with increasing the tested concentrations with EC_{50} 49.3 and 315 µg/ml after 24 and 48 hours, respectively. Its mortal effect against C*ulex pipens* was increased systematically with LC_{50} 1276, 934 and 257 µg/ml after 1, 2 and 4 days exposure, respectively. It inhibited the treated larvae pupation systematically with 148.7 µg/ml EC_{50} value. The isolated compound differently inhibited the hyphal growth of plant pathogenic fungi such as *Rhizoctonia solani*, *Macrofomina phaseoli*, *Pythium debarianum* and *Fusarium calmorum* in a treated fungus and its exposure time dependant effect.

Key-words: *Melia azedarach* fruits, insecticidal, fungicidal, 7-(3'-furyl)-4-hydroxybenzo[3,4-c]-7H-oxol-2-one, limonoid.

INTRODUCTION

Botanical pesticides have long been used as attractive alternatives to synthetic compounds for pest management because of their little threat to the environment and human health comparing with synthetic pesticides harmful actions. So, public concerns over biological control as a replacement of chemicals have been growing (Matsuki *et al*., 1997; Sumathi, 2013). Pesticides from Meliaceae plants, in particular *Azadirachta indica* Juss and *Melia azedarach* L. are promising (Isman, 1999). Limonoids, mono-, di-, sesqui-, and triterpenoids, coumarins, chromones, lignans, flavonoids and other phenolics were isolated from its certain species with wide-ranging uses in ethnomedicine (Mulholland *et al*. 2000; Srinivasan *et al.*, 2014). Several pesticidally active compounds were identified from *M. azedarach* L. leaves including ring limonoids (Zhou *et al*., 2004). Strong antifeeding activity against *Pieris rapae* was referred to melianoninol, melianol, meliciandiol, meliantriol, vanillic acid, agnillin and toosendanin from *M. azedarach* fruits (Wuang *et al*., 1994). From its root, 1-methyl acryloyl-3- acetyl-11-methoxymeliacarpinin and 1-(2-methylpropanoyl)-3-acetyl-11-methoxymeliacarpinin with significant lethality on the brime shrimp (Fukyama *et al*., 2000) and salannal and meliacarpinin as potent insect antifeedant (Huang *et al*., 1996) were isolated. Several other *M. azedarach* extracts showed their insecticidal activity as larvicidal, pupicidal, adulticidal, antiovipositional, antifeedant, chitin biosynthesis inhibitor, repellent and other effects (Kamarai *et al*., 2010; Andrade-Coelho, 2009; Rachokarn *et al*., 2008; Prophiro *et al*, 2008; Nathan *et al*., 2006; Nakalani *et al*., 1995), fungicidal (Capinella *et al*. 2003 and 2005), antibacterial (Saleem *et al*., 2002), antiviral (Bueno *et al*., 2009 & 2010), acaricidal (Borges *et al*., 2003) and antihelminthic activities (Szewezuk *et al*., 2006; Abdul Hamid *et al.*, 2012). In preliminary tests that have been previously done we found the ability of this plant fruits to kill the mites as mixing 5 gm of its powder around a plant of sweet corn reducing *Cryptostigmata* and *Mesostigmata* mites' populations in soil.

So, this work was carried out to evaluate an isolated compound from its fruits for its pesticidal effects on certain pests and plant pathogens.

MATERIALS AND METHODS

Reagents and consumables: Lannete (Methomoyl), S-methyl-N-(methyl carbamoyloxy)thioacetimidate was obtained from Kafr El-Zayat Company for pesticides, Egypt. Metalaxyl (Radomil), N-(2,6-dimethylphenyl-N-methoxyacetyl)-DL-alaninemethylester was obtained from El-Wataneya Company for pesticides, Egypt. All

other used chemicals and solvents were purchased from El-Gomhouria Drug Company, Egypt.

The infra-red (I.R.) spectroscopy measurements were done using Perkin-Elmer 1430 Ratio recording IR Spectrophotometer. Ulta-Violet (U.V.) spectroscopy measurements were carried on Perkin-Elmer Lambada 4B UV/VIS Spectrophotometer. IR and UV spectroscopy measurements were carried out in the Central Laboratory Unit, faculty of Science, Alexandria University, Alexandria, Egypt.

The NMR spectra were recorded on Varian Mercury-VX-300 NMR Spectrometer in DMSO-d6 using tetramethylsilane (TMS) as a standard. EI-Mass spectra were recorded on a Shimadzu MS-5988 mass spectrometer at 70 eV. NMR and MS spectroscopy measurements were done in Micro-analytical Center, Faculty of Science, Cairo University, Giza, Egypt.

