Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2018; 10(4); 123-131
doi: 10.25258/phyto.10.4.1
ISSN: 0975-4873

Research Article

Phytotoxicity of Essential Oils from Culinary Herbs against Seed Germination and Seedling Growth of Selected Weeds

M Dolores Ibáñez, M Amparo Blázquez*

Departament de Farmacologia, Facultat de Farmàcia, Universitat de València, Avda. Vicent Andrés Estellés s/n 46100 Burjasot, Valencia, Spain.

Received: 15th Nov 17; Revised 9th Feb, 18, Accepted: 11th Mar, 18; Available Online:25th Apr, 18

ABSTRACT

Chemical composition of *Rosmarinus officinalis* L. and *Ocimum basilicum* L. ssp. *basilicum* essential oils as well as their phytotoxic effects against seed germination and seedling growth of *Portulaca oleracea, Lolium multiflorum* and *Echinochloa crus-galli* has been investigated. Seventy-eight compounds accounting between 98.10-99.15% of the total commercial oils were identified by GC/MS analysis. The oxygenated monoterpenes 1,8-cineole (24.95±0.11%) and camphor (20.45±0.05%) were the main compounds of rosemary essential oil, whereas large amounts of the aromatic compound methyl chavicol (79.09±0.29%) was found in basil essential oil. Rosemary essential oil significantly inhibited the seed germination of *L. multiflorum* and *E. crus-galli* and the seedling growth of the three weed, whereas basil essential oil only showed significant effects in hypocotyl and/or radicle length depending of the weed and dose.

Keywords: rosemary, basil, essential oils, GC-MS, weed control.

INTRODUCTION

In agriculture, the visible consequences of climate change such as the Earth's rising temperature, extreme weather events, shifting seasons as well as scarcity in precipitations[1] have led to the proliferation and propagation of weeds, pests and diseases that lessen crop yields and therefore increase production, collection and industrial processing costs[2]. Food and Agriculture Organization (FAO) especially warns about the increasing presence of invasive weeds, which interfere and compete against cultivated plants for light, water and nutrients[3,4]. Simultaneously, they are indirect transmitters of pests as they are hosts of viruses and insect vectors that help them propagating. According to FAO member expert, 'weeds are the principal enemy of farmers' due to the fact that they cause a higher number of losses in crop production than any other disaster. These damages reach numbers of 95000 million dollars in global food production, representing 380 million tons of wheat[5]. Despite the most affected countries are the developing ones, weeds also disturb developed countries invading pathways, gardens, historical monuments as well as causing allergies, fires, etc. So, one of the most important challenges nowadays is to fight against invasive exotic species, which constitute a growing threat for native plants[6].

In this sense, *Echinochloa* genus, especially barnyard grass (*Echinochloa crus-galli* (L.) Beauv) mimics rice plants, producing inevitable crop yield losses once *E. crus-galli* can be easily recognized[7]. Another weed species to take into consideration is the well-known common purslane (*Portulaca oleracea* L.), annual weed which although is edible and consumed in several countries, it is also considered a spontaneous weed of orchards, pathways and gardens in many others[8]. Purslane infests a wide variety of crops, such as sweet maize, tomato, sunflower, rice and cotton among others due to its opportunistic properties[3,9]. Manual weeding as innocuous and unique method to eliminate them results non-viable, leading to the increased use of synthetic herbicides with problems of toxicity as well as the appearance of long-term resistance.

Regarding resistance, Italian ryegrass (*Lolium multiflorum* Lam.) has shown 10-fold levels of resistance to glyphosate, the world's most widely used herbicide since 1974[10,11], in comparison to a susceptible population due to its constant application after many years[12,13]. *L. multiflorum* resistance to glyphosate has been recorded in orchards spread through the American continent, Spain and more recently in Japan[10,14]. Although the mechanism of developing resistance is not completely known, it has been demonstrated that a proline 106 to serine amino acid substitution of EPSP synthase decreases glyphosate binding and confers moderate levels of glyphosate resistance[13]. Furthermore, additional concerns related to synthetic agro-chemicals are the potential biodiversity damages[15], especially against human health: it causes skin and mucosa irritation, head and stomach ache, vomiting, unconsciousness, etc., through the intoxication, ingestion of contaminated food, inhalation and direct contact[16]. World Health Organization (WHO) warns about certain synthetic herbicides because can cause human cancer. In this sense, glyphosate produces cancer in lab animals as well as chromosome and human DNA damage, being consequently classified as 'probable carcinogenic' (Group 2A) substance[17].

**Author for Correspondence: amparo.blazquez@uv.es*

Therefore, it is necessary to reduce the dependence on synthetic weed killers and give priority to other natural compounds that neither damage both environment and living organisms beings nor promote resistance appearance[18]. Between these natural alternatives, essential oils are being employed to control crop pests[19,20] apart from their very well-known anti-inflammatory, anticancer, antiviral, repellent, antibacterial, antifungal or antioxidant activities and wide employment in perfumery, cosmetics,

Table 1: Chemical composition of rosemary and basil essential oils.

