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Allelopathic Potential of Essential Oils Isolated from Aromatic Plants on *Silybum marianum* L.

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The essential oils from fourteen Egyptian plants were extracted using the hydrodistillation method. Gas chromatography/mass spectroscopy (GC-MS) analysis of the isolated essential oils showed that the major constituents of the essential oils were α-pinene in *Cupressus sempervirens*, *Syzygium cumini* and *Thuja occidentalis*, 1,8-cineole in *Callistemon viminals* and *Rosmarinus officinals*, sabinene in *Pituranthous tortuosus* and *Schinus terebinthifolius*, *β*–thujone, capillene, terpinen-4-ol, pulegone, *β*-citronellol, α-phellandrene and *trans*-caryophyllene in *Artemisia judaica*, *A. monosperma*, *Cupressus macrocarpa*, *Origanum vulgare*, *Pelargonium graveolens*, *S. molle* and *Vitex aguns-castus*, respectively. The essential oils were tested for their inhibitory effects on seed germination and seedling growth of *Silybum marianum*. The phytotoxic assay results showed that the essential oil *P. graveolens* was the most potent inhibitor of seed germination, followed by the oils of *A. monosperma*, *O. vulgare* and *A. judaica*. The oil of *S. terebinthifolius* showed the lowest reduction of germination at all tested concentrations. On the other hand, the essential oils of *P. graveolens*, *A. judaica* and *A. monosperma* exhibited the highest inhibitory effect of root growth with EC_{50} values of 76.1, 639.3 and 698.2 mg/L, respectively. In contrary, the oils of *S. molle*, *S. terebinthifolius* and *R. officinals* caused stimulant effect on root growth of *S. marianum*. Moreover, the isolated essential oils revealed a significant shoot growth reduction at all of the tested concentrations with *P. graveolens* and *A. judaica* being the most potent growth inhibitors. The results obtained suggest that, in addition to their known activities, essential oils can also serve as natural weed control products.

Keywords: Essential oils, Egyptian plants, Phytotoxic effect, *Silybum marianum*

INTRODUCTION

Allelopathy offers an important tool for selective biological weed management throw production and release of allelochemicals from leaves, flowers, seeds, stems and roots of plants (Weston 1996). Natural products released from allelopathic plants may help to reduce the use of synthetic herbicides for weed management and therefore, cause less pollution, safer agricultural products as well as alleviate human health concerns (Khan *et al*. 2008).

Essential oils released from aromatic plants provide a number of ecological advantages to the plant. For example, they act as pollinator attractants, determinants of vegetation patterning, provide protection against predators

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and other enemies, and mediate plant–plant interactions including allelopathy (Batish *et al.* 2008; Singh *et al.* 2009). Earlier studies have documented that essential oils and their constituents are seed germination inhibitors and retard plant growth (Barney *et al.* 2005; Batish *et al*. 2006; Ens *et al.* 2009; Mutlu *et al.* 2010; Krifa *et al.* 2011; Batish *et al.* 2012).

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. Milk thistle is grown commercially as a medicinal plant in Europe, Egypt, China, and Argentina (Holm *et al.* 1997).

The aim of the present study was to investigate the chemical compositions of essential oils isolated from fourteen Egyptian plants, namely, *Artemisia judaica*, *A. monosperma*, *Callistemon viminals*, *Cupressus macrocarpa*, *C. sempervirens*, *Pelargonium graveolens, Pituranthous tortuosus, Origanum vulgare*, *Rosmarinus officinalis*, *Syzygium cumini*, *Schinus molle*, *S. terebinthifolius*, *Thuja occidentalis* and *Vitex agnus-castus*. The effects of the isolated essential oils on the germination and seedling growth of a dicots weed milk thistle, *S. marianum*, were evaluated.