Extraction of plant sample and isolation of the studied compound: Fruits of *Melia azedarach*, Meliaceae (280 gm) were collected from Antoniades garden in Alexandria, Egypt and completely grinded and soaked in 1.0 l of 50% aqueous acetone for three weeks in the dark at room temperature. The extracted materials were filtered under vacuum in 0.8 l of brown filtrate. The plant debris was re-soaked in 0.75 l of 75% aqueous acetone for further two weeks under the same conditions and filtered to 0.6 l. The combined filtrates were concentrated under vacuum to 300 ml containing 37.5 gm oven dried extract (13.4% of the un-extracted sample). The extracted materials were fractionated three times with 200 ml of water saturated n-butanol using a separating funnel. The organic layers were combined and concentrated using the rotary evaporator. The concentrate was dried in a petri dish by air dryer to give bright yellow powder (Fraction I, 6.5 gm, 2.32%). The aqueous layer was partitioned with chloroform (200 ml, three times) and the organic layers were combined and concentrated under reduced pressure, then dried to fraction II (3.2 gm, 1.14%) of dark brown powder. The aqueous layer was concentrated under vacuum to give a viscous liquid, which was dried over sodium hydroxide and phosphorus pentoxide (P_2O_5) to fraction III (24.5 gm, 8.75%) of brownish powder. Fractions I, II and III, were separated with 89.9% recovery. Fraction I (n-BuOH separated fraction) was further fractionated as 2 gm was dissolved in 100 ml of ethanol gradually under warming and then filtered through a centered glass filter. The ethanol soluble sub-fraction was evaporated at room temperature and re-crystallized from ethanol with 80.4 % recovery to **c**ompound 1 (0.232 gm, 0.27% of the un-extracted sample) as white amorphous powder. The ethanol insoluble sub-fraction was freed from the solvent and separated as pale yellow amorphous powder (1.375 gm, 1.6%) of the un-extracted sample) with 96% recovery. This sub-fraction (0.5 gm) was recrystallized from methanol to give **c**ompound 2 (0.20 gm, 0.64%) of methanol soluble yellowish white powder and **c**ompound 3 (0.28 gm, 0.89%) as methanol insoluble white amorphous powder. Extraction and isolation procedure is shown in Figure (1).

Insecticidal Activity: The 4th instar larvae of both cotton leaf worm, *Spodoptra littoralis* (Boisid), Lepidoptera and *Culex pipens*, Culicidae, Diptera was used. They were cultured in the breeding sector of Pesticide Chemistry and Technology Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

The treated cotton leaf worm, *S. littoralis* (Boisid) 4th instar larvae were reared on castor bean leaves (Eldefrawi *et al.*, 1964). Twenty larvae were treated in each replicate and three replicates were considered one treatment. The tested compound was used at 5, 25, 50, 100, 200, 500, 1000 and 2000 µg/ml using the leaf dipping technique (Kubo and Nakanishi, 1977). The castor bean leaves were cut into equivalent circles 2.5 cm in diameter and immersed in the tested solutions for 30 seconds and dried before introducing to insects in plastic pots sealed with a piece of cloth and fixed with a rubber band. The experiment was carried out at 25± 2ºC and 70% relative humidity. Control was concurrently conducted. After 24 and 48 hours exposure, the alive larval number was counted and mortality percents were calculated (Abbot, 1925) at each concentration. Antifeeding activity was revealed by comparing the average consumed food of each larva in control and treatment at 5, 25, 50, 100, 200, 500 and 1000 µg/ml, determining the percentage of feeding inhibition according to the following formula (Abivandl and Benz, 1984). The obtained results were compared with methomyl (lanette) as a standard insecticide.

$$\% FI = 100 \times (C-T) \div C$$

FI, feeding inhibition; **C,** consumed food of control larva; **T,** consumed food of treated larva

The treated larvae of *Culex pipens* were treated in water at concentrations of 5, 10, 50, 100, 200, 500, 1000 and 2000 µg/ml of the isolated compound. Sixty larvae of each treatment were divided in three replicates. Number of alive larvae was distinguished from dead and mortality percent was calculated after 1, 2 and 4 days exposure (Abbot, 1925). Another population was exposed for 5 days to the isolated compound for checking its effect on pupation. Treated larvae were noticed and pupation percents were calculated comparing with control at concentrations of 10, 50, 100, 200, 500, 1000 and 1500 µg/ml. Lanette (Methomoyl), S-methyl-N-(methylcarbamoyloxy) thioacetimidate was used as comparative insecticide.

Fungicidal Activity: Measurements were carried out by using radial growth test (poison food technique) according to Torgeson (1967). A definite volume of the well-known Czapek-Dox medium (12 ml) containing

agar (4.5 gm/100 ml water) was sterilized in conical flasks. Citrate-Phosphate buffer solution (3 ml) was autoclaved separately; both solutions were mixed in a sterile conical flask. The tested compounds (7-(3'-furyl)-benzo [3,4-c]-7H-oxol-2-one and the standard fungicide) were dissolved in dimethyl-sulfoxide (DMSO) and finally added to be 1, 10, 50, 100, 200 and 500µg/ml of the poisoned fungal growth medium. The contents of each flask (36 ml) were distributed in three sterilized Petri-dishes and considered as one treatment. All additions were done under aseptic conditions. After solidification, the inoculum disc (7 mm in diameter) of each tested fungus was located in the center of the dish. Control in the presence of the calculated volume of dimethylsulfoxide only to be 1 % as its final concentration was concurrently conducted. The results were recorded by measuring the diameter of the hyphal growth at different times and the inhibition percent of the hyphal growth were calculated (Topps and Wain, 1957). EC_{50} (the effective concentration caused 50% inhibition in the hyphal growth) were determined in µg/ml for each compound and fungus. The obtained results were compared with Metalaxyl (Radomil), N-(2,6-dimethylphenyl-N-methoxyacetyl)-DL-alaninemethylester as a standard fungicide. Data were analyzed using probit analysis according to Finney (1971).

