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CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF FOUR *CITRUS* ESSENTIAL OILS

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ABSTRACT

Essential oils of four *Citrus* species*, Citrus aurantifolia*, *C. limon*, *C. paradisi* and *C. sinensis*, were extracted from fruit peels by hydrodistillation. The chemical composition of the isolated oils was analyzed by gas chromatography/mass spectrometry (GC-MS). Effect of the isolated essential oils on seed germination and seedling growth of *Silybum marianum* was evaluated. The essential oils were also tested for their molluscicidal and insecticidal activities against the adults of *Theba pisana* snails and the fourth instar larvae of *Spodoptera littoralis*. Chemical analysis showed that *dl*-Limonene was the major component in the four essential oils and represented 40.19, 56.30, 74.29 and 89.23% of *C. aurantifolia*, *C. limon*, *C. paradisi* and *C. sinensis*, respectively. In general, the four oils were characterized to be rich in monoterpene hydrocarbons and oxygenated monoterpenes. Allelopathic experiment on *S. marianum* revealed that the tested oils reduced the germination percentages at all of the tested concentrations. The oil of *C. aurantifolia* caused the highest germination reduction at concentration of 10 µL/ Petri dish where 26.7 % of seeds were germinated. The oil of *C. sinensis* was the most potent inhibitor for root and shoot growth of *S. marianum* with EC_{50} values of 2.5 and 6.97 µL/Petri dish, respectively. The tested oils were more effective in reducing the growth of roots than shoots, except for *C. paradise* which stimulated the root growth. On the other hand, the tested oils showed strong fumigant toxicity against the adults of *T. pisana* snail with *C. sinensis* oil (LC_{50} = 14.2 µL/L) being the most potent one. Also, the tested oils possessed a remarkable toxic effect against the fourth instar larvae of *S. littoralis* and the oils of *C. aurantifolia* and *C. sinensis* had the highest toxic effect with LC_{50} values of 6.84 and 6.88 µL/L, respectively. The findings of the present study suggest that the essential oils have a potential to be used for pest control.

Keywords: *Citrus* essential oils; allelopathic activity; molluscicidal activity; insecticidal activity; *Silybum marianum*; *Theba pisana*; *Spodoptera littoralis*

INTRODUCTION

Essential oils are liquid, volatile, secondary metabolites of aromatic plants. They are lipid soluble and soluble in organic solvents and characterized by a strong odour. Essential oils are complex natural mixtures which can contain about 20–60 components at quite different concentrations. They are characterized by two or three major components at high concentrations (20–70%) compared to other components present in traces. In general these major components are responsible for the biological activities of the essential oils (Croteau et al., 2000; Betts, 2001; Bowles, 2003; Pichersky et al., 2006). Essential oils have been widely used as bactericidal, fungicidal, antiparasitical, insecticidal, pharmaceutical and cosmetic since the middle ages. They are also used nowadays in pharmaceutical, sanitary, cosmetic, agricultural and food industries. Essential oils are usually extracted by steam or hydro-distillation first developed in the middle ages by Arabs.

The extraction product can vary in quality, quantity and in composition according to climate, soil composition, plant organ, age and vegetative cycle stage (Masotti et al., 2003; Angioni et al., 2006; Bakkali et al., 2008).

There are great efforts to use plant natural products as alternatives of synthetic pesticides since they are more safe for human and environment and give new modes of action to overcome resistance phenomena of old pesticides. In this regard great attention is toward the use of essential oils for pest managements because they causes little or no mammalian toxicity and they do not persist in soil or contaminate ground water (Isman 2000) and also seem to have no specific cellular targets because the great number of constituents (Carson et al., 2002).

The citric industry is one of the world's largest agro industries, and the juice manufacturing process creates enormous amount of residues. Essential oils from fruit peels and seeds of *Citrus* species may be recommended as a cheap, easily available at farmer level, eco-friendly with low mammalian toxicity and good alternative to synthetic pesticides. It could further reduce the use of synthetic pesticides (Sharma and Tripathi 2006; Ozmen and Tulay, 2007; Tsai 2008; Celikel and Kavas, 2008; Prabuseenivasan, et al., 2006).