RI	Compound	Rosemary	Basil
	Monoterpene hydrocarbons	**38.27±0.13**	**0.25±0.02**
926	Tricyclene	0.31±0.00	-
939	α-Pinene	16.70±0.12	0.04±0.01
944	β-Fenchene	0.13±0.01	-
955	Camphene	9.94±0.04	-
976	Sabinene	-	0.01±0.00
979	β-Pinene	6.14±0.01	0.03±0.00
992	Myrcene	1.27±0.01	0.03±0.01
1005	α-Phellandrene	0.11±0.00	-
1012	δ-3-Carene	0.05±0.00	-
1020	α-Terpinene	0.12±0.00	-
1027	*p*-Cymene	3.12±0.03	0.01±0.00
1031	Limonene	-	0.05±0.01
1043	*cis*-Ocimene	0.03±0.01	-
1052	*trans*-Ocimene	-	0.08±0.01
1063	γ-Terpinene	0.19±0.00	-
1090	Terpinolene	0.17±0.01	-
	Oxygenated monoterpenes	**57.70±0.09**	**16.20±0.36**
1033	1,8-Cineole	24.95±0.11	0.17±0.01
1070	*cis*-Sabinene hydrate	0.03±0.00	-
1075	*cis*-Linalool oxide	-	0.15±0.01
1089	*trans*-Linalool oxide	-	0.13±0.01
1098	*trans*-Sabinene hydrate	0.06±0.01	-
1101	Linalool	1.13±0.02	14.58±0.26
1116	α-Fenchol	0.03±0.01	-
1123	β-Fenchol	0.02±0.01	-
1142	1-Terpineol	0.07±0.02	-
1149	Camphor	20.45±0.05	-
1155	Menthone	-	0.05±0.01
1160	Isoborneol	1.33±0.01	-
1164	*iso*-Menthone	-	0.01±0.00
1165	*neo*-Menthol	-	0.01±0.00
1169	Borneol	3.02±0.04	-
1175	Menthol	-	0.37±0.02
1179	Terpinen-4-ol	0.20±0.15	-
1183	*p*-Cymen-8-ol	0.02±0.01	-
1190	α-Terpineol	2.50±0.18	-
1197	γ-Terpineol	0.33±0.01	-
1222	α-Fenchyl acetate	0.02±0.01	-
1244	Neral	-	0.20±0.14
1258	Geraniol	-	0.04±0.01
1260	Linalool acetate	0.32±0.00	-
1273	Geranial	-	0.46±0.02
1288	Bornyl acetate	3.23±0.03	-
1294	Menthyl acetate	-	0.02±0.01
1382	Geranyl acetate	-	0.01±0.00
	Sesquiterpene hydrocarbons	**3.08±0.03**	**1.62±0.17**
1351	α-Cubebene	0.04±0.00	-
1376	α-Ylangene	0.02±0.00	-
1376	α-Copaene	0.10±0.01	0.03±0.01
1388	β-Bourbonene	-	0.01±0.00
1403	Longifoleno	0.07±0.00	-

1415	*cis*-α-Bergamotene	-	0.01±0.00
1419	β-Caryophyllene	2.31±0.02	0.34±0.04
1430	β-Copaene	0.02±0.00	-
1437	*trans*-α-Bergamotene	-	0.56±0.06
1439	Aromadendrene	0.01±0.00	-
1444	*cis*-β-Farnesene	-	0.05±0.01
1454	α-Humulene	0.27±0.00	0.15±0.02
1459	*trans*-β-Farnesene	-	0.19±0.02
1481	γ-Muurolene	0.05±0.01	0.17±0.02
1500	α-Muurolene	0.02±0.00	-
1508	β-Bisabolene	0.01±0.01	0.07±0.01
1514	γ-Cadinene	0.03±0.00	-
1524	δ-Cadinene	0.12±0.00	0.03±0.01
	Oxigenated sesquiterpenes	**0.06±0.00**	**0.20±0.02**
1577	Spathunelol	-	0.04±0.00
1582	Caryophyllene oxide	0.06±0.00	0.10±0.01
1607	Humulene epoxide	-	0.03±0.01
1684	α-Bisabolol	-	0.02±0.00
	Aromatic compounds	**0.03±0.01**	**79.69±0.22**
1209	Methyl Chavicol	-	79.07±0.29
1255	*p*-Anis aldehyde	-	0.03±0.01
1256	Chavicol	-	0.02±0.01
1286	*trans*-Anethole	-	0.01±0.00
1359	Eugenol	0.01±0.01	0.03±0.01
1405	Methyl Eugenol	0.02±0.00	0.02±0.01
1567	*p*-Methoxy Cinnamaldehyde	-	0.51±0.06
	Others	**-**	**0.14±0.02**
853	3-Hexen-1-ol	-	0.01±0.01
971	3,7-Dimethyl-2-octene	-	0.05±0.01
986	6-Methyl-5-hepten-2-one	-	0.07±0.00
1003	Octanal	-	0.03±0.01
1009	Hexenyl acetate	-	0.01±0.00
	Total identified	**99.15±0.01**	**98.10±0.27**

RI, retention index relative to C_8-C_{32} *n*-alkane on HP-5MS column; values are means ± standard deviation of three samples, *only detected in a sample

pharmaceutical and food industry[21-23].