RESULTS AND DISCUSSION

Identification of the isolated compound: ^{1}H NMR spectrum indicated the aromatic protons at δ 7.2-7.6 region. The integration explained 1: 3: 1: 2 protons. From the chemical shift, the aromatic benzene ring is substituted with an electron donating group. Substituted furyl moiety distributed its protons peaks at δ 7.21 (1H, dd, C_4-H, *J*= 5.4, 3.1, 3.8 Hz), 7.3 (1H, d, C_2-H, *J*= 1.9 Hz) and 7.6 (1H, d, C_5-H, *J*= 7.7 Hz), respectively. Their multiplicity was referred to their coupling each other and differences in their chemical shift are owed to their position to the heteroaromatic oxygen atom. Benzofuran ring differently distributed its protons peaks as a function of their position to the lactonized carbonyl group in furanone moiety. C_7-H proton gave its peak at δ 7.27 (1H, d, C_7-H, *J*= 3.9 Hz) due to coupling with C_6-H proton. Protons of C_3, C_5 and C_6 carbons were differently affected with C_4-OH distributing their peaks at δ 7.28 (1H, t, C_5-H, *J*= 6.5, 3.1 Hz) due to *ortho* coupling with C_6-H proton and *meta* coupling with C_3-H proton, 7.36 (1H, t, C_6-H, *J*= 6.9, 7.6 Hz) and 7.7 (1H, d, C_3-H, *J*= 7.65 Hz). C_5-H peak was up-field shifted to both C_3-H and C_6-H protons because of their *ortho* position to the hydroxyl group. C_3-H appeared down field to C_6-H by the lactonized carbonyl group.

^{13}C NMR spectrum, C-H carbons gave their signals at different chemical shifts due to their positions. While C-7 gave its signal at 127.1 ppm, both C-3 and C-4' superimposed at 128.3 ppm and C-5 superimposed with C-2' at 128.7 ppm. C-5' and C-6 gave their signals at 128.8 and 131.3 ppm, respectively. Non substituted carbons (C-8 and C-9) arranged their signals at 137.6 and 136.1 ppm referring to different effect of the carbonyl group on each of them. The other non-substituted C-3' was shifted to 175.8 ppm due to the conjugation with oxygen non shared electrons with the double bond. However C_4-OH gave its signal at 177.9 ppm, C-2 showed its signal at 185.9 ppm (lactonized carbony group).

C-H COSY spectrum helped and ensured the identification and correlation between carbon and hydrogen as it explained C-5 connection to both C-3 and C-6 carbons, while C-6 was connected to C-5 only emphasizing their peaks multiplicity. C-7 carbon is connected to C-6 carbon in the benzofuran-2-one ring. Coupling among C-2', C-4' and C-5' was noticed explaining their proton multiplicity.

Mass spectrum showed the parent molecular ion at m/z 216 (21.7%). It was fragmented through fission of the hetero-aromatic rings to m/z 184 (32.7%) fragment, which loses $C_4H_3^+$ ion at m/z 51 (12.8%) to m/z 133 fragment that subsequently loses CO to 105 m/z (15.2%) fragment. The molecular ion de-protonated to m/z 215 fragment that was fragmented around the carbonyl group to fragments at m/z 67 (7.5%), 95 (35.3%) and 120 (0.9%) that loses CO to m/z 92 (54.0%) and 91 (7.5%), which was subjected to phenyl ring fission to give m/z 78 (19.0%), 65 (7.5%) and 54 (6.6%) fragments, respectively by losing one, two or three CH^+ ions. The molecular ion may also be fragmented through the furan-2-one ring fission to m/z 172 fragment that gave hydroxy-tropilium ion at m/z 108, which loses the hydroxyl group to m/z 91 (11.9%) fragment. The m/z 59 (100%) may be due to $^+CH_2$-COOH ion through benzofuran-2-one ring fission. The illustrated fragmentation pathways are briefly presented in Figure (2).

Qualitatively the structure was ascertained through UV and IR spectroscopy as follows:

Ultra–Violet (UV) spectroscopy measurements**,** Electron absorption spectra of the isolated compound were characterized by several maxima stretching from 227-238 nm (log ε= 2.05-2.08) since conjugation of phenyl ring with the carbonyl group in the furan-2-one ring. It also contains non-shared electron pairs of oxygen atom. Their spectra were identical with two maxima: K-bands due to $\prod$ -$\prod^*$ transition because of high conjugation degree and high extinction coefficient and R-bands in 282-293 nm regions ((log ε= 0.52-0.92) due to n -$\prod^*$ transition at longer wavelength because of non-shared electrons and conjugation between CO and –O- in

furan-2-one moiety. Besides σ-σ* transition at 198-208 nm, n-σ* transition absorbed at 260-271 nm. Presence of a chromophoric group (CO) attached to aromatic ring results in B-bands at longer wavelength.

Infra-red (IR) spectroscopy spectrum ensured the isolated compound finger print and functional groups with the strong discriminatory band at 1742-1728 cm^{-1} for the carbonyl group and the band at 1691-1559 cm^{-1} indicated its conjugation with the phenyl ring as well as the band at 1485cm^{-1} explained its lactonized structure. Some spectral data are shown in (Table 1).

From the previous mentioned results, the isolated compound was identified as 7-(3'-furyl)-benzo[3,4-c]-7H-oxol-2-one (Figure 3). This result goes with Dambrosio and Guerriero (2002) and Nakatani *et al*. (1998) as they proved the presence of the same skeletal structure and they isolated some limonoids other than our identified derivative from the roots of *M. azedarach*.

