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Herbicidal Activity of Volatiles from Coriander, Winter Savory, Cotton Lavender, and Thyme Isolated by Hydrodistillation and Supercritical Fluid Extraction

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The volatiles from *Coriandrum sativum* L., *Satureja montana* L., *Santolina chamaecyparissus* L., and *Thymus vulgaris* L. were isolated by hydrodistillation (essential oil) and supercritical fluid extraction (volatile oil). Their effect on seed germination and root and shoot growth of the surviving seedlings of four crops (*Zea mays* L., *Triticum durum* L., *Pisum sativum* L., and *Lactuca sativa* L.) and two weeds (*Portulaca oleracea* L. and *Vicia sativa* L.) was investigated and compared with those of two synthetic herbicides, Agrocide and Prowl. The volatile oils of thyme and cotton lavender seemed to be promising alternatives to the synthetic herbicides because they were the least injurious to the crop species. The essential oil of winter savory, on the other hand, affected both crop and weeds and can be appropriate for uncultivated fields.

KEYWORDS: Coriander; cotton lavender; hydrodistillation; natural herbicides; supercritical fluid extraction; thyme; winter savory

INTRODUCTION

In agricultural fields, weeds can interfere with crops causing the reduction of both quality and quantity of crop production. As a consequence, there are economic losses to farmers. According to Anaya (*1*), weeds are responsible for the loss of 12% of the world crop production. Thus, there are different approaches to control the germination and development of weeds, the use of synthetic herbicides being the most widespread. All over the world, about 60% of the sold pesticides are herbicides, and, in 2002, global herbicide sales were nearly U.S. $28 billion (*1*, *2*).

However, the indiscriminate application of synthetic herbicides during the past decades had a negative impact, namely, soil and groundwater contamination and toxicity for living organisms, including humans (*1*, *3*−*6*). Moreover, the number of weeds resistant to the available herbicides increased, which led to the search of new efficient compounds with herbicidal activity (*1*, *2*, *4*, *5*, *7*). Until now, 272 resistant weed biotypes from 172 species have been identified (*4*).

In nature, secondary metabolites (e.g., phenolic compounds and terpenoids) produced by one species can affect the germination and development of other species, and these properties have been explored for agrosystem management purposes (*8*−*11*). The search for new natural-based herbicides relies on some advantages shown by secondary metabolites over synthetic herbicides. Synthetic herbicides have a limited number of modes of action, whereas natural products, due to their structural diversity, may have novel action target sites, different from those of the already known synthetic herbicides (*4*, *5*, *11*, *12*).

Essential oils are among the most studied plant secondary metabolites. Due to their volatility they do not accumulate in soils and groundwater. Moreover, they have little or no mammalian toxicity (*1*, *2*, *4*, *11*). Mono- and sesquiterpenes are known to affect physiological processes in weeds, namely, photosynthesis and chlorophyll synthesis. Accumulation of lipid globules in the cytoplasm as well as the reduction in organelles, namely, mitochondria and nuclei, by membrane organelles disruption was also reported (*12*, *13*).

Hydrodistillation (HD) and steam distillation are the conventional methods for extracting plant volatiles. To avoid hydrolysis reactions, as well as possible thermal degradation of the most thermolabile compounds, supercritical fluid extraction with CO_2 (SFE) is a good alternative, because it can operate at moderate temperatures (usually between 40 and 60 °C). The most used supercritical fluid is carbon dioxide due to its safety, availability, and low cost. It allows supercritical operations at relatively low pressure (critical pressure, P_c = 73.8 bar) and moderate temperatures (critical temperature, T_c = 31.1 °C). Moreover, the extract is practically solvent free, because CO_2 is a gas at NPT conditions (*14*, *15*). According to the European Pharmacopoeia (*16*), essential oils are extracts isolated from aromatic plants by distillation processes only, such as hydrodistillation. Other extracts obtained from aromatic plants, by other methods, even containing volatile components, should not be considered essential oils.

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In view of this, an alternative name (volatile oils) has been adopted throughout this paper to refer to the extracts isolated by CO_2 SFE.