Among Lamiaceae family[24-26], rosemary (*Rosmarinus officinalis* L.) is a quite famous aromatic plant due to its numerous health effects such as digestive, anti-inflammatory, anti-nociceptive, diuretic, antihepatotoxic, antispasmodic or neuroprotective against Alzheimer and Parkinson diseases[27]. Its essential oil has been tested against *Sporothrix brasiliensis* and S. schenckii isolated from humans, cats, dogs and environmental soils, being a promising product for treatment of sporotrichosis in refractory cases to itroconazole[28]. In addition, it has been combined with thyme essential oil for the control of *Listeria monocytogenes* in mortadella packaging[29] as well as part of the active packaging of refrigerated beet meat for its preservation prolonging the shelf-life until day 15[30], being also used as potential bio-fumigant in control of *Callosobruchus maculatus* (F.) in chickpea seeds without affecting the food product[31]. According to its phytotoxic potential, rosemary essential oil supressed germination rate of *Avena sterilis* and *Sinapis arvenis*, weed species commonly found in wheat growing areas, affecting in a lower extent wheat cultivars[32].

Another interesting culinary spice belonging also to the Lamiaceae family is basil (*Ocimum* spp.), popularly used as food additive to prevent microbial arise[33]. *Ocimum tenuiflorum* extracts produced a reduction in seedling growth of Italian ryegrass (*L. multiflorum*), barnyard grass (*E. crus-galli*) between other weeds[34]; similarly, *Ocimum basilicum* L. ssp. *basilicum* essential oil is highly phytotoxic against ferns, gingers and delicate flowers when used as insecticide against *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) causing leaves losses of more than 50%[35].

So, the aims of this work was analyze by Gas Chromatography-Mass Spectrometry the chemical composition of rosemary (*Rosmarinus officinalis* L.) and basil (*Ocimum basilicum* L. ssp. *basilicum*) essential oils, two widely culinary spices in order to determine through the seed germination and seedling growth of *P. oleracea*, *L. multiflorum* and *E. crus-galli* the potential bioherbicide effects of its species mainly used in several dishes of the Mediterranean diet.

MATERIAL AND METHODS

Plant material

Commercial samples of rosemary (*Rosmarinus officinalis* L.) (Batch 0037337) essential oil purchased from Guinama (Valencia, Spain), and *Ocimum basilicum* L. ssp.

Table 2: *In vitro* effects of rosemary and basil essential oils against *P. oleracea*, *L. multiflorum* and *E. crus galli* seed germination.

Concentration (µl/ml)	*P. oleracea*	
	Rosemary	Basil
Control	83.00±5.83 a	85.00±5.24 a
0.125	78.00±5.83 a	77.00±2.55 a
0.25	76.00±4.30 a	81.00±3.67 a
0.5	74.00±4.58 a	86.00±4.85 a
1	73.00±2.00 a	77.00±4.36 a
Concentration (µl/ml)	*L. multiflorum*	
	Rosemary	Basil
Control	73.00±3.39 a	73.00±3.39 a
0.125	68.00±5.83 a,b	73.00±3.39 a
0.25	69.00±3.32 a,b	71.00±3.32 a
0.5	64.00±4.30 a,b	63.00±4.90 a
1	53.00±3.39 b	62.00±5.61 a
Concentration (µl/ml)	*E. crus-galli*	
	Rosemary	Basil
Control	86.00±6.00 a	86.00±6.00 a
0.125	22.00±7.35 b	83.00±2.56 a
0.25	18.00±9.17 b	83.00±3.39 a
0.5	13.00±8.31 b	83.00±2.55 a
1	1.00±1.00 b	78.00±1.23 a

Values are mean of five replications ± error deviation after 14 days of incubation. Means followed by different letters in the same column indicate that are significantly different at $p < 0.05$ according to T3 Dunnet and Tukey tests.

basilicum essential oil (Batch 0F22144) supplied by Pranaròm International, were stored at 4 ºC until chemical analysis and phytotoxic studies.

Weeds

Mature seeds of annual weeds of *Portulaca oleracea* L., *Lolium multiflorum* Lam. and *Echinochloa crus-galli* (L.) Beauv., were purchased from Herbiseed (website: www.herbiseed.com).

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis was carried out with a 5973N Agilent apparatus, equipped with a capillary column (95 dimethylpolysiloxane- 5 % diphenyl), Agilent HP-5MS UI (30 m long and 0.25 mm i.d. with 0.25 µm film thickness). The column temperature program was 60 ºC during 5 min, with 3 ºC/min increases to 180 ºC, then 20 ºC/min increases to 280 ºC, which was maintained for 10 min. The carrier gas was Helium at a flow-rate of 1 mL/min. Split mode injection (ratio 1:30) was employed. Mass spectra were taken over the m/z 30-500 range with an ionizing voltage of 70 eV.

Identification

The individual compounds were identified by MS and their identity was confirmed by comparison of their Kovat's retention index calculated using standard hydrocarbons relative to C8-C32 n-alkanes, and mass spectra with reference samples or with data already available in the NIST 2005 mass spectral library and in the literature[36].

Herbicidal activity

Sets of 20 seeds each with five replicates per treatment were homogenously distributed in Petri dishes (9 cm diameter) between two layers of filter paper (Whatman No.1) moistened with 4 mL of distilled water and with 0 (control), 0.125, 0.250, 0.5, and 1µl/ml of rosemary and basil essential oils. Petri dishes were sealed with parafilm and incubated in a germination chamber Equitec EGCS 301 3SHR model, according to previous assays[37] alternating 30.0 ± 0.1ºC 16 h in light and 20.0 ± 0.1ºC 8 h in dark and with (*E. crus-galli*) and without (*P. oleracea, L. multiflorum*) humidity.