Insecticidal effects: The isolated compound exhibited moderate lethal effects on the treated *S. littoralis* 4th larval instar with dramatically increased mortality percent to 20.3 and 28.6 % after 24 and 48 hours at 200-2000 µg/ml concentration range comparing with the used standard insecticide, which caused mortality increased with increasing the tested concentrations reaching complete killing the treated population at 200 µg/ml (Table 2). It reduced the treated larvae palatability to the used castor leaves increasingly with increasing the tested concentrations. The average consumed food for each larva was ranged from 81.8 % to 25.2% of the value of the untreated larva with effective concentration of 50% equaled 49.3 µg/ml after 24 hours exposure, while the antifeeding effect was decreased to 81.7% - 43.1% range of the untreated larva value achieving 315 µg/ml EC_{50} value after 48 hours, which may due to decreasing the palatability of the castor leaves to both treated and un-treated larvae (Table 3). So, the compound 7-(3'-furyl)-4-hydroxybenzo[3,4-c]-7H-oxol-2-one maybe active as antifeedant more than as lethal compound in agreement with Wuang *et al*. (1994), who reported the antifeeding effect of *M. azedarach* fruits, while the roots and bark extracts were lethal to *S. littoralis* and *S. exigwa* at high doses only (Srivastava and Srivastava, 1996). The resulted insecticidal activity against *S. littoralis* may be referred to inhibition of carboxylesterase and glutathione-s-transferase (Rachokarn *et al*., 2008).

Table 1. Identification of the isolated compound, 7-(3'-furyl)-4-hydroxybenzo[3,4-c]-7H-oxol-2-one

IR spectrum		* NMR sectrum	Ms spectrum (m/z) **
Assignment	**Wave number (cm^{-1})**		
Ar. C-H **Str**	3055-3026	**^{1}H NMR spectrum**, δ **7.21** (1H, dd, C_4-H, *J*= 5.4, 3.1, 3.8 Hz), **7.3** (1H, d, C_2-H, *J*= 1.9 Hz), **7.6** (1H, d, C_5-H, *J*= 7.7 Hz), **7.27** (1H, d, C_7-H, *J*= 3.9 Hz), **7.28** (1H, t, C_5-H, *J*= 6.5, 3.1 Hz), **7.36** (1H, t, C_6-H, *J*= 6.9, 7.6 Hz), **7.7** (1H, d, C_3-H, *J*= 7.65 Hz).	216 (M+) (21.7**), 184 (32.7), 124 (96.5), 120 (0.9), 119 (9.2), 105 (15.2), 95 (35.3), 92 (54.0), 91 (11.9), 78 (19.0), 67 (7.5), 65 (7.5), 54 (6.6), 51 (12.8), 59 (100)
Ar. C-H (IP) bending	1272-1070		
Ar. C-C **Str**	1691-1581		
Ar. C=C **Str**	1581-1559		
C-H (OOP) bending	808-653		
C=O Str. (**s, St**)	1742-1728		
Conjugated C=O	1691-1559	**^{13}C NMR spectrum**, C-2 (185.9), C-3 (128.3), C_4-OH (177.9), C-5 (128.7), C-6 (131.3), C-7 (127.1), C-8 (137.6), C-9 (136.1), C-2' (128.7), C-3' (175.8), C-4' (128.3), C-5' (128.8)	
Lactonized C-O	1485		
O-H (IP) bending	1400-1300		
Free O-H **Str** (**Br.**)	3450		

Str, stretching**; IP,** in plane**; OOP,** out of plane**; s,** sharp**; St,** strong**; Br,** broad band**, * s,** singlet**; d,** doublet**; t,** triplet**; ( ),** chemical shift in ppm; ** ( ), % relative abundance

Although the isolated compound was less effective against the treated *C. pipens* 4th larval instar than the used standard insecticide in its lethal activity, it caused mortality percents as a function of the tested concentration and the exposure time to the tested compound. Its mortal effect was increased systematically to 54.5, 60.3 and 71.6 mortality percents within the used concentration range with lethal concentration of 50% treated population (LC_{50}) equaled 1276, 934 and 257 µg/ml after 1, 2 and 4 days exposure, respectively (Table 4). On the other hand, after continuous five consecutive days exposure, the tested compound affected the pupation of the treated *C. pipens* larvae systematically with increasing the tested concentration to 7.17% of control at 1000 µg/ml followed by complete stopping pupation of the treated population at 1500 µg/ml with 148.7 µg/ml EC_{50} value (Table 5). These results agreed with Prophiro *et al*. (2008) as they reported the mortal effect of *M. azedarach* leaf extract against *Aedes egyptii* (Diptra, Culicidae) after 24 and 48 hours as well as its pupicidal activity against *A. egyptii* was exhibited by Nathan *et al.* (2006).

Fungicidal activity: The isolated compound differently affected the hyphal growth of some plant pathogenic fungi comparing with the standard fungicide, metalaxyl.

Table 2. Mortality effects of the compound 7-(3'-furyl)-4-hydroxybenzo[3,4-c]-7H-oxol-2-one on *Spodoptera littoralis.*

Compound	Time (hrs)	Mortality percents at different concentrations (µg/ml)								
		0	5	25	50	100	200	500	1000	2000
Methomyl (Lannete)	24	0	0	56.2± 2.1	69.3± 3.4	80.5± 2.4	100			
	48	0	0	65.3± 2.3	75.1± 2.6	90.2± 4.1	100			
The isolated compound	24	0	0	0	0	0	5.2± 1.2	5.9± 1.8	10.6± 2.1	20.3± 3.2
	48	0	0	0	0	0	5.6± 0.9	10.9± 1.5	20.7± 3.1	28.6± 35

Data are shown as mortality percents ± SD.