The aim of this work was to assess the inhibitory activity of the essential oils and volatile oils from coriander seeds (*Coriandrum sativum* L.), aerial flowering parts of winter savory (*Satureja montana* L.), flower heads of cotton lavender (*Santolina chamaecyparissus* L.), and aerial flowering parts of thyme (*Thymus vulgaris* L.) on seed germination and seedling growth of four crops (sweet corn, *Zea mays* L.; durum wheat, *Triticum durum* L.; dwarf pea, *Pisum sativum* L.; and lettuce, *Lactuca sativa* L.) and two weeds (common purslane, *Portulaca oleracea* L.; and common vetch, *Vicia sativa* L.). The herbicidal effect of the essential oils of winter savory and thyme, obtained by hydrodistillation (*17*), and coriander, isolated using steam distillation (*13*), was already studied. On the other hand, to the best of our knowledge, this is the first attempt to compare the herbicidal activity of volatiles obtained by hydrodistillation and SFE. The results are also compared with those obtained with two synthetic herbicides, Agrocide [2-methyl-4-chlorophenoxyacetic acid (MCPA)] and Prowl [2,6-dinitro-*N*-(1-ethylpropyl)-3,4-xylidine (pendimethalin)].

MATERIALS AND METHODS

Chemicals. Carbon dioxide (99.995% purity) was supplied by Air Liquide (Lisbon, Portugal). Synthetic herbicides, Agrocide and Prowl, were purchased from Agroquisa, Agroquímicos, S.A. (Portugal). C_9-C_{36} *n*-alkanes were obtained from Supelco (Bellefonte, PA), and standards for GC-MS analysis were purchased from Extrasynthese (CAS 1) (Barcelona, Spain), Sigma-Aldrich-Fluka (CAS 2) (Steinheim, Germany), Riedel-de Haën (CAS 3) (Seelze, Germany), and Alltech Associates, Inc. (CAS 4) (Deerfield, IL).

For the synthesis of the oil components quoted in **Table 1**, all reagents were of analytical grade: dimethyl sulfoxide, potassium hydroxide, acetic anhydride, formic acid, and sodium borohydride were purchased from Merck (Darmstadt, Germany) and iodomethane, pyridine, 4-dimethylaminopyridine (DMAP), isovaleryl chloride, isopentanol, propionic anhydride, and butyric anhydride were purchased from Sigma-Aldrich-Fluka (Steinheim, Germany).

Synthesis of Standards. *Synthesis of Isoborneol.* In a test tube, a reaction mixture containing 50 mg of camphor dissolved in 500 μL of methanol and 50 mg of sodium borohydride was heated at 60 °C for 5 min and then allowed to cool to room temperature. The residue was washed twice with cooled water (3 mL) and isoborneol dissolved with ethyl ether (1 mL). *m*/*z* (%), 154 (M^+, 0.8), 139 (7.1), 136 (9.8), 121 (13.3), 110 (16.5), 96 (9.8), 95 (100.0), 93 (20.4), 92 (9.0), 83 (7.1), 82 (8.6), 81 (7.4), 79 (7.4), 71 (7.4), 69 (11.4), 67 (18.8), 55 (20.4), 53 (8.6), 43 (28.2), 41 (39.2). RI (1270 ± 5).

Borneol, Geraniol, Isoborneol, and Isopentanol Acylation. In a derivatization vial, the alcohol to be acylated (0.3 mmol) was added to 100 μL of pyridine containing 0.4 mmol of acylating agent (acetic anhydride, propionic anhydride, or butyric anhydride) and DMAP (0.15 mmol). The solution was heated at 60 °C for 1 h. For the synthesis of isopentyl isovalerate, using isopentanol and isovaleryl chloride, the reaction mixture did not contain DMAP and the reaction was carried out at room temperature. The corresponding esters were obtained after the conventional workup.

Isopentyl Isovalerate. *m*/*z* (%), 172 (M^+, 0.7), 131 (4.7), 113 (7.1), 103 (15.3), 95 (7.1), 85 (51.0), 81 (7.4), 73 (6.3), 71 (34.9), 70 (100.0), 60 (15.3), 59 (9.0), 57 (49.8), 56 (8.6), 55 (38.0), 51 (5.5), 43 (70.2), 42 (26.6), 41 (61.2). RI (1217 ± 5).