To evaluate the herbicidal activity of the essential oils, the number of germinated seeds was counted and compared with those of untreated seedlings. Emergence of the radicle (≥1 mm) was used as an index of germination and seedling length (hypocotyl and/or radicle) data were recorder after 3, 5, 7, 10 and 14 days in each replicate.

Statistical analysis

Experiments were made with five replicates. Resulting data were subjected to one-way analysis of variance with SPSS statistics 22 software. Tukey's post hoc test was used when variances remained homogeneous (Levene's test) and T3 Dunnett's post hoc one was employed if not, assuming equal variances. Differences were considered to be significant at $p \leq 0.05$.

RESULTS

Essential oil composition

Seventy-eight compounds accounting between 98.10-99.15% of the total commercial rosemary and basil essential oils were identified by GC/MS analysis. Components are clustered (Table 1) in homologous series of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, aromatic compounds and others and listed according to Kovat's retention index calculated in GC on apolar HP-5MS column.

Seventeen oxygenated monoterpenes (57.70±0.09%), 13 monoterpene hydrocarbons (38.27±0.13%) 13

Tabla 3: Effects of rosemary and basil essential oils on seedling length (hypocotyl and radicle) of *P. oleracea*, *L. multiflorum* and *E. crus-galli*.

Concentration (µl/ml)	*P. oleracea* Rosemary		Basil	
	Hypocotyl	Radicle	Hypocotyl	Radicle
Control	9.60±1.03 a	11.60±1.69 a	11.60±1.69 a	11.60±1.69 a
0.125	6.60±0.68 b	6.60±0.68 b	7.60±0.60 b	14.40±1.97 a
0.25	6.60±0.25 b	6.60±0.25 b	7.80±0.86 b	12.40±0.68 a
0.5	6.20±0.66 b	4.80±0.37 b	7.40±0.40 b	12.60±1.36 a
1	4.80±0.80 b	4.20±0.20 b	6.00±0.32 b	11.20±0.86 a
Concentration (µl/ml)	*L. multiflorum* Rosemary		Basil	
	Hypocotyl	Radicle	Hypocotyl	Radicle
Control	48.50±3.35 a	39.20±2.14 a	48.50±3.35 a	39.20±2.14 a
0.125	34.99±2.18 b	30.04±1.75 a,b	40.90±1.10 a,b	31.36±0.78 b
0.25	29.14±1.43 b,c	31.95±2.44 a,b	35.36±1.42 b	35.37±1.88 a,b
0.5	23.19±0.54 c,d	27.01±4.33 b	35.21±1.80 b	24.98±1.82 c
1	19.90±1.61 d	22.18±0.94 b	25.01±2.04 c	20.85±1.57 c
Concentration (µl/ml)	*E. crus-galli* Rosemary		Basil	
	Hypocotyl	Radicle	Hypocotyl	Radicle
Control	23.66±3.80 a	20.78±1.46 a	23.66±3.80 a	20.78±1.46 a
0.125	18.60±3.53 a	4.00±1.64 b	12.32±0.67 b	12.78±0.31 b
0.25	7.00±1.14 b	4.00±2.45 b	8.72±0.31 b	12.92±0.38 b
0.5	6.40±0.40 b	2.00±2.00 b	6.32±0.86 b	8.58±0.28 c
1	2.00±0.00 b	0.00±0.00 b	5.68±0.45 b	5.60±0.41 d

Values are mean of five replications ± error deviation after 14 days of incubation. Means followed by different letters in the same column indicate that are significantly different at $p<0.05$ according to T3 Dunnet and Tukey tests.

sesquiterpene hydrocarbons (3.08±0.03%), one oxygenated sesquiterpene (0.06%) and two aromatic components (0.03±0.01%) were the compounds identified in rosemary essential oil. Monoterpene compounds with the oxygenated monoterpenes 1,8-cineole (24.95±0.11%) and camphor (20.45±0.05%) followed by the monoterpene hydrocarbons α-pinene (16.70±0.12%), camphene (9.94±0.04%) and β-pinene (6.14±0.01%) were the principal components. Between the sesquiterpene fraction β-caryophyllene with 2.31±0.02% was the main compound, being caryophyllene oxide (0.06%) the only oxygenated sesquiterpene identified. The phenylpropanoids, eugenol and methyl eugenol, were detected in low amount among the compounds biosynthesized by the shikimic biogenetic pathway.

Aversely, aromatic fraction (79.69±022%) with seven identified compounds, was the main phytochemical group of basil essential oil, followed by the oxygenated monoterpenes (16.20±0.36%) and sesquiterpene hydrocarbons (1.62±0.0.17%) with 13 and 11 compounds, respectively. The phenylpropanoid methyl chavicol was the most abundant compound in basil essential oil (79.07±0.29%), followed by the oxygenated monoterpene linalool (14.58±0.26%). No higher percentages than 0.1% were found between the seven monoterpene hydrocarbons and only β-caryophyllene (0.34%), α-*trans*-bergamotene (0.56%), α-humulene (0,15%), β-*trans*-farnesene (0.19%), γ-muurolene (0.17%) and *p*-methoxy-cinnamaldehyde (0.51%) reached percentages higher than 0.1% in the sesquiterpene hydrocarbons and aromatic fractions respectively.