Table 3. Anti-feeding effect of the compound 7-(3'-furyl)-4-hydroxybenzo[3,4-c]-7H-oxol-2-one on *Spodoptera littoralis.*

Time (hrs)	Average consumed food of each larva (gm ò 10^{-4})								EC_{50} 95% CL	Slope ± SE	i^2
	0	5	25	50	100	200	500	1000			
24	210.6± 12.3	170.2±10.3	120.0 ± 8.7	98.2 ± 9.2	74.0 ± 6.7	62.9 ± 2.9	58.9 ± 4.2	53.0 ± 5.1	49.3 34.3 –70.3	0.67 ± 0.005	8.2
48	88.4 ± 6.7	72.2 ± 5.6	59.9 ± 4.5	54.1 ± 3.2	50.0 ± 4.8	47.1 ± 2.1	42.3 ± 3.4	38.1 ± 2.3	315 174 –582	0.44 ± 0.005	1.3

Data are averages of three replicates ± SD.

Table 4. Lethal effect of the compound 7-(3'-furyl)-4-hydroxybenzo[3,4-c]-7H-oxol-2-one on *Culex pipens* larvae

Comp.	Day	Mortality% at different concentrations (µg/ml)									LC_{50} 95% CL	Slope ± SE	i^2
		0	5	10	50	100	200	500	1000	2000			
Lanette	1	0	63.7 ±0.6	71.7 ±2.1	90.7 ±1.2	100					< 5.0		
The isolated compound	1	0	0.0	5.4 ±0.4	12.2 ±0.9	19.5 ±2.5	28.9 ±1.0	33.9 ±0.9	47.3 ±2.4	54.5 ±3.4	1276 863-1901	0.83± 0.006	4.2
	2	0	0.0	12.8 ±0.9	17.2 ±1.0	22.8 ±0.9	31.6 ±2.9	38.9 ±1.0	51.7 ±1.7	60.3 ±1.7	934 632-1390	0.75± 0.005	10
	4	0	6.6 ±1.8	30.0 ±1.7	34.4 ±1.0	38.3 ±1.7	46.8 ±1.4	54.1 ±1.3	65.0 ±1.7	71.6 ±1.6	257 179-369	0.61± 0.003	13

Mortality percents are averages of three replicates ± SD.

Table 5. Effect of the compound 7-(3'-furyl)-4-hydroxybenzo[3,4-c]-7H-oxol-2-one on *Culex pipens* larvae pupation.

Pupation% at different concentrations (µg/ml)								EC_{50} (95%CL)	Slope± SE	χ^2
0	10	50	100	200	500	1000	1500			
100	100	79.67± 1.53	65.63± 2.52	33.67± 1.15	20.33± 0.58	7.17± 0.76	0.0	148.7 (128.5 – 172)	1.97±0.02	9.05

Data are represented as averages of three replicates after 5 days exposure.

Against *Rhizoctonia solani*, it was not significantly different from the standard fungicide in its effect although they exhibited 443 and 234 µg/ml EC_{50} values, respectively after 2 days exposure. It was less effective on *Macrofomina phaseoli* with EC_{50} values equaled > 1000 µg/ml after 2 and 4 days exposure. The isolated compound, 7-(3'-furyl)-4-hydroxybenzo[3,4-c]-7H-oxol-2-one inhibited the hyphal growth of the fungus, *Pythium debarianum* exhibiting EC_{50} values of 608, 466 and 560 µg/ml after 2, 4 and 6 days exposure, respectively with no significance between either the exposure times or the used standard fungicide with EC_{50} values of 596, 334 and 355 µg/ml after 2, 4 and 6 days exposure respectively. While *Fusarium calmorum* was affected with the isolated compound after 2 days exposure more that after 4 and 6 days exposure, there was no significant difference between the last two exposure times. It was less effective than the standard fungicide with EC_{50} values of 292 and 131µg/ml, respectively after 6 days exposure (Table 6). These results are in agreement with Capinella *et al*. (2003

and 2005) who found the fungicidal activity of seeds and kernels of *M. azedarach* against *F. solani*, *F. oxysporum* and *Aspergillus flavus*.

Table 6. Fungicidal effect of the compound, 7-(3'-furyl)-4-hydroxybenzo[3,4-c]-7H-oxol-2-one; shown as EC_{50} values

Treated fungus	Days	Tested compound	EC_{50}	95%C.L	Slope± SE	¡ ^{2}
Rhizoctonia solani	2	The isolated compound	443	323 - 609	0.97 ± 0.012	4.3
		Metalaxyl (Redomil)	234	156-354	0.78 ± 0.012	1.4
Macrofomina phaseoli	2	The isolated compound	> 1000			
		Metalaxyl (Redomil)	221	152 - 323	0.84 ± 0.012	1.0
	4	The isolated compound	> 1000			
		Metalaxyl (Redomil)	175	135 - 243	0.78 ± 0.016	1.6
Pythium debarianum	2	The isolated compound	608	449 - 826	1.14 ± 0.017	2.5
		Metalaxyl (Redomil)	596	423 - 759	1.32 ± 0.020	3.6
	4	The isolated compound	466	354 - 613	1.15 ±0.015	9.3
		Metalaxyl (Redomil)	334	196 - 579	0.68± 0.012	1.6
	6	The isolated compound	560	399 - 789	0.98 ± 0.013	1.5
		Metalaxyl (Redomil)	355	190 - 502	0.86 ±0.018	2.5
Fusarium calmorum	2	The isolated compound	136	107- 171	1.27 ±0.012	3.2
		Metalaxyl (Redomil)	-			
	4	The isolated compound	321	247 - 419	1.08 ± 0.013	4.9
		Metalaxyl (Redomil)	-			
	6	The isolated compound	292	234 - 365	1.29 ±0.015	1.5
		Metalaxyl (Redomil)	131	75 - 233	0.47±0.010	1.3