MATERIALS AND METHODS

1. Plant materials

The aerial parts of *Artemisia judaica* L. and *Origanum vulgare* L., and the leaves of *Artemisia monosperma* Del., *Callistemon viminals* (Sol.ex Gaertn.) G. Don, *Cupressus macrocarpa* Hartw. ex Gordon, *Cupressus sempervirens* L., *Pelargonium graveolens* L'Her*, Pituranthous tortuosus, Rosmarinus officinalis* L., *Syzygium cumini* L. Skeels, *Schinus molle* L., *Schinus terebinthifolius* Raddi, *Thuja occidentalis* L. and *Vitex agnus-castus* L. were collected from different locations of Alexandria, Behira and Matrouh Governorates, Egypt between August, 2010 and April, 2011, when the specimens were in the middle of their flowering period. The plant materials were identified by Prof. FathAllah Zaitoon of Plant Pathology Department, Faculty of Agriculture, Alexandria University. Voucher specimens have been deposited in Department of Chemistry of Pesticides, Faculty of Agriculture, Alexandria University.

2. Isolation of essential oils

The plant materials were dried under shade for one week before extraction. Essential oils were extracted 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.

3. 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.

4. Tested seeds

Silybum marianum (L.) Gaertn. (milkthistle) field biotype seeds 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 75%.

5. Seed germination and seedling growth bioassay

Essential oils volumes of 0, 3, 5, 10 and 15 µl were dissolved in 1ml diethyl ether and placed in 9.0 cm Petri dishes lined with filter papers. The solvent was allowed to evaporate and 5ml distilled water was added to give concentrations of 0 (control), 600, 1000, 2000 and 3000 mg/L, as three micro-liter of essential oil per Petri dish is equivalent to 600 mg/l. Twenty seeds of *S. marianum* were placed in each Petri plate (Krifa *et al.* 2011). Three replicates of each concentration were prepared. The Petri dishes were kept on a germination cabinet at 20±1ºC with 12 h photoperiod, 3.3 µmol $m^{-2}s^{-1}$. After 9 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; where 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).

6. Statistical analysis

The concentration–response data were subjected to Probit analysis (Finney 1971) to obtain the EC_{50} values

Table 1. Major constituents of the isolated essential oils

Plant oil	Compound (%)	Plant oil	Compound (%)
A. judaica	*β* –Thujone (49.83)	*P. tortuosus*	Sabinene (32.09)
	Chrysanthenone (10.88)		Terpinen-4-ol (20.31)
	α- Thujone (8.21)		Myristicine (6.84)
	1,8-Cineole (4.91)		Dillapiole (5.72)
A. monosperma	Capillene (36.86)	*R. officinals*	1,8-Cineole (19.60)
	2,4-Pentadiynylbenzene (14.68)		Camphor (17.01)
	γ-Terpinene (12.46)		α-Pinene (15.12)
	β-Pinene (7.85)		Verbenone (9.55)
C. viminals	1,8-Cineole (71.77)	*S. cumini*	α-Pinene (17.26)
	α-Pinene (11.47)		α-Terpineol (13.88)
	Terpinen-4-ol (3.18)		β-Pinene (11.28)
	Octadecanoic acid (3.08)		cis-Ocimene (11.27)
C. macrocarpa	Terpinen-4-ol (20.29)	*S. molle*	α-Phellandrene (29.87)
	Sabinene (18.67)		β-Phellandrene (21.08)
	β-Citronellol (13.01)		Elemol (13.00)
	γ-Terpinene (7.59)		τ-Muurolol (5.35)
C. sempervirens	α-Pinene (37.88)	*S. terebinthifolius*	Sabinene (14.93)
	δ-Carene (20.05)		γ-Elemene (13.18)
	α-Terpinolene (6.91)		β-Elemene (6.63)
	β-Myrcene (5.47)		α-Candiol (6.61)
O. vulgare	Pulegone (77.45)	*T. occidentalis*	α-Pinene (35.49)
	Menthone (4.86)		δ-3-Carene (25.42)
	cis-Isopulegone (2.22)		α-Cedrol (9.05)
	Piperitenone (2.13)		α-Terpinolene (6.76)
P. graveolens	β-Citronellol (35.92)	*V. aguns-castus*	*trans*-Caryophyllene (15.19)
	trans-Geraniol (11.66)		1,8-Cineole (13.04)
	Citronellylformate(11.40)		*trans*-β-Farnesene (8.35)
	Linalool (9.63)		4-Terpineol (7.45)

using the SPSS 12.0 software program (Statistical Package for Social Sciences, USA). The values of EC_{50} were considered to be significantly different, if the 95% confidence limits did not overlap. 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.