Isobornyl Acetate. *m*/*z* (%), 154 (M − 42, 7.8), 136 (43.1), 121 (41.9), 110 (14.1), 109 (11.7), 108 (21.1), 107 (9.4), 95 (89.0), 93 (42.3), 92 (11.4), 82 (12.1), 79 (11.7), 69 (14.1), 67 (18.0), 55 (21.1), 53 (10.6), 43 (100.0), 41 (42.7). RI (1405 ± 5).

Bornyl Propionate. *m*/*z* (%), 210 (M^+, 2.3), 154 (9.0), 136 (43.1), 121 (47.8), 110 (13.3), 109 (27.8), 108 (25.1), 107 (13.3), 95 (100.0), 93 (67.5), 92 (18.8), 81 (16.1), 80 (21.5), 79 (15.3), 69 (19.2), 67 (18.4), 57 (97.7), 55 (20.0), 43 (18.0), 41 (38.0). RI (1483 ± 5).

Geranyl Acetate. *m*/*z* (%), 196 (M^+, 0.4), 154 (1.6), 136 (12.5), 121 (15.3), 107 (4.3), 94 (6.3), 93 (24.3), 85 (8.2), 81 (6.7), 80 (12.5), 70 (6.7), 69 (100.0), 68 (41.5), 67 (17.2), 54 (1.6), 53 (11.0), 43 (80.4), 41 (75.7). RI (1513 ± 5).

Geranyl Butyrate. *m*/*z* (%), 154 (M − 70, 2.0), 136 (13.7), 121 (22.3), 107 (6.7), 94 (7.8), 93 (38.0), 92 (9.4), 81 (9.0), 80 (18.8), 71 (52.6), 69 (100.0), 68 (50.6), 67 (19.2), 55 (5.5), 53 (11.4), 43 (47.4), 42 (7.1), 41 (81.2). RI (1684 ± 5).

Thymol Formylation. In a test tube, 150 μL of a stock formylating mixture (100 mmol of acetic anhydride and 200 mmol of formic acid) was added to 250 μL of pyridine containing 0.3 mmol of thymol. After 24 h at room temperature, 2 mL of pentane/ethyl ether (95:5, v/v) was added to the solution, and following treatment with cold 5% aqueous NaOH and cold 5% aqueous HCl, thymyl formate was obtained after solvent evaporation. *m*/*z* (%), 178 (M^+, 16.5), 163 (3.9), 150 (3.5), 149 (16.1), 136 (10.6), 135 (100.0), 133 (5.1), 121 (3.1), 117 (7.8), 116 (3.9), 115 (14.9), 107 (9.4), 105 (7.1), 91 (23.1), 79 (6.3), 78 (3.1), 77 (10.2), 65 (6.3), 63 (3.1), 53 (3.9), 51 (5.1), 41 (6.3). RI (1412 ± 5).

Synthesis of Thymyl and Carvacryl Methyl Ethers. In a test tube, thymol (0.3 mmol) or carvacrol (0.3 mmol) and iodomethane (0.3 mmol) were added to 1.5 mL of DMSO containing 0.4 mmol of powdered KOH and stirred vigorously for 2 min. After 1 h at room temperature with occasional stirring, water (5 mL) was added and the reaction mixture extracted with pentane/ethyl ether (95:5, v/v). The corresponding ethers were obtained after solvent evaporation.

Thymyl Methyl Ether. *m*/*z* (%), 164 (M^+, 25.9), 150 (11.4), 149 (100.0), 134 (7.8), 133 (3.5), 119 (12.9), 117 (8.6), 115 (7.8), 105 (6.3), 103 (3.9), 91 (19.6), 79 (4.7), 78 (3.1), 77 (8.2), 65 (4.7), 63 (3.1), 51 (4.3), 41 (4.7). RI (1371 ± 5).