Metalaxyl (Redomil), N-(2,6-dimethylphenyl-N-methoxyacetyl)-DL-alaninemethylester χ^2 is Chi^2

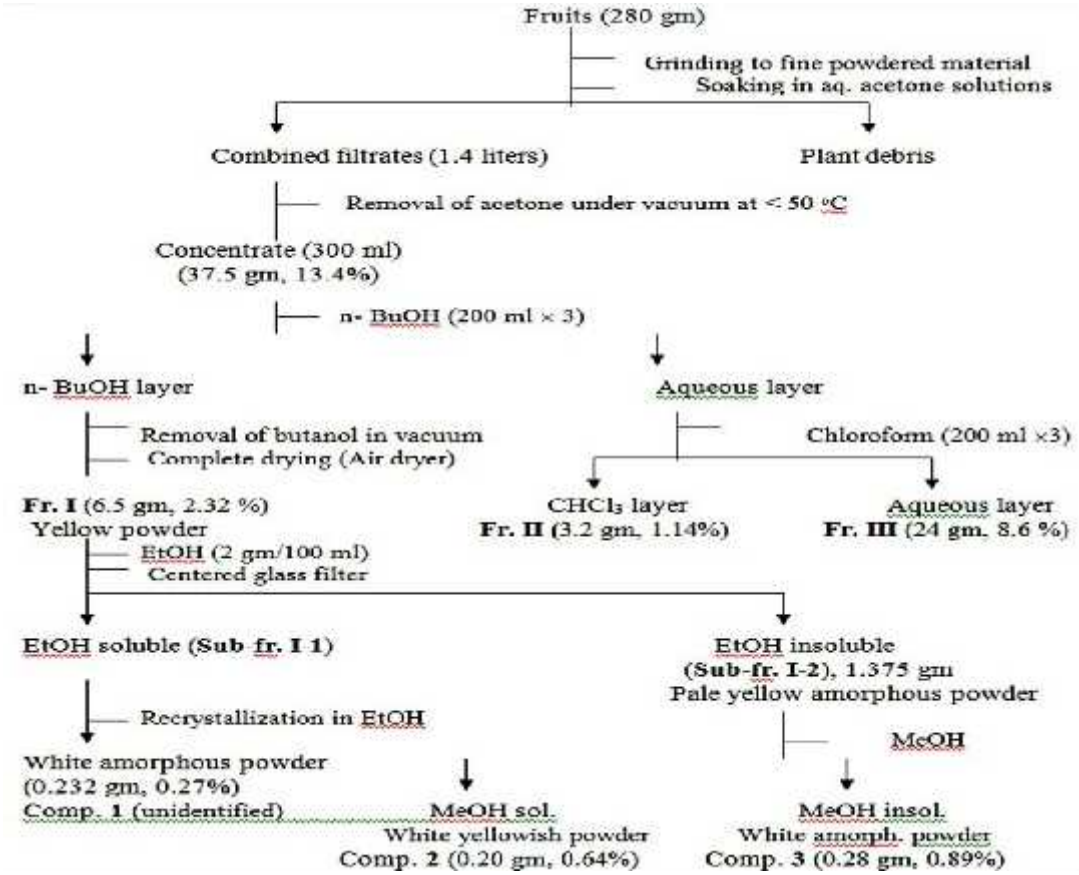

Figure 1. Extraction and isolation of 7-(3'-furyl)-benzo[3,4-c]-7H-oxol-2-one (3) from of *M. azedarach* L fruits