RESULTS AND DISCUSSION

1. Chemical composition of the isolated essential oils

The essential oils of fourteen plants grown in north Egypt were obtained by hydrodistillation. The chemical compositions of the isolated oils were identified by GC/MS analysis. The main components of the essential oils are displayed in Table 1. The major constituents of the essential oils were *β*- thujone (49.83%) in *A. judaica*, *α*-pinene (11.47%, 37.88%, 15.12%, 17.26% and 35.49%) in *C. viminals, C. sempervirens, R. officinalis, S. cumini* and *T. occidentalis*, respectively, 1,8-Cineole (71.77%, 19.60%, 13.04% and 4.9%) in *C. viminals, R. officinalis, V. agnus-castus* and *A. judaica*, respectively, capillene (36.86%) in *A. monosperma,* terpinen-4-ol (20.29% and 7.45%) in *C. macrocarpa* and *V. agnus-castus,* pulegone (77.45%) in *O. vulgare, β*-citronellol (35.92% and 13.01%) in *P. graveolens* and *C. macrocarpa,* respectively, and *α*-phellandrene (29.87%) and *β*-phellandrene (21.08%) in *S. molle*. It can be notice that, some major components were found in more than one plant, such as *α*-pinene, *β*-pinene, 1,8-cineole, *β*-citronellol, sabinene and *γ*-terpinene

Table 2. Effect of essential oils on *Silybum marianum* germination 9 d after sowing[a]

Conc	Germination % ± SE				
mg/l	*A. judaica*	*A. monosperma*	*C. viminals*	*C. macrocarpa*	*C. sempervirens*
0	76.7±3.34a[b]	76.7±3.34a	76.7±3.34a	76.7±3.34a	76.7±3.34a
600	40.0±0.00b	73.3±3.34a	66.7±3.34ab	73.3±3.34a	66.7±3.34b
1000	30.0±0.00c	63.3±3.34b	63.3±3.34cb	50.0±5.80b	66.7±3.34b
2000	13.3±3.34d	0.0±0.0c	53.3±3.34cd	6.70±6.70c	40.0±0.0c
3000	0.0±0.0 e	0.0±0.0c	43.3±3.34d	0.0±0.0c	20.0±0.0d
Conc	Germination % ± SE				
mg/l	*O. vulgare*	*P. graveolens*	*P. tortuosus*	*R. officinalis*	*S. cumini*
0	76.7±3.34a	76.7±3.34a	76.7±3.34a	76.7±3.34a	76.7±3.34a
600	56.7±3.34b	10.0±5.80b	76.7±3.34a	56.7±3.34b	66.7±3.34ab
1000	26.7±3.34c	0.0±0.0b	63.3±3.34b	46.7±3.34c	63.3±3.34b
2000	0.0±0.0d	0.0±0.0b	43.3±3.34c	33.3±3.34d	56.7±3.34b
3000	0.0±0.0d	0.0±0.0b	20.0±0.0d	0.0±0.0e	53.3±3.34b
Conc	Germination % ± SE				
mg/l	*S. molle*	*S. terebinthifolius*	*T. occidentalis*	*V. agnus-castus*	
0	76.7±3.34a	76.7±3.34a	76.7±3.34a	76.7±3.34a	
600	66.7±3.34ab	76.7±3.34a	76.7±3.34a	66.7±3.34ab	
1000	63.3±3.34b	70.0±5.80a	70.7±3.34ab	56.7±3.34b	
2000	56.7±3.34b	66.7±3.34a	66.7±3.34b	43.3±3.34c	
3000	53.3±3.34b	63.3±3.34a	55.3±0.00c	33.3±3.34c	

[a] Data are expressed as means ± SE from experiments with three replicates of 20 seeds each.
[b] Means within a column sharing the same letter are not significantly different at the 0.05 probability level.