Carvacryl Methyl Ether. *m*/*z* (%), 164 (M^+, 35.3), 150 (11.4), 149 (100.0), 134 (9.4), 133 (5.1), 121 (4.7), 119 (11.7), 117 (9.8), 115 (7.1), 105 (5.9), 91 (20.8), 79 (5.5), 78 (4.3), 77 (9.4), 65 (5.1), 51 (5.1), 41 (5.5). RI (1381 ± 5).

Plant Material. Seeds from coriander (*C. sativum* L.; L'Ortolano, Cesena, Italy), aerial flowering parts of winter savory (*S. montana* L.; Centro de Investigación y Tecnología Agroalimentaria, Ejea, Spain), flower heads of cotton lavender (*S. chamaecyparissus* L.; Centro de Investigación y Tecnología Agroalimentaria, Villa Roya, Spain), and aerial flowering parts of thyme (*T. vulgaris* L.; Centro de Investigación y Tecnología Agroalimentaria, Ejea, Spain) with initial moisture contents of 8.5, 11.8, 12.2, and 10.9%, respectively, were frozen with liquid N_2 and ground using a commercial mill.

The seeds of four crops (sweet corn, *Z. mays* L.; durum wheat, *T. durum* L.; dwarf pea, *P. sativum* L.; and lettuce, *L. sativa* L.) and two weeds (purslane golden, *P. oleracea* L.; and common vetch, *V. sativa* L.) species were obtained from Sementes Hortelão - Soares & Rebelo, Lda., Portugal.

SFE Apparatus. The SFE apparatus was described in detail in a previous work (*18*). Briefly, the apparatus is composed by a diaphragm pump, an extraction vessel (1 L), and two separators (0.27 L), operating in series and allowing a fractional separation. The pressure of the extractor is regulated by a back-pressure regulator and measured with a Bourdon type manometer, and the preset temperature is reached with the aid of a water jacket. The total volume of CO_2 used in the extraction is determined with a dry test meter.

Extraction of Essential and Volatile Oils. Essential oils were isolated by hydrodistillation (HD), using 40 g of plant material, in a Clevenger-type apparatus, during 4 h (coriander seeds, flowering aerial parts of winter savory, and thyme) or 2 h (flower heads of cotton lavender).

Volatile oils were isolated by SFE, using 100 g of plant material, and the best extraction conditions were reported previously (*19*−*22*). With regard to the operational conditions of pressure, temperature, mean particle size, CO_2 flow rate, and amount of CO_2 consumed, the volatile oils from coriander seeds and from the flowering aerial parts of winter savory were as follows: 90 bar/40 °C/0.6 mm/1.1 kg h^{-1}/4.4 kg, respectively. For aerial flowering parts of thyme, the experimental conditions were 90 bar/40 °C/0.6 mm/1.1 kg h^{-1}/4.8 kg, respectively. Furthermore, the volatile oil from the flower heads of cotton lavender was isolated at 80 bar/40 °C/0.6 mm/1.1 kg h^{-1}/2.1 kg, respectively.

Fractionation steps were carried out at a pressure of 80 bar and a temperature of −8 °C, in the first separator, and at 20 bar and −15 °C, in