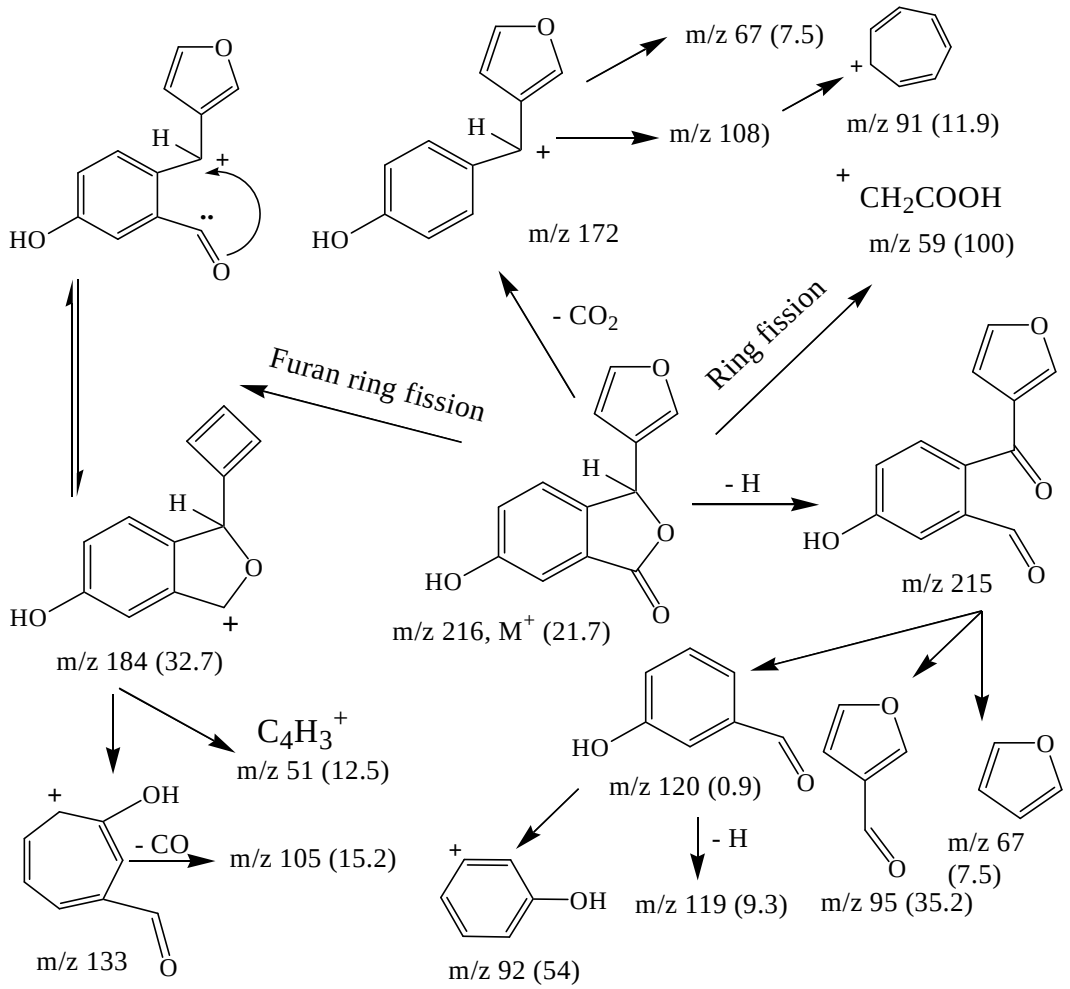

Figure 2. Fragmentation pathways of the isolated 7-(3'-furyl)-4-hydroxybenzo[3,4-c]-7H-oxol-2-one

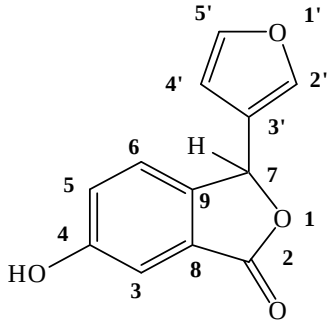

Figure 3.The chemical structure of 7-(3'-furyl)-4-hydroxybenzo[3,4-c]-7H-oxol-2-one

Acknowledgement: The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group "RGP 010".

REFERENCES

Abbot, W. W. (1925). A method of computing the effectiveness of an insecticide. J. Eco Entomol., 18: 265-267.

Abdul Hamid, Z., S.B. Budin, N.W. Jie, A. Hamid, K. Husain, J. Mohamed (2012). Nephroprotective effects of *Zingiber zerumbet* Smith ethyl acetate extract against paracetamol-induced nephrotoxicity and oxidative stress in rats. J Zhejiang Univ-Sci B (Biomed & Biotechnol). 13(3):176-185.

Abivandl, C., G. Benz (1984). Tests with the extracts of 21 medicinal plants for anti-feedant activity against larvae of *Pieris brassicae* L. (Lep, Ppieridae). Bull. Soc. Entomol. Swisse., 57: 383-392.

Andrade- Coelho, C. A., N. A. Souza; C. Gouveia, V. C. Silva, M. S. Gonzalez, E. F. Rangel (2009). Effect of fruit and leaves of Meliaceae plants (*Azadirachta indica* and *Melia azedarach*) on

the development of *Lutzomyia longipalpis* larvae (Diptera: Psychodidae) under experimental conditions. J. Med. Entomol., 46 (5): 1125-1130.

Borges, L. M., P. H. Ferri, W. J. Silva, W. C. Silva and J. G. Silva (2003). In vitro efficacy of extracts of *Melia azedarach* against the tick *Boophilus microplus*. Med. Vet. Entomol., 17 (2): 228-231.

Bueno, C. A., A. A. Barquero, H. Dicansoli, M. S. Maier, L. E. Alch (2009). A natural tetranortriterpenoid with immunomodulating properties as a potential anti HSV agent. Virus Res., 141 (1): 47-54.

Bueno, C. A.; L. E. Alch, A. A. Barquero (2010). 1-Cinnamoyl-3, 11-dihydroxy-meliacarpin delays glycoprotein transport restraining virus multiplication without cytotoxicity. Biochem. Biophys. Res. Commun., 393 (1): 32-37.

Capinella, M. C.; C. G. Ferrayoli and S. M. Palacios (2005). Antifungal synergistic effect of scopoletin, a hydroxycoumarin isolated from *Melia azedarach* L.fruits. J. Agric. Food Chem., 53 (8): 2922-2927.

Capinella, M. C.; L. M. Giorda; C. G. Ferrayoli, S. M. Palacios (2003). Antifungal effects of different organic extracts from *Melia azedarach* L. on phytopathogenic fungi and their isolated active components. J. Agric. Food Chem., 51 (9): 2506-2511.

D'Ambrosio, M., A. Guerriero (2002). Degraded limonoids from *Melia azedarach* and biogenetic implications. Phytochem., 60 (4): 419-424.

Eldefrawi, M. E., A. Toppozada, N. Mansour, M. Zeid (1964). Toxocological studies on the Egyptian cotton leaf worm *Prodenia litura* (F.) I-Susceptibility of different larval instars of *Prodenia* to insecticides. J. Econ. Entomol., 57: 591-593.