Silybum marianum (L.) Gaertn. (milk thistle, family Asteraceae) is a serious weed in many areas of North and South America, Africa, Australia, and the Middle East (Holm et al., 1997). Milk thistle is grown commercially as a medicinal plant in Europe, Egypt, China, and Argentina (Anonymous 1995). White garden snails, *Theba pisana* Muller (Mollusca: Gastropoda: Helicidae), feed on a wide variety of plants, including cereals, vegetables, fruits, herbs, and many ornamentals, destroying seeds and seedlings, stunting growth, and reducing yields. Not only do they directly damage the plants they feed on but the wounds they create allow plant pathogenic fungi to infect plants. The snails can also be vectors of various plant pathogens, and their mucus trails can contaminate grains, vegetables, fruits, and herbs. In large numbers, their bodies and shells can be contaminants of mechanically harvested crops (Godan, 1983; Barker, 2002). The white garden snail is currently a serious agricultural pest in many areas of the world, including Europe, the USA, the Mediterranean region and Australia, particularly in wet seasons. In Egypt, this snail is a destructive agricultural animal pest of several economic crops, including tree fruits, vegetables and ornamental plants (El-Okda, 1983). The Egyptian cotton leafworm, *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae), is a well-known polyphagous pest of various crops (e.g. cotton, soybeans, alfalfa, pepper, eggplant, tomato, lettuce, strawberry), widely distributed in the Mediterranean region, the Middle East, and North and East Africa (Hosny et al., 1986; Pineda et al., 2007). *S. littoralis* larvae feed mainly on leaves and stems and can seriously retard growth or reduce crop production.

The objectives of this study were to determine the chemical composition of the essential oils isolated from four *Citrus* species, namely *Citrus aurantifolia*, *C. limon*, *C. paradisi* and *C. sinensis*, and to evaluate the allelopathic activity of these oils against milk thistle, *S. marianum.* In addition,

the molluscicidal and insecticidal activities of these oils were also examined on *T. pisana* and *S. littoralis*.

MATERIALS AND METHODS

Plant materials

The fruits of four *Citrus* species, namely *Citrus aurantifolia* (Christm.) Swingle *Citrus limon* (L.) Burm.f., *Citrus paradisi* Macfad and *Citrus sinensis* (L.) Osbeck were purchased from Alexandria main fruits and vegetables market, Alexandria, Egypt in February, 2011. Fresh fruit peels were used for extraction of essential oils.

Isolation of essential oils

Essential oils were extracted from the fresh fruit peels by hydrodistillation in a Clevenger-type apparatus for 3 h. The oils were dried over anhydrous sodium sulfate, and stored at 4°C until used for GC-MS analysis and biological activity tests.

Analysis of essential oils

Essential oils were diluted in diethyl ether and 0.5 µl was injected into the gas chromatography (Hewlwett Packard 5890)/mass spectrometry (Hewlwett Packard 5989B) (GC-MS) apparatus. The GC column was a 30 m (0.25 mm i.d., film thickness 0.25 µm) HP-5MS (5% diphenyl) dimethylpolysiloxane capillary column. The GC conditions were as follows: injector temperature, 240°C; column temperature, isothermal at 70°C for 2 min, then programmed to 280°C at 6°C/min and held at this temperature for 2 min; ion source temperature, 200°C; detector temperature, 300°C. Helium was used as the carrier gas at the rate of 1 ml/min. The effluent of the GC column was introduced directly into the ion source of the MS. Spectra were obtained in the EI mode with 70 eV ionization energy. The sector mass analyzer was set to scan from 40 to 400 amu for 5 s. The oil components were identified by comparison of their retention indices and mass spectra with the NIST Mass Spectral Library.