Table 1. Composition (in Percentage) of the Essential and Volatile Oils

			coriander		winter savory		cotton lavender		thyme	
ident[a]	component	RI[b]	HD	SFE	HD	SFE	HD	SFE	HD	SFE
RO 1, RO 2	α-thujene	1010	t[c]	t	0.6	0.3		t	0.5	0.2
CAS 1, RO 1	α-pinene	1016	2.5	1.5	0.6	0.3	0.1	0.2	0.8	0.4
CAS 1, RO 1	camphene	1027	0.3	0.2			0.8	0.9	0.7	0.4
CAS 1, RO 1	sabinene	1060	0.1	0.1	0.2	0.1				
CAS 2	1-octen-3-ol	1062			0.1	0.1			0.2	0.2
CAS 1, RO 1, RO 2	β-pinene	1063	0.4	0.2	0.8	0.6	1.3	1.6	0.9	0.7
RO 3	1,8-dehydrocineole	1080					0.2	t		
CAS 2	3-octanol	1081							0.4	0.2
CAS 2, RO 1, RO 2	β-myrcene	1081	2.8	1.0	1.0	0.6			0.4	0.2
CAS 2	2-pentylfuran	1085					0.3	t		
CAS 2, RO 1, RO 2	α-phellandrene	1093			0.2	0.2			0.1	0.1
RO 3	Yomogi alcohol	1095					0.3	0.7		
CAS 2, RO 1, RO 2	δ-3-carene	1100			0.1	t			0.1	t
CAS 2, RO 1, RO 2	α-terpinene	1108			1.7	1.2	2.4	1.9	0.9	0.5
CAS 2, RO 1, RO 2	*p*-cymene	1117	1.3	0.8	12.8	10.1	2.0	2.1	34.7	28.6
CAS 2, RO 1, RO 2	1,8-cineole	1122			0.5	0.4	24.8	38.3	0.3	0.2
CAS 1, RO 1, RO 2	β-phellandrene	1122			0.5	0.4			0.3	0.2
CAS 2, RO 1, RO 2	limonene	1124	3.1	1.4	0.4	0.3			0.7	0.6
CAS 2, RO 1, RO 2	*cis*-β-ocimene	1125	t	t		0.1				
CAS 2, RO 1, RO 2	*trans*-β-ocimene	1146	1.7	0.4					t	t
CAS 2	artemisia ketone	1153					0.3	t		
CAS 2, RO 1, RO 2	γ-terpinene	1157	6.8	5.0	8.9	4.3			7.0	4.1
CAS 2	*trans*-sabinene hydrate	1166	t	0.1	0.5	0.7	3.1	1.7	0.5	1.2
CAS 1	*cis*-linalool oxide	1173	t	t					0.1	0.1
CAS 2	*n*-octanol	1173	t	t						
CAS 1	*trans*-linalool oxide	1174							0.1	0.1
CAS 2, RO 1, RO 2	terpinolene	1194	0.9	0.5	0.2	0.1	1.4	2.1		
CAS 2	*cis*-sabinene hydrate	1201			0.1	0.2	0.8	0.6	0.2	0.3
CAS 2	*n*-nonanol	1202					0.4	0.6	t	t
CAS 2	linalool	1210	67.6	75.9	0.8	0.7	0.2	0.3	2.9	3.6
LSC	isopentyl isovalerate	1217					0.2	t		
RO 3	α-campholenal	1225					0.3	0.7		
RO 3	*trans*-*p*-menthen-1-ol	1238					0.7			
CAS 2	camphor	1254	3.0	3.1			7.4	10.7	0.7	0.9
CAS 2	citronellal	1265	0.1	0.1						
CAS 2	borneol	1279	0.1	0.1	0.7	0.7	8.3	3.8	1.1	1.2
RO 3	thuj-3-en-10-al	1289					0.8	0.8		
CAS 2, RO 1, RO 2	terpinen-4-ol	1292	0.2	0.2	0.7	0.4	7.4	1.9	0.8	0.7
CAS 2	*cis*-dihydrocarvone	1299			0.2	0.2			0.2	0.3
CAS 2	myrtenal	1300					0.4	0.4		
CAS 2, RO 1, RO 2	α-terpineol	1305	0.4	0.1			0.2	t	0.2	0.2
CAS 2	myrtenol	1311					1.7	1.7		
CAS 2	*trans*-carveol	1345					0.2	t		
CAS 2	citronellol	1360	t	t						
CAS 2	carvone	1366				0.2				
CAS 2	thymoquinone	1371			0.2	2.9				3.5
LSC	thymyl methyl ether	1371							0.2	t
LSC	carvacryl methyl ether	1381			0.1				1.0	t
RO 3	*p*-cymen-7-ol	1393					0.3	0.2		
LSC	isobornyl acetate	1405					1.5	1.2		
CAS 2	geraniol	1407	2.7	2.9					0.1	t
LSC	thymyl formate	1412							0.3	0.2
CAS 3, RO 1	thymol	1419			11.0	10.9	0.1	0.2	35.4	36.8
RO 3	*trans*-ascaridol	1426					t			
CAS 2, RO 1	carvacrol	1431			52.2	52.7	0.3		2.6	2.6
CAS 2	*trans*,*trans*-2,4-decadienal	1465					0.2	0.4		
LSC	bornyl propionate	1483							t	0.1
RO 4	β-bourbonene	1507			0.1	0.1			0.1	0.2
LSC	geranyl acetate	1513	2.8	3.5						
RO 4	bicycloelemene	1536					t	0.5		
CAS 2, RO 1, RO 2	*trans*-β-caryophyllene	1551			1.3	1.5			1.0	1.4
CAS 2	β-copaene	1561							0.2	1.4
CAS 2, RO 1, RO 2	α-humulene	1570							0.1	0.1
CAS 2	α-aromadendrene	1571					1.3	1.4		
RO 4	*trans*-bergamotene	1579			0.2	0.2				
RO 2	γ-muurolene	1585			0.1	0.1			0.2	0.4
RO 1, RO 2	germacrene D	1606			0.2	0.3	2.7	2.9	t	0.1