Finney, D. J. (1971). Probit analysis. 3rd Fukuyama, Y.; M. Ogawa; H. Takahashi and H. Minami (2000). Two new meliacarpins from the roots of *Melia azedarach*. Chem. Pharm. Bull., 48 (2):301 303.

Fukayama, Y., M. Ogawa, H. Takahashi, H. Minami (2000). Two new meliacarpinins from the roots of *Melia azedarach*. Chem. Pharm. Bull. (Jpn), 48 (2): 301-303.

Huang, R. C., K. Tadera, F. Yagi, Y. Minami, H.Okamura, T. Iwagawa and M. Nakatani (1996). Limonoides from *Melia azedarach*. Phytochem., 43 (3): 581-583.

Isman, M. B. (1999). Neem and related natural products. In Methods in biotechnology, Biopesticides: use and delivery Ed. F. R. Hall and J. J. Menn, 5, Humana Press Inc., Totowa, NJ, pp 139-153.

Karamarai, C., A. A. Rahuman, A. Bagavan, M. J. Mohamed, G. Elango, G. Rajakumar, A. A. Zahir, T. Santhoshkumar and S. Marimuthu (2010). Ovicidal and larvicidal activity of crude extracts of *Melia azedarach* against *Haemonchus contortus* (Strongylida). Parasitol. Res.. 106 (5): 1071-1077.

Kubo, I., K. Nakanishi (1977). In "Host plant resistance to pests." Hedin. P. A. Ed., American Chemical Society: Washington, ACS Symp. Ser. No. 62, P.165.

Matsuki, T., H. Negishi, M. Honda and T. Fujimori (1997). An improved method for preparation and preservation of conidia for *Nimbya scirpicola*, pathogenic fungus for *Eleocharis kuroguwai*. J. Pestic. Sci, 22: 39-41.

Mulholland, D. A., B. Parel, P. H. Coombes (2000). The chemistry of the Meliaceae and Ptaeroxylaceae of southern and eastern Africa and Madagascar. *Current Org.* Chem., 4 (10):1011-1054.

Nakatani, M., R. C. Huang, H. Okamura, T. Iwagawa, K. Tadera (1998). Degraded limonoids from *Melia azedarach*. Phytochemistry, 49 (6): 1773- 1776.

Nakatani, M., R. C. Huang, H. Okamura, T. Iwagawa, K. Tadera, H. Naoki (1995). Three ntifeeding meliacarpinins from chinese *Melia azedarach.* Tetrahydron, 51 (43): 11731-11736.

Nathan, S. S., G. Savitha, D. K. George, A. Narmadin, L. Suganya, P. G. Chung (2006). Efficasy of *Melia azedarach* L. extract on the malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). Bioresour. Technol., 97 (11): 1316-1323.

Prophiro, J. S., J. C. Rossi, M. F. Pedroso, L. A. Kanis, O. S. Silva (2008). Leaf extracts of *Melia azedarach* Linnaeus (Sapidales: Meliaceae) act as larvicide against *Aedes aegyptii* (Linnaeus, 1762) (Diptera: Culicidae). Rev. Soc. Bras. Med. Trop., 41 (6): 560-564.

Rachokran, S., N. Piyasaengthong, V. Bullangpoti (2008). Impact of botanical extracts derived from leaf extracts of *Melia azedarach* L. (Meliaceae) and *Amaranthus viridis* L. (Amaranthaceae) on populations of *Spodoptera exigua* (Lepidoptera: Noctuidae) and detoxification enzyme activities. Commin. Agric. Appl. Biol. Sci., 73 (3): 451-457.

Saleem, R., S. I. Ahmed, S. M. Shamim, S. Faizi, B. S. Siddiqui (2002). Antibacterial effect of *Melia azedarach* flowers on rabbits. Phytother. Res., 16 (8): 762-764.

Srivastava, S. D., S. K. Srivastava (1996). Insect antifeedant novel limonoids from the roots of *Melia azedarach*. J. Indian. Chem. Soc., 73 (9): 467-469.

Srinivasan, V., Panneerselvam, R., Gunasekaran, S., Palani, S. (2014). Ethanolic extract of Melia Azadirachta against Acetaminophen induced Nephrotoxicity. International J. PharmTech Research. 6 (1): 70-79.

Sumathi, A, (2013). Evaluation of physicochemical and phytochemical parameters of Melia Azedarach. Leaves (family: meliaceae), International J. Pharmacy and Pharmaceutical Sciences. 5, Suppl 2.

Szewezuk, V. D., E. R. Mongelli, A. B. Pomilio (2006). In vitro anthelmintic activity of *Melia azedarach* naturalized in Argentina. Phytother. Res., 20 (11): 993-996.

Topps, J. H., R. L. Wain (1957). Investigation on fungicides. III. The fungitoxicity of 4- and 5-alkyl salicylanilide and p-chloroanilines. Ann. Appl. Biol., 45 (3): 506-11.

Torgeson, D. C. (1967). Fungicides. 1, Agricltural and industerial applications environmental interactions. Academic Press New York and London

Wang, W., Y. Wang, S. Chiu (1994). The toxic chemical factors in the fruits of *Melia azedarach* and their bio-activities toward *Pieris rapae*. Kunchong Xuebao, 37 (1): 20-24.

Zhou, H., A. Hamazaki, J. D. Fontana, H. Takahashi, T. Esumi, C. B. Wandscheer, H. Tsujimoto and Y. Fukuyama (2004). New ring C-seco limonoids from Brazilian *Melia azedarach* and their cytotoxic activity. J. Nat. Prod., 67 (9): 1544-1547.