Test organisms

Silybum marianum (L.) Gaertn. (milk thistle) field biotype were collected from Alexandria Desert Research Station Farm, Alexandria, Egypt. All undersized or damaged seeds were discarded, and the seeds of uniform size were selected. Germination tests were carried out before experiments and the germination percent was 85% for milk thistle. Adult terrestrial snails (16 ± 0.5 mm shell diam.), *Theba pisana* (Muller), were collected from the Faculty of Agriculture Garden, Alexandria, Egypt in April, 2013. The snails were kept under laboratory conditions at 26 ± 2°C in ventilated glass jars for two weeks before bioassay and were fed a diet of fresh lettuce leaves (*Lactuca sativa* L.). A susceptible strain of *Spodoptera littoralis* (Boisd) was obtained from the Bioassay Laboratory, Faculty of Agriculture, Alexandria University. The colony was reared under laboratory conditions on caster bean leaves, *Ricinus communis* L. (Euphorbiaceae), at 26 ± 2°C and 70 ± 5% r.h. (El-Defrawi et al., 1964).

Phytotoxic assay on *S. marianum*

Aliquots of 0, 3, 5 and 10 µl essential oils of were dissolved in 1ml diethyl ether and placed in 9.0 cm Petri dishes lined with filter paper. The solvent was allowed to evaporate and 5ml distilled water was added to obtain concentrations of 0 (control), 600, 1000 and 2000 mg/L. Three micro-liter of essential oil per Petri dish is equivalent to 600 ppm. Twenty seeds of *Silybum marianum* were placed in the Petri plates (Krifa et al. 2011). The Petri dishes were kept on a germination cabinet at 20±1ºC with 12 h photoperiod, 3.3 µmol m^{-2} s^{-1}. After 8 days of sowing, germination and root and shoot lengths were determined. The growth inhibition percentages of root and shoot lengths were calculated from the following equation: I(%)= [1- T/C] x 100; T is the length of treatment (cm) and C is the length of control (cm). The concentrations causing 50% inhibition (EC_{50}s) of root and shoot growth were calculated from a probit analysis (Finney, 1971).

Fumigant toxicity assay on *T. pisana*.

Glass jars of one liter capacity were used as exposure chambers to test the toxicity of essential oils vapors against adults of *T. pisana*. The essential oils were applied to Whatman no. 1 filter paper pieces (2x2 cm) attached to the undersurface of the screw caps of the glass jars. The tested concentrations were 5, 10,15, 20, 25, 30, 40 and 50 µl/l. After the addition of essential oils the jars were sealed. A similar set-up but without essential oils served as a control. For each treatment, four replicates with 5 snails on each one were maintained. Mortality was determined after 24-h exposure. Test snails were considered dead if no response was observed after being touched with a thin needle (WHO, 1965). LC_{50} (lethal concentration to kill 50% of the population relative to control) values were calculated by probit analysis (Finney, 1971).

Fumigant toxicity assay on *S. littoralis*.

Fourth instar larvae of *S. littoralis* were used to assess the fumigant toxic action of the tested essential oils. Ten larvae were placed in each glass jars of 0.4 l capacity. The tested essential oils were applied to Whatman no.1 filter paper pieces (2x2 cm) attached to the undersurface of screw caps of the glass jars. Caps were then screwed tightly onto the jars. The essential oils were tested at a series of concentrations ranging from 5, 10, 15, 20, 25, 30, 40 and 50 µl/l. For each treatment, three replicates with ten larvae on each one were maintained. The mortality percentages were recorded after 24-h treatment. LC_{50} values were calculated as previously described.

Statistical analysis

Germination percentages, root lengths and shoot lengths were subjected to one-way analysis of variance followed by Student–Newman–Keuls test (Cohort software Inc. 1985) to determine significant differences among mean values at the probability level of 0.05. For the snail and larvae bioassays the mortality of each concentration was calculated after 24-h treatment as the mean of three replicates. The mortality data were subjected to probit analysis (Finney, 1971) to obtain the LC_{50} values, using SPSS 12.0 (SPSS, Chicago, IL, USA). The values of LC_{50} were considered significantly different if the 95% confidence limits did not overlap.

RESULTS AND DISCUSSION

Chemical composition of the isolated essential oils

The essential oils of *Citrus* species obtained by hydrodistillation were analyzed using GC-MS. The chemical composition of the essential oils are given in Table 1.