but others were specific to the plant species. The major constituents of the essential oils mainly belonged to four chemical groups: oxygenated monoterpenes (e.i., *α*-thujone, *β*-thujone, chrysanthenone, terpinen-4-ol, linalool, pulegone, *β*-citronellol and camphor), monoterpene hydrocarbons (e.i., sabinene, *γ*-terpinene, *β*-pinene, *β*-carene, phellandrene and *α*-pinene), sesquiterpene hydrocarbons (e.i., *α*-elemene, *β*-elemene and *trans*-caryophyllene) and oxygenated sesquiterpenes **(**e.i., cedrol and elemol).

The chemical compositions of the isolated essential oils from *C. viminals*, *C. sempervirens*, *S. molle*, *C. macrocarpa*, *P. graveolens*, *R. officinals* are in accordance with those previously reported (Chanegriha *et al.* 1997; Malizia *et al.* 2000; Srivastava *et al.* 2003; Bendaoud *et al.* 2010). On the other hand, the major constituents of the essential oils isolated from *A. monosperma*, *T. occidentalis*, *O. vulgare* and *A. judaica* were completely differed with those previously reported on the chemistry of these oils (Şahin et al. 2004; Mohamed, and Abdelgaleil 2008; Tsiri et al. 2009; Khan et al 2012). Some of the major constituents of the essential oils of *P. tortuosus*, *V. agnus-castus*, *S. terebinthifolius* and *S. cumini* were similar to those previously reported for the oils isolated from plants growing in Egypt and other countries around the world (Gundidza *et al.* 2009; Şahin *et al.* 2004; Stojković *et al.* 2011). However, the percentages of constituents are differed. The chemical composition of essential oils of the same plants may vary widely depending on geographical location, season, environmental conditions and nutritional status of the plants (Perry *et al.* 1999; Hussain *et al.* 2008).

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

The results in Table 2 showed a significant reduction of seed germination of *S. marianum* treated with all the tested essential oils at concentrations of 1000, 2000 and 3000 mg/L except for *S. terebinthifolius* oil. The essential oil *P. graveolens* was the most potent inhibitor of seed germination where a complete inhibition of seed germination was observed at concentrations of 1000, 2000 and 3000 mg/L and germination percent was 10% at concentration 600 mg/L. Three oils, *A. monosperma*, *O. vulgare* and *P. graveolens* caused complete seed germination inhibition at concentration of 2000 mg/L. These three oils and the oils of *A. judaica*, *C. macrocarpa*

and *R. officinals* showed complete seed germination inhibition at concentration of 3000 mg/L. The oil of *S. terebinthifolius* did not display significant germination reduction at all tested concentrations. This result was in agreement with the finding of Barbosa *et al.* (2007) who reported that *S. terebinthifolius* essential oil at a concentration of 10,000 µg/mL did not cause significant inhibition of seed germination of *Lactuca sativa* and *Cucumis sativus* L. However, Pawlowski et al. (2012) found that the essential oils of *S. terebinthifolius* significantly reduced the percentage germination of lettuce by 65.2%. These variations in allelopathic activity could be attributed to differences in oil chemical composition as Barbosa *et al.* (2007) verified that *S. terebinthifolius* essential oil consisted mainly of sesquiterpenes, while in the present work, the oil was found to mainly contain monoterpenes. It has been reported that the oils of *A. judaica*, *R. officinals*, *O. vulgare* and *P. graveolens* possessed inhibitory effect on seed germination of wheat (Dudai *et al.* 1999). In addition, the inhibitory effect of essential oils from other plants on seed germination was previously reported (Singh *et al.* 2005; Barbosa *et al.* 2007; Paudel and Gupta 2008; Kordali *et al.* 2009; Zahed *et al.* 2010).