Table 1. Continued

			coriander		winter savory		cotton lavender		thyme	
ident[a]	component	RI[b]	HD	SFE	HD	SFE	HD	SFE	HD	SFE
RO 5	α-muurolene	1616							t	0.1
RO 3	bicyclogermacrene	1621					1.4	1.0		
RO 1, RO 2	β-bisabolene	1636			2.0	2.5				
RO 2	γ-cadinene	1642			0.2	0.2	0.2	0.2	0.1	0.1
RO 2	*trans*-calamenene	1647							0.3	0.4
RO 2	δ-cadinene	1654					0.3	t	0.3	0.5
RO 4	thymohydroquinone	1666			0.4	0.5				
RO 3, RO 5	α-calacorene	1666					t	t		t
LSC	geranyl butirate	1684							t	t
RO 1, RO 2	spathulenol	1701					3.1	0.6		
CAS 2, RO 1, RO 2	β-caryophyllene oxide	1707			0.2	0.2	1.0	t	0.8	1.5
CAS 2	globulol	1717					0.1	t		
RO 3	viridiflorol	1717					0.1	t		
RO 3	anhydro-oplopanone	1725					1.0	t		
RO 5	10-epi-γ-eudesmol	1750							t	0.1
RO 2	epi-α-cadinol	1767					1.1	0.2	0.1	0.3
CAS 2	α-bisabolol	1776				t				
RO 2	α-cadinol	1792							0.1	0.1
CAS 2	hexadecanoic acid	1921					0.2	t		
CAS 4	*n*-heneicosane	1996					t	0.3		
CAS 4	*n*-tricosane	2300					t			
CAS 4	*n*-tetracosane	2399					0.2	0.1		
CAS 4	*n*-octacosane	2800					0.1	t		
CAS 4	*n*-hexatriacosane	3600					0.1	0.3		

[a] Ident, identification procedure: RO 1, reference oil (*Thymus caespititius*); RO 2, reference oil (*Achillea milefollium*); RO 3, reference oil (*Santolina chamaecyparissus*); RO 4, reference oil (*Satureja montana*); RO 5, reference oil (*Thymus vulgaris*); CAS 1, commercial standard (Extrasynthese); CAS 2, commercial standard (Sigma-Aldrich-Fluka); CAS 3, commercial standard (Riedel-de Haën); CAS 4, commercial standard (Alltech); LSC, laboratory-synthesized components. [b] RI, retention indices relative to C9−C36 *n*-alkanes on a DB-5 column. [c] t = trace (<0.05%).

the second one. Under these experimental conditions, a volatile oil free of waxes was obtained in the second separator.

Gas Chromatography (GC). Quantitative analyses of the volatile and essential oils were performed in a Hewlett-Packard 5890 gas chromatograph (HP, Waldbronn, Germany), equipped with a flame ionization detector (FID) and a fused-silica DB-5 capillary column (J&W; 30 m × 0.25 mm i.d., film thickness = 0.25 μm; Folsom, CA). Oven temperature was held isothermal at 40 °C, for 2 min, then programmed to 230 at 3 °C/min and subsequently at 5 °C/min to 310 °C, and then held isothermal for 15 min. Injector and detector temperatures were 310 °C, and the carrier gas was helium, adjusted to a linear velocity of 24 cm/s. The samples were injected using a split ratio of 1:50. The volume of injection was 0.1 μL. The percentage composition of the oils was computed by the normalization method from the GC peak areas without using response factors.