Table 1. Chemical composition of essential oil isolated from *Citrus* plant species

Citrus aurantifolia **Compound (%)**	***Citrus limon*** **Compound (%)**	***Citrus paradisi*** **Compound (%)**	***Citrus sinensis*** **Compound (%)**
α-Thujene (0.36)	α-Thujene (0.33)	α-Pinene (1.17)	α-Pinene (0.44)
α-Pinene (1.79)	α-Pinene (1.34)	dl-Limonene (74.29)	Sabinene (0.23)
Camphene (0.11)	Sabinene (0.75)	Linalool oxide (4.18)	β-Myrcene (1.77)
Sabinene (1.29)	β-Pinene (8.81)	L-Linalool (4.61)	Octanal (1.28)
β-Pinene (19.65)	β-Myrcene (1.30)	*trans-P*-mentha-2,8-dienol (0.58)	σ-Carene (0.23)
β-Myrcene (0.88)	α-Terpinene (0.26)	β-Fenchyl alcohol (1.99)	dl-Limonene (89.23)
α-Terpinene (0.26)	dl-limonene (56.30)	Decanal (1.45)	Linalool (2.98)
dl-limonene (40.19)	β-Phellandrene (0.27)	β-Citral (2.66)	Citronellal (0.16)
β-Ocimene (0.33)	γ-Terpinene (6.42)	Eugenol (0.72)	Teroinen-4-ol (0.25)
γ-Terpinene (6.34)	α-Terpinolene (0.50)	Geraniol (0.91)	α-Terpineol (0.51)
α-Terpinolene (0.54)	Linalool (1.78)	Caryophyllene (0.91)	Decanal (0.47)
Linalool (1.41)	L-Camphor (0.20)	α-Humulene (0.13)	*trans*-Geraniol (0.25)
Citronellal (0.23)	Citronellal (0.30)	Germacrene-D (0.25)	β-Citral (0.29)
Borneol (0.19)	4-Terpineol (2.25)	Valencene (0.41)	Citral (0.37)
Terpinen-4-ol (2.62)	α-Terpineol (3.38)	δ-Cadinene (0.17)	Valencene (0.26)
α-Terpineol (3.71)	Nerol (2.29)	Junipene (1.59)	
L-Citronellol (1.87)	β-Citral (3.83)	Farnesol (0.64)	
α-Citral (8.14)	α-Citral (4.96)	Nootkatone (1.78)	
trans-Geraniol (2.11)	Neryl acetate (1.11)		
σ-Elemene (0.26)	α-Bergamotene (0.51)		
Neryl acetate (0.44)	Valencene (0.56)		
Geranyl acetate (1.00)	β-Bisabolene (0.73)		
β-Selinene (0.24)			
trans-Caryophyllene (0.32)			
α-Bergamotene (0.85)			
α-Humulene (0.16)			
Germacrene D (0.86)			
β-Bisabolene (2.15)			Monoterpene hydrocarbons (91.9)
Monoterpene hydrocarbons (71.74)	Monoterpene hydrocarbons (76.28)	Monoterpene hydrocarbons (75.46)	Oxygenated monoterpenes (4.81)
Oxygenated monoterpenes (21.72)	Oxygenated monoterpenes (20.1)	Oxygenated monoterpenes (17.10)	Sesquiterpene hydrocarbons (0.26)
Sesquiterpene hydrocarbons (4.84)	Sesquiterpene hydrocarbons (1.8)	Sesquiterpene hydrocarbons (3.46)	Oxygenated sesquiterpenes (0.0)
Oxygenated sesquiterpenes (0.0)	Oxygenated sesquiterpenes (0.0)	Oxygenated sesquiterpenes (2.42)	Others (1.53)
Total identified (98.3)	Total identified (98.18)	Total identified (98.44)	Total identified (98.47)

The major constituents of the essential oils were dl-limonene (40.19%), β-pinene (19.65%) and α-citral (8.14%) in *C. aurantifolia* and dl-limonene (56.30%), β-pinene (8.81%) and γ-terpinene (6.42%) in *C. limon* and dl-limonene (74.29%), L-linalool (4.61%) and linalool oxide (4.18%) in *C.*

paradisi and dl-limonene (89.23%) and linalool (2.98%) in *C. sinensis*. The results showed that dl-limonene was the major component in the four oils. However, the highest concentration was observed in *C. sinensis* and the lowest one was observed in *C. aurantifolia*. The identified constituents of the essential oils belonged to four groups: oxygenated monoterpenes, monoterpene hydrocarbons, sesquiterpene hydrocarbons and oxygenated sesquiterpenes.