Seed germination and seedling growth inhibition against P. oleracea, L. multiflorum and E. crus-galli

The effect of rosemary and basil essential oils against seed germination and seedling growth of *P. oleracea*, *L. multiflorum* and *E. crus-galli* is shown in Tables 2-3 and Figures 1, 2 and 3, respectively. Despite *R. officinalis* essential oil did not exert significant weed killer capacity against *P. oleracea* germination at none of the tested doses, there was a significant difference between those dishes containing the highest dose (1 µl/ml) of rosemary essential oil and control ones with *L. multiflorum* (Table 2); and remarkable significant differences between the seed germination of *E. crus-galli* control plates and all doses assayed, meaning rosemary essential oil was obtained (Table 2).

On the other hand, no significant effect in the seed germination of the three weeds resulted after basil essential oil exposure at all the doses applied (Table 2).

Regarding seedling growth, rosemary essential oil showed at all doses assayed significant inhibitory effect with respect to control in both hypocotyl and radicle of *P. oleracea* and at the doses of 0.25-1 µl/ml and all dose of hypocotyl and radicle respectively of *E. crus-galli* (Table 3, Figures 1a and 2a), showing also significant differences in a dose-dependent manner in hypocotyl and radicle of *L. multiflorum* (Table 3, Figure 3a).

Basil essential oil showed only significant differences between control and treated hypocotyl length, showing no

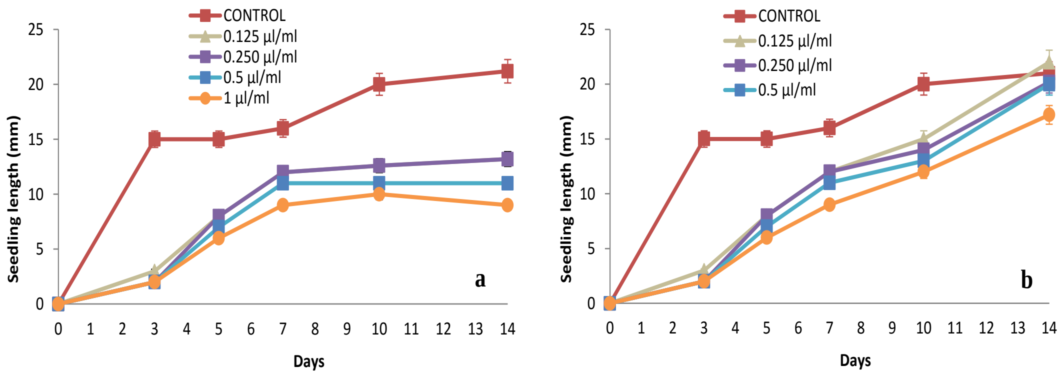

Figure 1: Values of seedling length (mm) (mean ± s.e.) of *P. oleracea* control and treated with rosemary (a) and basil (b) essential oils at 0.125, 0.25, 0.5 and 1 µl/ml measured over 14 days.

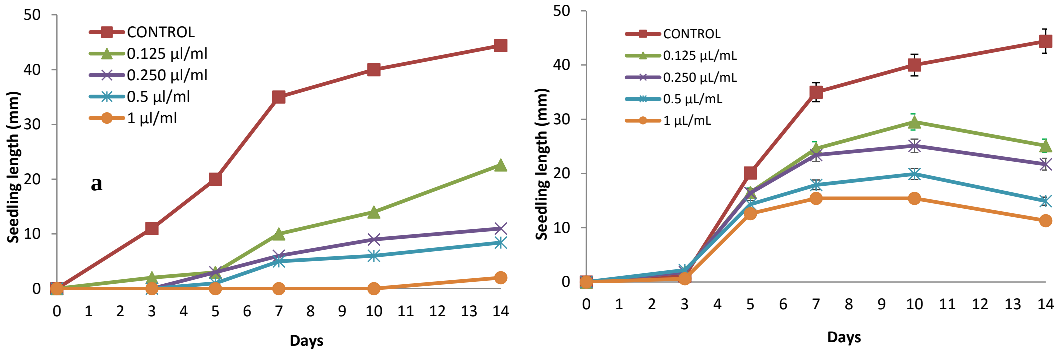

Figure 2: Values of seedling length (mm) (mean ± s.e.) of *E. crus-galli* control and treated with rosemary (a) and basil (b) essential oils at 0.125, 0.25, 0.5 and 1 µL/mL measured over 14 days.

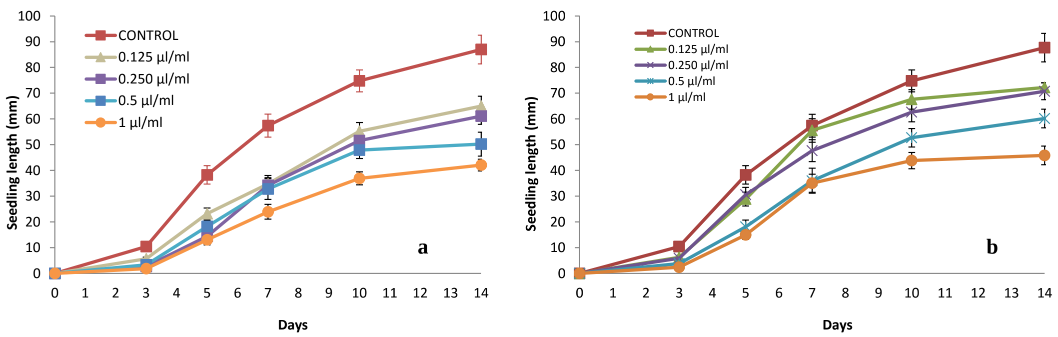

Figure 3: Values of seedling length (mm) (mean ± s.e.) of *L. multiflorum* control and treated with rosemary (a) and basil (b) essential oils at 0.125, 0.25, 0.5 and 1 µl/ml measured over 14 days.

significant effects, even a slight stimulating effect on radicle elongation against *P. oleracea* (Table 3, Figure 1b). Significant differences between hypocotyl and radicle control and all doses applied were found against *L. multiflorum* (Table 3, Figure 3b), being this essential oil able to inhibit significantly at all doses assayed both hypocotyl and radicle elongation of *E. crus-galli* (Table 3, Figure 2b).