3. Effect of essential oils on *S. marianum* root growth

The inhibitory effects of the isolated oils on the root growth of *S. marianum* are shown in Table 3. There were great differences between the essential oils inhibitory effects on root growth. The essential oils of *A. judaica*, *A. monosperma*, *C. viminals*, *O. vulgare*, *P. graveolens*, *P. tortuosus* and *S. cumini* caused a significant reduction of root growth at all concentrations with EC_{50} values of 639.3, 698.2, 1086.4, 691.5, 76.1, 723.9 and 2878.5 mg/L, respectively. The most effective essential oil was *P. graveolens*. This oil caused complete inhibition of root growth at 1000 mg/L. The essential oils of *C. macrocarpa*, *C. sempervirens, R. officinalis* and *V. agnus-castus* stimulated the root growth of *S. marianum* at the lowest concentration (600 mg/L) and then caused inhibition of root growth at higher concentrations with EC_{50} values of 1118.0, > 2000, 978.7 and 1662.0 mg/L, respectively. The essential oils of *S. molle* and *S. terebinthifolius* and *T. occidentalis* stimulated the root growth of *S. marianum* at all concentrations except at the highest concentration (3000 mg/L) in which they caused weak root inhibition. Moreover, the oil of *T. occidentalis* stimulated the root growth of *S. marianum* at all concentrations. Among the isolated oils, only four essential oils of *C. sempervirens*, *P. tortuosus*, *S. molle* and *S. terebinthifolius* were reported to possess allelopathic effect (Zahed *et al.* 2010; Krifa *et al.* 2011; Pawlowski *et al.* 2012; Ismail *et al.* 2013). However, the inhibitory effects of essential oils on seedling growth were previously described (Duke *et al.* 2000; Dayan *et al.* 2008; Yang *et al*. 2012; Ribeiro and Lima 2012).

4. Effect essential oils on *S. marianum* shoot growth

The results in Table 4 showed that the tested oils revealed a significant shoot reduction at all of the tested concentrations compared with control except the oil of *C. macrocarpa* at the lowest concentration (600 mg/L). Essential oils of *P. graveolens* (EC_{50} = 459.7 mg/L), *A. judaica* (EC_{50} = 746.0 mg/L), *O. vulgare* (EC_{50} = 792.6 mg/L), *A. monosperma* (EC_{50} = 926.0 mg/L) and *P. tortuosus* (EC_{50} = 925.5 mg/L) exhibited the highest shoot reduction with *P. graveolens* oil being the most effective one. Essential oils of *C. macrocarpa*, *C. sempervirens, R. officinalis, S. terebinthifolius* and *V. agnus-castus* showed a moderate reduction of shoot growth with EC_{50} values ranged between 1000 and 3000 mg/L. Finally the essential oils of *C. viminals, S. cumini, S. molle* and *T. occidentalis* showed a weak inhibition of shoot growth with EC_{50} values more than 3000 mg/L. It is noteworthy to mention that the oil of *S. molle* changed leaf colour of *S. marianum* to brown. The leaf brownness was directly proportional with oil concentration.

The allelopathic effect of the tested essential oils is in agreement with previous studies demonstrating the phytotoxicity of essential oils (Duke *et al.* 2000; Angelini *et al.* 2003; Scrivanti *et al.,* 2003; Singh *et al.* 2009; Mutlu *et al.* 2010). On the other hand, the results revealed a correlation between the chemical composition of the essential oils and their effects on germination and seedling growth. Almost all the effective oils had high percentage of oxygenated monoterpenes and this was in agreement with previous work of De Almeida *et al.* (2010) and Vokou *et al.* (2003). They stated that the potent phytotoxic activity was linked to the present of high percentage of oxygenated monoterpenes. Similarly, Scrivanti *et al.* (2003) and Lopez *et al.* (2009) demonstrated that the essential oils with high percentages of oxygenated compounds were more active than essential oils with high percentages of hydrocarbon compounds.