The chemical compositions of the isolated essential oils are in accordance with those previously reported (Lota et al., 2001; Ahmed et al., 2006; Sokovic` et al., 2007; Viuda-Martos et al., 2009; Kamal et al., 2011; Pistelli et al., 2012). However, the percentages of constituents slightly differed. The differences in essential oil compositions could be due to several factors, such as geographical location, season, environmental conditions, nutritional status of the plants and other factors (Perry et al., 1999).

Effect of essential oils on seed germination of *S. marianum*

The effect of four essential oils on the reduction of seed germination of *S. marianum* is shown in Table 2. The tested oils reduced the germination percentages at all of the tested concentrations. At the concentration of 5 µL/Petri dish, the oil of *C. paradisi* showed the highest reduction of seed germination, while the oil of *C. sinensis* revealed the lowest reduction of seed germination as the germination percents were 63.3 and 76.7%, respectively. The oil of *C. aurantifolia* was the most effective at concentration of 10 µL/Petri dish, whereas the oil of *C. lemon* was the less effective one. Although there were no reported studies on the effects of four tested *Citrus* oils on seed germination, some of other essential oils were reported to cause a reduction of seed germination (Fujihara and Shimizu, 2003; Singh et al., 2005; Paudel and Gupta, 2008; Kordali et al., 2009; Verdeguer et al., 2009).

Table 2. Effect of *Citrus* essential oils on *Silybum marianum* germination 8 d after sowing[a]

Conc. (µL)[b]	Germination %			
	C. aurantifolia	***C. limon***	***C. paradisi***	***C. sinensis***
0	86.7 ± 3.33a	86.7 ± 3.33a	86.7 ± 3.33a	86.7 ± 3.33a[c]
3	83.3 ± 3.33a	76.7 ± 3.33b	73.3 ± 3.33b	83.3 ± 3.33a
5	73.3 ± 3.33a	73.3 ± 3.33b	63.3 ± 3.33c	76.7 ± 3.33ab
10	26.7 ± 3.33b	70.0 ± 0.0b	50.0 ± 0.0d	66.6 ± 3.33 b

[a] Data are expressed as means ±SE from experiments with three replicates of 20 seeds each.

[b] Concentration by µL/ Petri dish.

[c] Means within a column sharing the same letter are not significantly different at the 0.05 probability level.

Effect of essential oils on root and shoot growth of *S. marianm*

The results showed the essential oils caused a strong reduction of root growth of *S. marianm* in a concentration-dependent manner (Table 3). The oil of *C. sinensis* was the most potent, followed by the oils of essential oils of *C. lemon* and *C. aurantifolia* with EC_{50} values of 2.5, 4.19 and 6.13 µL/ Petri dish, respectively. In contrast, the essential oil of *C. paradisi* showed no reduction effect on root growth but this oil stimulated the root growth.

On the other hand, all the essential oils caused reduction of shoot growth as shown in Table 4. The essential oil of *C. sinensis* (EC_{50} = 6.97 µL/ Petri dish) and *C. aurantifolia* (EC_{50} = 7.24 µL/Petri dish) were more effective than the oils of *C. limon* and *C. paradisi* which the EC_{50} value of the two later oils was greater than 10 µl/Petri dish. In general, the results showed that the inhibitory effects of the tested essential oils on root growth were greater than on shoot growth with the exception of *C. paradisi*. In addition, the tested oils showed higher inhibition effect on root and shoot growth than on seed germination. Similar findings were observed on the effects of other natural compounds on seedling growth of weeds (Leather and Einhellig, 1985; Chung and Miller, 1995; Abdelgaleil et al., 2009; Saad et al., 2012).