Gas Chromatography−Mass Spectrometry (GC-MS). The GC-MS unit consisted of a Perkin-Elmer Autosystem XL gas chromatograph (Perkin-Elmer, Shelton, CT) equipped with a DB-1 fused silica column (30 m × 0.25 mm i.d., film thickness = 0.25 μm; J&W Scientific Inc., Agilent Technologies, Santa Clara, CA) and interfaced with a Perkin-Elmer Turbomass mass spectrometer (software version 4.1; Perkin-Elmer). Oven temperature was programmed from 45 to 175 °C, at 3 °C/min and subsequently at 15 °C/min to 300 °C and then held isothermal for 10 min. The injector temperature was 280 °C, the transfer line temperature was 280 °C, and the ion source temperature was 220 °C. The carrier gas, helium, was adjusted to a linear velocity of 30 cm/s. The split ratio was 1:40, the ionization energy, 70 eV, the scan range, 40−300 u, and the scan time, 1 s.

The identity of the components was assigned by comparison of their retention indices, relative to C_9−C_{36} *n*-alkanes and GC-MS spectra of a library composed by components of reference oils (RO; RO 1, *Thymus caespititius*; RO 2, *Achillea milefollium*; RO 3, *Santolina chamaecyparissus*; RO 4, *Satureja montana*; RO 5, *Thymus vulgaris*), laboratory-synthesized components (LSC), and commercially available standards (CAS 1−CAS 4).

Bioassay. Crop and weed seeds were surface-disinfected in a mixture of distilled water/sodium hypochlorite (3:1) during 20 min and then soaked in distilled water during 4 h. Seeds were then sown in Petri dishes (9 cm), on three layers of filter paper (Whatman no. 1) wetted with 8 mL of distilled water. To evaluate the inhibitory effect of the oils on seed germination, 0 (control), 2, 10, and 20 μL of pure oils were tested. Immediately following oil application on the center of the filter paper, Petri dishes were sealed with parafilm and incubated at 22.5 ± 2 °C, 48 ± 8% relative humidity, and a 16 h day/8 h night photoperiod (Osram L8W/840 Active Daywhite tube). No additional water was added to the Petri dishes during the incubation period. A similar approach was followed to assess the herbicidal activity of the synthetic herbicides. Amounts of 2, 10, and 20 μL of Agrocide solution, corresponding to 0.8, 4.0, and 8.0 mg of MCPA, or the same volumes of a Prowl solution, corresponding to 0.7, 3.3, and 6.6 mg of pendimethalin, respectively, were used.

Eight seeds were sown per Petri dish for corn, dwarf pea, and common vetch, 10 seeds for durum wheat, 20 seeds for purslane golden, and 15 seeds for lettuce.

After an incubation period of 7 days, the inhibitory effect of the essential and volatile oils as well as of synthetic herbicides, expressed as ED_{50} values (μL oil or herbicide/Petri dish), were assessed by measuring germination percentage and root and shoot lengths. The two last parameters were assessed by measuring all of the germinated seedlings.

Statistical Analysis. All experiments were done in triplicate. Germination percentages and root and shoot lengths were subjected to one-way analysis of variance (ANOVA) followed by the Tukey post hoc test (Graphpad Prism 5.1) to determine significant differences among mean values at the probability level of 0.05.

RESULTS AND DISCUSSION

The chemical composition of the essential (HD) and volatile (SFE) oils isolated from coriander, winter savory, cotton lavender, and thyme is reported in **Table 1**. Both extracts (HD and SFE) isolated from all of the species were dominated by the monoterpene fraction, oxygen-containing monoterpenes and monoterpene hydrocarbons representing 47−86 and 8−48% of the total oils, respectively. The sesquiterpene fraction was rather small (5−12%).