Although the mode of action of the essential oils was not investigated in the present study, it has been reported that essential oils and their constituents inhibited cell division in growing root tips and interfered with DNA synthesis in growing meristems (Romagni *et al.* 2000; Nishida *et al.* 2005). Moreover, the essential oils inhibited plant growth through disruption of membrane integrity (Tworkoski 2002; Singh *et al.* 2009). Other studies documented that essential oils and their constituents induced oxidative stress, inhibited root growth, enhanced lipid peroxidation and hydrogen peroxide accumulation, and increased electrolyte leakage in root tissue (Scrivanti *et al.* 2003; Singh *et al.* 2006). The tested essential oils may cause

Table 3. Effect of essential oils on *Silybum marianum* root growth 9 d after sowing[a]

Conc	*A. judaica*		*A. monosperma*		*C. viminals*		*C. macrocarpa*	
mg/l	Root length (cm)	I (%)[b]	Root length (cm)	I (%)	Root length (cm)	I (%)	Root length (cm)	I (%)
0	8.1±0.18a[c]	0.0	8.1±0.18a	0.0	8.1±0.18a	0.0	8.1±0.18a	0.0
600	4.7±0.15b	42	4.0±0.12b	50.6	4.8±0.15b	40.7	8.5±0.15a	-4.9
1000	2.4±0.15c	70.3	3.6±0.15c	55.6	4.0±0.12c	50.6	5±0.12b	38.3
2000	0.8±0.07d	90.1	0.0±0.0d	100	3.5±0.15d	56.8	0.5±0.5c	93.8
3000	0.0±0.0e	100	0.0±0.0d	100	2.8±0.15e	65.4	0.0±0.0c	100
EC_{50}[d]	639.3		698.2		1086.4		1118.0	
Conc	*C. sempervirens*		*O. vulgare*		*P. graveolens*		*P. tortuosus*	
mg/l	Root length (cm)	I (%)	Root length (cm)	I (%)	Root length (cm)	I (%)	Root length (cm)	I (%)
0	8.1±0.18a	0.0	8.1±0.18a	0.0	8.1±0.18a	0.0	8.1±0.18a	0.0
600	9.8±0.12a	-21	4.9±0.07b	39.5	0.5±0.5b	93.8	4.1±0.21b	49.4
1000	9.5±0.15a	-17.3	1.9±0.09c	76.5	0.0±0.0b	100	3.8±0.12b	53.1
2000	4.5±0.25b	44.4	0.0±0.0d	100	0.0±0.0b	100	2.7±0.18c	66.7
3000	2.2±0.1c	72.8	0.0±0.0d	100	0.0±0.0b	100	1.5±0.15d	81.5
EC_{50}	-		691.5		76.1		723.9	
Conc	*R. officinalis*		*S. cumini*		*S. molle*		*S. terebinthifolius*	
mg/l	Root length (cm)	I (%)	Root length (cm)	I (%)	Root length (cm)	I (%)	Root length (cm)	I (%)
0	8.1±0.18a	0.0	8.1±0.18a	0.0	8.1±0.18	0.0	8.1±0.18	0.0
600	10.1±0.09a	-24.7	7.0±0.12b	13.6	9.5±0.29	-17.3	11.4±0.23	-40.7
1000	3.8±0.15b	53.1	6.0±0.15c	25.9	10.9±0.13	-34.6	9.2±0.17	-13.6
2000	1.1±0.06c	86.4	5.0±0.15d	38.3	9.7±0.36	-19.8	8.8±0.06	-8.6
3000	0.0±0.0d	100	3.9±0.13e	51.9	7.5±0.2	7.4	7.8±0.12	3.7
EC_{50}	978.7		2878.5		-		-	
Conc	*T. occidentalis*		*V. agnus-castus*					
mg/l	Root length (cm)	I (%)	Root length (cm)	I (%)				
0	8.1±0.18	0.0	8.1±0.18a	0.0				
600	8.8±0.12	-8.6	8.4±0.23a	-8.6				
1000	9.2±0.15	-13.6	5.6±0.09b	30.7				
2000	9.8±0.12	-21	3.6±0.20c	55.6				
3000	10.2±0.19	-25.9	2.1±0.09d	74.1				
EC_{50}	-		1662.0					

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

[b] I = inhibition.

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

[d] EC_{50} = concentration of compound causing 50% root growth inhibition.