Table 3. Effect of *Citrus* essential oils on *Silybum marianum* root growth 8 d after sowing[a]

Conc (µL)[b]	*C. aurantifolia*		*C. limon*		*C. paradisi*		*C. sinensis*	
	Root length (cm)	I (%)[c]	Root length (cm)	I (%)	Root length (cm)	I (%)	Root length (cm)	I (%)
0	6.3 ± 0.12a[d]	0.0	6.3 ± 0.12a	0.0	6.3 ± 0.12c	0.0	6.3 ± 0.12a	0.0
3	4.9± 0.15b	22.2	3.9 ± 0.09b	38.1	6.8± 0.15b	-7.9	2.7 ± 0.06b	57.0
5	4.4± 0.12c	30.2	2.4 ± 0.06c	61.9	8.5± 0.29a	-34.9	2.6 ± 0.09b	58.7
10	1.4±0.12d	77.8	2.2 ± 0.12c	65.1	8.3± 0.39a	-31.7	1.3 ± 0.06c	79.4
EC_{50}[e] (µL)	6.13		4.19		—		2.5	

[a] **Data are expressed as means ± SE from experiments with three replicates of 20 seeds each.**
[b] **Concentration by µL/ Petri dish.**
[c] **I = inhibition.**
[d] **Means within a column sharing the same letter are not significantly different at the 0.05 probability level.**
[e] **EC_{50} = concentration of compound causing 50% root growth inhibition.**

Among the isolated oils, only the essential oil of *C. limon* was reported to possess allelopathic effect against *Euphorbia heterophylla* and *Ipomoea grandifolia* (Ribeiro and Lima, 2012). However, the inhibitory effects of essential oils of different plant species on seedling growth were reported (Duke et al., 2000; Dayan et al., 2009; Krifa et al., 2011; Yang et al., 2012).

Table 4. Effect of *Citrus* essential oils on *Silybum marianum* shoot growth 8 d after sowing[a]

Conc (µL)[b]	*C. aurantifolia*		*C. limon*		*C. paradisi*		*C. sinensis*	
	Root length (cm)	I (%)[c]	Root length (cm)	I (%)	Root length (cm)	I (%)	Root length (cm)	I (%)
0	2.9 ± 0.06a[d]	0.0	2.9 ± 0.06a	0.0	2.9 ± 0.06a	0.0	2.9 ± 0.06a	0.0
3	2.2± 0.03b	24.1	2.4 ± 0.07b	17.2	2.2± 0.07b	24.1	1.97 ± 0.03b	32.1
5	1.7± 0.06c	41.4	2.2 ± 0.03c	24.1	1.9± 0.07c	34.5	1.7 ± 0.09c	41.4
10	1.2±0.07d	58.6	1.7 ± 0.06d	41.4	1.9± 0.03c	34.5	1.2 ± 0.07d	58.6
EC_{50}[e] (µL)	7.24		> 10.0		> 10.0		6.97	

[a] **Data are expressed as means ± SE from experiments with three replicates of 20 seeds each.**
[b] **concentration by µL/ Petri dish.**
[c] **I = inhibition.**
[d] **Means within a column sharing the same letter are not significantly different at the 0.05 probability level.**
[e] **EC_{50} = concentration of compound causing 50% root growth inhibition.**

Fumigant toxic effect of essential oils on *T. pisana*

The results in Table 5 showed that the essential oils had remarkable fumigant toxicity against the white garden snail, *T. pisana.* The most effective oils were *C. sinensis* and *C. aurantifolia* with LC_{50} values of 14.2 and 15.5 µl/L, respectively. The oils of *C. limon* and *C. paradisi* showed closely molluscicidal effect with LC_{50} values of 23.0 and 25.0 µl/L respectively. The molluscicidal activity of isolated essential oils were more potent than those of *Mentha microphylla* and *Eucalyptus camaldulensis* oils evaluated against the same snail, and comparable to those of *Schinus terebenthifolius* and *Lantana camara* oils, but less active than that of *Citrus reticulata* oil (El-Aswad and Abdelgaleil, 2008). In addition, the isolated oils were less effective than their major monoterpene and limonene (Abdelgaleil, 2010). On the other hand, some essential oils, such as *E. camaldulensis*, *Lavandula entate*, *Ruta chalepensis*, *M. microphylla* and *Lantana camara* were described to possess contact toxicity against adults of *T. pisana* snails (Hussein, 2005; Abdelgaleil and Badawy, 2006).