DISCUSSION

The chemical composition of commercial rosemary and basil essential oil has been analyzed in order to determine their phytotoxic effect against food weeds.

In relation to rosemary essential oil, the commercial sample here analysed is comparable to rosemary growing in Tunisia[38], rich in the oxygenated monoterpenes 1,8-cineole (24.95±0.11%) and camphor (20.45±0.05%), but like in other aromatic plants, the geographic location affects significantly their chemical composition; for instance, α-pinene (16.70±0.12%), which is the third major compound in our commercial sample (Table 1), is the main component with 40.55-45.10% of Brazilian rosemary essential oil[39], affecting the pharmacological activity of the essential oil employed: rosemary essential oil rich in 1,8-cineole has shown promising antibacterial properties against *Staphylococcus aureus*[40] as well as against other multi-drug-resistant microorganisms[41], while α-pinene, common in pines and cedar, has been recently studied for its pharmacological effects on central nervous system activity[42] as well as for its human physiological relaxation[43]. More specific researchers have reported numerous activities of rosemary essential oil against many agricultural pest of worldwide importance, like the acaricidal effect against twospotted spider mite on greenhouse tomato[44] at not phytotoxic concentrations to the host plant. However, rosemary essential oil is also able to decrease the germination percentage, shoot lengths of prickly lettuce and radish[45]. Our results demonstrated that rosemary essential oil has a selective herbicidal effect because seed germination of *P. oleracea* was not inhibited, whereas *L. multiflorum* seed germination only was significantly reduced at the higher dose applied (1 µl/ml) and *E. crus-galli* was the most sensible weed to rosemary essential oil with significant inhibitory effects at all doses (0.125, 0.25, 0.50 and 1 µl/ml) tested (Table 2).

The second selected essential oil to test food weed control was basil essential oil (*Ocimum basilicum* L. ssp. *basilicum*) with high content in the phenylpropanoid methyl chavicol with 79.07±0.29%, followed by linalool (14.58±0.26%). According to the main components in *Ocimum basilicum* essential oil, there exist several chemotypes in which methyl chavicol-rich and linalool-rich are included[46]. Depending on the cultivar of *O. basilicum*, methyl chavicol and linalool appear in higher or lower amounts, and in this sense, in *O. basilicum* var. *purpureum*, methyl chavicol represents 57.30% and linalool, 18.00%; whereas in *O. basilicum* var. *thyrsiflora* methyl chavicol achieves 20.00% and linalool, 68.00%[47]. In previous studies, *O. basilicum* essential oil has been able to display herbicidal effect against *Solanum lycopersicum* root and hypocoyl length, with 85% and 78.8% inhibition, respectively, so it could be used as post-emergence treatment[48]. In our study no significant seed germination inhibition has been found, showing basil essential oil significant inhibitory effect at all the doses assayed on *P. oleracea* hypocotyl, and in both hypocotyl and radicle elongation of *L. multiflorum* and *E. crus-galli*,, with a percentage of inhibition of 48.4% and 46.8% for *L. multiflorum* (hypocotyl and radicle, respectively) and 76.03% and 73.10%, against *E. crus-galli*, that corroborate, a post-emergence treatment.

CONCLUSION

The phytotoxic activity of rosemary was correlated with the high content of oxygenated monoterpenes. Rosemary essential oil showed a selective effect against *E. crus-galli* seed germination. Significant inhibitory effects on seedling length obtained with both rosemary and basil essential oils against the tree food weeds could be employed as a post-emergence treatment. Further studies *in vivo* conditions are needed to determine no phytotoxic effects on crops.

ACKNOWLEDGMENTS

The authors thank the Central Service for Experimental Research of the University of Valencia (SCSIE) for providing the Gas Chromatography–Mass Spectrometry equipment.