Table 4. Effect of essential oils on *Silybum marianum* shoot growth 9 d after sowing[a]

Conc	*A. judaica*		*A. monosperma*		*C. viminals*		*C. macrocarpa*	
mg/l	Root length (cm)	I (%)[b]	Root length (cm)	I (%)	Root length (cm)	I (%)	Root length (cm)	I (%)
0	2.6±0.1a[c]	0.0	2.6±0.1a	0.0	2.6±0.1a	0.0	2.6±0.1a	0.0
600	1.6±0.06b	38.5	1.9±0.06b	26.9	2.1±0.07b	19.3	2.5±0.15a	3.9
1000	0.8±0.03c	69.2	1.6±0.07c	38.5	1.8±0.06c	30.8	1.6±0.1b	38.5
2000	0.5±0.0d	80.8	0.0±0.0d	100	1.6±0.03cd	38.5	0.3±0.3c	88.5
3000	0.0±0.0e	100	0.0±0.0d	100	1.4±0.07d	46.2	0.0±0.0c	100
EC_{50}[d]	746.0		926.0		> 3000		1162.9	
Conc	*C. sempervirens*		*O. vulgare*		*P. graveolens*		*P. tortuosus*	
mg/l	Root length (cm)	I (%)	Root length (cm)	I (%)	Root length (cm)	I (%)	Root length (cm)	I (%)
0	2.6±0.1a	0.0	2.6±0.1a	0.0	2.6±0.1a	0.0	2.6±0.1a	0.0
600	2.1±0.12b	19.3	1.7±0.07b	34.6	0.6±0.35b	76.9	1.3±0.03b	50
1000	1.8±0.06c	30.8	1.1±0.03c	57.7	0.0±0.0b	100	1.2±0.03b	53.5
2000	1.5±0.03c	42.3	0.0±0.0d	100	0.0±0.0b	100	0.8±0.0c	69.2
3000	1.0±0.12d	61.5	0.0±0.0d	100	0.0±0.0b	100	0.5±0.03d	80.8
EC_{50}	2210.7		792.6		459.7		925.5	
Conc	*R. officinalis*		*S. cumini*		*S. molle*		*S. terebinthifolius*	
mg/l	Root length (cm)	I (%)	Root length (cm)	I (%)	Root length (cm)	I (%)	Root length (cm)	I (%)
0	2.6±0.1a	0.0	2.6±0.1a	0.0	2.6±0.1a	0.0	2.6±0.1a	0.0
600	2.2±0.15b	15.4	2.3±0.15b	11.5	2.2±0.12b	15.4	2.3±0.12b	11.5
1000	1.4±0.03c	46.2	2.1±0.06b	19.3	2.1±0.1b	19.3	1.7±0.07c	34.6
2000	1.0±0.03d	61.5	2.0±0.07b	23.1	2.0±0.09b	23.1	1.5±0.06cd	42.3
3000	0.0±0.0e	100	1.9±0.06b	26.9	1.6±0.07c	38.5	1.3±0.07d	50.0
EC_{50}	1194.6		> 3000		> 3000		2697.8	
Conc	*T. occidentalis*		*V. agnus-castus*					
mg/l	Root length (cm)	I (%)	Root length (cm)	I (%)				
0	2.6±0.1a	0.0	2.6±0.1a	0.0				
600	1.7±0.09b	34.6	2.2±0.03b	15.4				
1000	1.7±0.03b	34.6	1.8±0.06c	30.8				
2000	1.6±0.06cb	38.5	1.4±0.06d	46.2				
3000	1.4±0.06c	46.2	1.1±0.03e	57.7				
EC_{50}	> 3000		2245.3					

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

[b] I = inhibition.

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

[d] EC_{50} = concentration of compound causing 50% root growth inhibition.

their phytotoxic effects via one or more of these modes of action.

From the present study, it could be concluded that *Artemisia judaica*, *A. monosperma*, *C. viminals*, *O. vulgare*, *P. graveolens* and *P. tortuosus* essential oils possessed strong phytotoxicity against *S. marianum*. These oils could be useful for management of *S. marianum*. However, more studies are required to evaluate the herbicidal potential of these essential oils under field and greenhouse conditions and determine the effects on non-target species and safety.

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