Table 5. Fumigant toxicity of *Citrus* essential oils against the adults of *Theba pisana*

Oil	LC_{50}[a] (µl/L)	95% confidence limits (mg/L)		Slope[b] ± SE	Intercept[c] ± SE	(χ^2)[d]
		Lower	Upper			
C. aurantifolia	15.50	14.29	16.98	4.25±0.64	-5.06±0.75	0.38
C. limon	23.00	20.90	24.64	4.88±1.04	-6.65±1.45	0.87
C. paradisi	25.36	23.94	26.40	11.00±1.51	-15.44±2.20	0.13
C. sinensis	14.20	11.99	16.28	2.97±0.32	-3.42±0.42	1.48

[a]**The concentration causing 50% mortality.**
[b]**Slope of the concentration-inhibition regression line ± standard error.**
[c]**Intercept of the regression line ± standard error.**
[d]**Chi square value.**

Fumigant toxic effect of essential oils on *S. littoralis*

Table 6 shows the fumigant toxic effect of the tested essential oils against the fourth instar larvae of *S. littoralis*. The results demonstrated that the essential oils had strong insecticidal activity. The oil of *C. aurantifolia* and *C. sinensis* showed the highest toxic effect as the LC_{50} values were 6.84 and 6.88 µl/L, respectively. The oils of *C. limon* and *C. paradisi* revealed pronounced insecticidal activity as the LC_{50} values were 15.32 and 18.01 µl/L, respectively. The insecticidal activity of *C. aurantifolia* and *C. sinensis* was higher than the oils of *C. reticulata*, *Schinus terebenthifolius*, *Mentha microphylla, Lantana camara* and *Eucalyptus camaldulensis* tested against the third instar larvae of *S. littoralis* (El-Aswad and Abdelgaleil, 2008). On the other hand, the isolated oils were described to possess insecticidal activity against stored product insects (Abdelgaleil et al., 2012; Abbas et al., 2012; Saleem et al., 2013).

Table 6. Fumigant toxicity of *Citrus* essential oils against the fourth instar larvae of *Spodoptera littoralis*

Oil	LC_{50}[a] (µl/L)	95% confidence limits (mg/L)		Slope[b] ± SE	Intercept[c] ± SE	(χ^2)[d]
		Lower	Upper			
C. aurantifolia	6.84	5.63	7.72	3.90 ± 0.63	-3.25 ± 0.62	0.04
C. limon	15.32	14.49	16.22	6.67 ± 0.73	-7.91 ± 0.86	0.41
C. paradisi	18.01	9.23	22.51	8.76 ± 0.80	-11.00 ± 1.04	9.34
C. sinensis	6.88	5.41	7.96	3.04 ± 0.47	-2.54 ± 0.49	0.56

[a]**The concentration causing 50% mortality.**
[b]**Slope of the concentration-inhibition regression line ± standard error.**
[c]**Intercept of the regression line ± standard error.**
[d]**Chi square value.**

Comparing the toxicity of essential oils on *T. pisana* and *S. littoralis* revealed that the essential oils were more toxic against *S. littoralis* than *T. pisana*. These findings were in agreement with data obtained by El-Aswad and Abdelgaleil (2008) using different essential oils against the above two pests.

Insecticidal and molluscicidal activities of the essential oils investigated in the present study may be attributed to their major constituents of monoterpenes. Since some major constitutes of the tested oils, such as limonene, γ-terpinene, linalool and pinene possessed insecticidal and molluscicidal effects (Lee et al., 2001; Lee et al., 2003; Garcìa et al., 2005; Abdelgaleil et al., 2009; Abdelgaleil, 2010). Monoterpenes act as neurotoxicants against different insect species (Coats et al., 1991; Enan, 1998). Monoterpenes have been shown to inhibit both GABA receptor (Coats, 1990) and acetylcholinesterase (AChE) (Grundy and Still, 1985; Ryan and Byrne, 1988). It was also reported that monoterpenes may inhibit cytochrome P450-dependent monooxygenases (De-Oliveira et al., 1997). Therefore, it is suggested that the tested oils may cause insecticidal and molluscicidal activity via one or more of these modes of action.