REFERENCES

1. Nelson GC, Rosegrant MW, Koo J, Robertson R, Sulser T, Zhu T, Ringler C, Msangi S, Palazzo A, Batka M, Magalhaes M, Valmonte-Santos R, Ewing M, Lee D. Climate change: Impact on agriculture and costs of adaptation. International Food Policy Research Institute 2009; 21:1-30.
2. Environmental European Agency, Agriculture and climate change. Signals 2015; 1-10.
3. Dadkhah A. Phytotoxic potential of sugar beet (*Beta vulgaris*) and eucalyptus (Eucalyptus camaldulensis) to control purslane (Portulaca olearacea) weed. Acta Agriculturae Scandinavica Section B-Soil Plant Science, 2013; 63:46-51.
4. Food and Agriculture Organization of the United Nations. AGP- Pest and pesticide management. www.fao.org/agriculture/crops/corethemes/theme/pests/en/ (Accessed May 17, 2017).
5. Bozoglu F. In: Environmental Security and Ecoterrorism; Alpas H, Berkowicz S, Ermakova I. Ed.; Springer: Dordrecht 2011; pp. 73-82.
6. Oduor AMO, Leimu R, van Kleunen M. Invasive plant species are locally adapted just as frequently and at least as strongly as native plant species. Journal of Ecology 2016; 104:957-968.
7. Awan TH, Chauhan BS. Effect of emergence time, inter- and intra- specific competition on growth and fecundity of *Echinochloa crus-galli* in dry-seeded rice. Crop Protection 2016; 87: 98-107.
8. Kamal Uddin MD, Shukor Juraimi A, Sabir Hossain Md, Altaf Un Nahar M, Eaqub Ali Md, Rahman MM. Purslane weed (*Portulaca oleracea*): A prospective plant source of nutrition, omega-3 fatty acid, and antioxidant attributes. The Scientific World Journal 2014; 2014:1-6.
9. Feng L, Chen GQ, Tian XS, Yang HM, Yue MF, Yang CH. The hotter the weather, the greater the infestation of *Portulaca oleracea*: opportunistic life-history traits in a serious weed. Weed Research 2015; 55:396-405.
10. Niinomi Y, Ikeda M, Yamashita M, Ishida Y, Asai, M, Shimono Y, Tominaga T, Sawada H. Glyphosate-resistant Italian ryegrass (*Lolium multiflorum*) on rice paddy levees in Japan. Weed Biology and Management 2013; 13:31-38.
11. Powles SB, Yu Q. Evolution in action: plants resistant to herbicides. Annual Review of Plant Biology 2010; 61:317-347.
12. Ghanizadeh H, Harrington KC, James TK, Woolley DJ, Ellison NW. Restricted herbicide translocation was found in two glyphosate-resistant Italian ryegrass (*Lolium multiflorum* Lam.) populations from New

Zealand. Journal of Agricultural Science and Technology 2016; 18:1041-1051.
13. Perez-Jones A, Park KW, Polge N, Colquhoun J, Mallory-Smith CA. Investigating the mechanisms of glyphosate resistance in *Lolium multiflorum*. Planta 2007; 226:395-404.
14. Heap I. Global perspective of herbicide-resistant weeds. Pest Management Science 2014; 70:1306-1315.
15. Parajouli R, Kristensen IS, Knudsen MT, Mogensen L, Corona A, Birkved M, Peña N, Graversgaard M, Dalgaard T. Environmental life cycle assessments of producing maize, grass-clover, ryegrass and winter wheat straw for biorefinery. Journal of Cleaner Production 2017; 142:3859-3871.
16. Badii MH, Landeros J. Plaguicidas que afectan a la salud humana y sustentabilidad; Cultura Científica y Tecnológica 2007; 4:21-34.
17. International Agency for Research on Cancer. Evaluation of five organophosphate insecticides and herbicides. Evaluation of Carcinogenic Risks to Humans, International Agence of Rerearch for Cancer Monographs: Lyon, 2015.
18. Dayan FE, Cantrell CL, Duke SO Natural products in crop protection. Bioorganic and Medicinal Chemistry 2009; 17:4022-4034.
19. Isman MB Plant essential oils for pest and disease management. Crop Protection 2000; 19:603-608.
20. Santamarina MP, Roselló J, Giménez S, Blázquez MA. Commercial *Laurus nobilis* L. and *Syzygium aromaticum* L. Merr. & Perry essential oils against post-harvest phytopathogenic fungi on rice. LWT-Food Science and Technology 2016; 65:325-332.
21. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils. A review. Food and Chemical Toxicology 2008; 46:446-475.
22. Blázquez M.A. Role of natural essential oils in sustainable agriculture and food preservation. Journal of Scientific Research and Reports 2014; 3:1843-1860.
23. Peris I, Blázquez MA. Comparative GC-MS analysis of bay leaf (*Laurus nobilis* L.) essential oils in commercial samples. International Journal of Food Properties 2015; 18:757-762.
24. Angelini LG, Carpanese G, Cioni PL, Morelli I, Macchia M, Flamini G. Essential oils from Mediterranean Lamiaceae as weed germination inhibitors. Journal of Agricultural and Food Chemistry 2003; 51:6158-6164.
25. Batish DR, Singh HP, Kaur M, Kohli RK, Singh S. Chemical characterization and phytotoxicity of volatile essential oil from leaves of *Anisomeles indica* (Lamiaceae). Biochemical Systematic and Ecology 2012; 41:104-109.
26. Ricci D, Epifano F, Fraternale D. The essential oil of *Monarda didyma* L. (Lamiaceae) exerts phytotoxic activity in vitro against various weed seeds. Molecules 2017; 22:222.
27. Moreira I, Costa F, Vieira O, Hitomi C. Development of mouthwash with *Rosmarinus officinalis* extract. Brazilian Journal of Pharmaceutical Sciences 2014; 50:851-858.
28. Waller SB, Madrid IM, Cleff MB, Santin R, Freitag RA, Meireless MCA, Mello JRB. Effects of essential oils of *Rosmarinus officinalis* Linn. and *Origanum vulgare* Linn. from different origins on *Sporothrix brasiliensis* and *Sporothrix schenckii* complex. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 2016; 68:991-999.
29. Giarratana F, Muscolino D, Ragonese C, Beninati C, Sciarrone D, Ziino G, Mondello L, Giuffrida A, Panebianco A. Antimicrobial activity of combined thyme and rosemary essential oils against *Listeria monocytogenes* in Italian mortadella packaged in modified atmosphere. Journal of Essential Oil Research 2016; 28:467-474.
30. Sirocchi V, Devlieghere F, Peelman N, Sagratini G, Maggi F, Vittori S, Ragaert P. Effect of *Rosmarinus officinalis* L. essential oil combined with different packaging conditions to extend the shelf life of refrigerated beef meat. Food Chemistry 2017; 221:1069-1076.
31. Güdek M, Çetin H. Fumigant toxicity on adults of *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) of essential oil from *Rosmarinus officinalis* L. and its side effects on chickpea grains. Journal of Essential Oil Bearing Plants 2017; 20:272-281.
32. Atak M, Mavi K, Uremis I. Bio-herbicidal effects of oregano and rosemary essential oils on germination and seedling growth of bread wheat cultivars and weeds. Romanian Biotechnologycal Letters 2016; 21:11149-11159.
33. Chorianopoulos N, Kalpoutzakis E, Aligiannis N, Mitaku S, Nychas GJ, Haroutounian SA. Essential oils of *Satureja*, *Origanum*, and *Thymus* species: chemical composiciton and antibacterial activities against foodborne pathogens. Journal of Agricultural and Food Chemistry 2004; 52:8261-8267.
34. Mominul Islam AKM, Kato-Noguchi H. Phytotoxic activity of *Ocimum tenuiflorum* extracts on germination and seedling growth of different plant species. The Scientific World Journal 2014; 2014:1-8.
35. Karamaouna F, Kimbaris A, Michaelakis A, Papachristos D, Polissiou M, Papatsakona P, Tsora E. Insecticidal activity of plant essential oils against the vine mealybug, *Planococcus ficus*. Journal of Insect Science 2013; 13:142.
36. Adams, R.P. Identification of essential oil components by gas chromatography/mass spectrometry, 4th ed.; Allure Publishing Corporation: Carol Stream, 2007.
37. Blázquez MA, Carbó E. Control of *Portulaca oleracea* by boldo and lemon essential oils indifferent soils. Industrial Crops and Products 2015; 76:515-521.
38. Hcini J, Sotomayor A, Jordan MJ, Bouzid S. Chemical composition of the essential oil of rosemary (*Rosmarinus officinalis* L.) of Tunisian origin. Asian Journal of Chemistry 2013; 25:2601-2603.
39. Atti-Santos AC, Rossato M, Pauletti GF, Rota DL, Rech JC, Pansera MR, Agostini F, Serafini LA, Moyna P. Physico-chemical evaluation of *Rosmarinus*