In conclusion, the results obtained in this study demonstrate that the *Citrus* essential oils have remarkable phytotoxic, molluscicidal and insecticidal effects against *S. marianum*, *T. pisana* and *S. littoralis*. Interestingly, these oils are extracted from fruit peels which are waste products of *Citrus* fruit juice industry. Converting these tons of waste products to safer natural pesticides is highly recommended. Based on the pesticidal activity of the *Citrus* oils demonstrated in this study, these oils could be used in integrated pest management (IPM) programs for pest control.

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المكونات الكيميائية والنشاط البيولوجى لأربعة من زيوت الموالح الطيارة

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تم عزل الزيوت الطيارة من قشور ثمار أربعة من أصناف الموالح وهى الليمون البنزهير والليمون الأضاليا والجريب فروت والبرتقال البلدى بطريقة التقطير المائى. التركيب الكيميائى للزيوت المعزولة تم تقديره بإستخدم جهاز كروماتوجرافى الغاز- مطياف الكتلة (GC/MS). وتم دراسة تأثير الزيوت المعزولة على إنبات ونمو المجموع الخضرى والجذرى لحشيشة الخرشوف البرى. وأيضا تم دراسة التأثير السام للزيوت الطيارة على الطور البالغ لقوقع الحدائق الأبيض والعمر اليرقى الرابع لدودة ورق القطن. نتائج التحليل الكيميائى للزيوت المعزولة أوضحت أن مركب الليمونين كان هو المركب الرئيسى فى كل الزيوت المعزولة وكان يمثل نسبة 40.19 و56.30 و 74.29 و 89.23% فى زيوت الليمون البنزهير والليمون الأضاليا والجريب فروت و البرتقال البلدى على الترتيب. بصفة عامة المركبات الموجودة فى الزيوت كانت تتبع مجموعتيى المونوتربينات الهيدروكربونية والمونوتربينات المؤكسدة. نتائج تأثير الزيوت على إنبات حشيشة الخرشوف البرى أظهرت أن كل الزيوت سببت خفضاً فى نسبة الإنبات على كل التركيزات المختبرة . زيت الليمون البنزهير هو الأكثر خفضاً فى الإنبات على تركيز 10 ميكرولتر/طبق بترى حيث كانت نسبة الإنبات 26.7% . زيت البرتقال البلدى كان أعلى الزيوت فى تثبيط نمو المجموع الجذرى والمجموع الخضرى لحشيشة الخرشوف البرى حيث كانت قيم التركيز المؤثر على 50% (EC_{50}) 2.5 و 6.97 ميكروليتر/طبق بترى على المجموع الجذرى والمجموع الخضرى على الترتيب. كل الزيوت كانت أكثر فاعلية فى تثبيط المجموع الجذرى عن المجموع الخضرى عدا زيت الجريب فروت والذى سبب تنشيط لنمو المجموع الجذرى. على الجانب الأخر فإن الزيوت المعزولة أظهرت سمية قوية بالتدخين ضد قوقع الحدائق الأبيض وكان زيت البرتقال البلدى هو أكثر الزيوت فاعلية حيث كان التركيز القائل لـ 50% من الأفراد المعاملة (LC_{50}) يساوى 14.2 ميكروليتر/لتر. نتائج الفاعلية الإبادية الحشرية على العمر اليرقى الرابع لدودة ورق القطن أظهرت أن الزيوت المعزولة لها فعل إبادى مميز وكان زيتى الليمون البنزهير و البرتقال البلدى هما الأعلى فاعلية حيث كانت قيم LC_{50} له تساوى 6.84 و 6.88 ميكروليتر/لتر على الترتيب. النتائج المتحصل عليها من هذه الدراسة توضح أن الزيوت المختبرة لها فاعلية جيدة ويمكن أن تستخدم فى تطوير منتجات جديدة لمكافحة الأفات.

قام بتحكيم البحث

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