officinalis L. essential oil. Brazilian Archives of Biology and Technology 2005; 48:1035-1039.

40. Neto NJG, Luz ID, Tavares AG, Honorio VG, Magnani M, de Souza EL. *Rosmarinus officinalis* L. essential oil and its majority compound 1,8-cineole at sublethal amounts induce no direct and cross protection in *Staphylococcus aureus* ATCC 6538. Foodborne Pathogen Disease 2012; 9:1071-1076.
41. Barreto HME, Filho CS, de O Lima E, Coutinho HDM, Morais-Braga MFB, Tavares CCA, Tintino SR, Rego JV, de Abreu APL, Lustossa M do CGR, Oliveira WG Citó AMGL, Lopes JAD. Chemical composition and possible use as adjuvant of the antibiotic therapy of the essential oil of *Rosmarinus officinalis* L. Industrial Crops and Products 2014; 59:290-294.
42. Goudarzi S, Rafieirad M. Evaluating the effect of alpha-pinene on motor activity avoidance memory and lipid peroxidation in animal model of Parkinson disease in adult male rats. Research Journal of Pharmacognosy 2017; 4:53-63.
43. Ikei H, Song C, Miyazaki Y. Effects of olfactory stimulation by alpha-pinene on autonomic nervous system. Journal of Wood Science 2016; 62:568-572.
44. Miresmailli S, Isman MB. Efficacy and persistence of rosemary oil as an acaricide against twospotted spider mite (Acari: Tetranychidae) on greenhouse tomato. Journal of Economic Entomology 2006; 99:2015-2023.
45. Alipour M, Saharkhiz MJ. Phytotoxic activity and variation in essential oil content and composition of Rosemary (*Rosmarinus officinalis* L.) during different phonological growth stages. Biocatalysis and Agricultural Biotechnology 2016; 7:271-278.
46. Lawrence BM. In: Flavors and fragrances: A world perspective; Lawrence LBM, Mookerjee BD, Willis BJ. Amsterdam, 1988; pp. 161–70.
47. Avetisyan A, Markosian A, Petrosyan M, Sahakyan N, Babayan A, Aloyan S, Trchounian A. Chemical composition and some biological activities of the essential oils from basil *Ocimum* different cultivars. BMC Complementary and Alternative Medicine 2017; 17:1-8.
48. Rolli E, Marieschi M, Maietti S, Sacchetti G, Bruni R. Comparative phytotoxicity of 25 essential oils on pre- and post-emergence development of *Solanum lycopersicum* L.: a multivariate approach. Industrial Crops and Products 2014; 60:280-290.