Essential and volatile oils were assessed for their ability in preventing seed germination and seedling growth of four crops

Table 2. Effect of Essential Oils, Volatile Oils, and Synthetic Herbicides on Seed Germination of Crop and Weed Species[a]

	crop species				weed species	
	sweet corn	durum wheat	dwarf pea	lettuce	common purslane	common vetch
coriander HD	13.5 a	4.5 a	10.5 ab	5.2 ab	5.4 a	8.8 a
coriander SFE	15.5 a	4.9 a	9.2 ab	5.4 ab	5.4 a	5.0 b
winter savory HD	6.4 b	5.1 a	6.0 a	4.0 ae	2.9 b	5.0 b
winter savory SFE	>20	5.6 ac	13.6 b	5.4 b	5.2 a	5.7 c
cotton lavender HD	>20	5.5 ac	>20	8.5 c	5.4 a	12.9 d
cotton lavender SFE	10.0 c	12.9 b	11.8 b	19.4 d	7.5 c	5.7 c
thyme HD	>20	5.2 a	>20	4.7 abe	5.1 a	5.3 bc
thyme SFE	>20	6.6 c	>20	8.6 c	5.1 a	10.7 e
Agrocide	14.7 a	10.2 d	13.3 b	3.6 e	16.9 d	5.7 c
Prowl	ni	10.9 d	>20	17.8 f	>20	>20
p (ANOVA)	***	***	**	***	***	***

[a] ED_{50} values (μL oil or herbicide/Petri dish) are expressed as mean of three independent assays. Within the same column, different lowercase letters denote statistically significant differences between the oils (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$). ni, no inhibition.

(sweet corn, durum wheat, dwarf pea, and lettuce) and two weeds (common purslane and common vetch) in comparison with Agrocide and Prowl, two synthetic herbicides. Agrocide is commercially available as a concentrate solution of MCPA (400 g/L). This herbicide, which can persist in the soil for 54 days, induces uncontrolled growth of meristematic tissues and alters DNA and protein synthesis (*23*). It is used to kill broadleaf plants. Prowl is available as a concentrate solution of pendimethalin (330 g/L) and is used to control annual grasses, as well as broadleaf weeds, inhibiting mitosis in developing root systems (*24*, *25*), and possesses a half-life of about 90 days. This herbicide is one of the most water-soluble and the least volatile on the market (*24*, *25*).

With regard to seed germination, coriander oils obtained either by SFE and or by HD showed similar ED_{50} values for all crops and weeds tested. In contrast, essential and volatile oils isolated from the other aromatic plants under study did not always affect the target species in the same way (**Table 2**). Sweet corn showed high susceptibility to the essential oil (HD) isolated from winter savory (ED_{50} = 6.4 μL/Petri dish), whereas the effect of the volatile oils (SFE) from this species as well as that from thyme, and the essential oils (HD) from cotton lavender and from thyme was less remarkable (ED_{50} > 20.0 μL/Petri dish) when compared with those induced by the synthetic herbicides (Agrocide, ED_{50} = 14.7 μL/Petri dish; and Prowl, no inhibition) (**Table 2**). A similar result was recorded for the germination of dwarf pea seeds (**Table 2**). The volatile oil (SFE) isolated from cotton lavender was the least injurious to the seed germination of durum wheat and lettuce (ED_{50} = 12.9 and 19.4 μL/Petri dish, respectively). For both target plants, this extract showed a lower inhibitory effect than Agrocide and Prowl (**Table 2**). The inhibition of common purslane seed germination was more efficient with all essential oils than with the synthetic herbicides (**Table 2**), winter savory essential oil (HD) being the most effective (ED_{50} = 2.9 μL/Petri dish). With regard to seed germination of common vetch, only the volatile oils (SFE) from coriander (ED_{50} = 5.0 μL/Petri dish) and the essential oil (HD) of thyme (ED_{50} = 5.3 μL/Petri dish) and from winter savory (ED_{50} = 5.0 μL/Petri dish) showed to be more harmful than Agrocide (ED_{50} = 5.7 μL/Petri dish) and Prowl (ED_{50} > 20.0 μL/Petri dish).

From our results, it seems clear that oil composition remarkably affects seed germination and that the role of the minor components should not be neglected in this process. Actually, the essential oil (HD) of winter savory showed to be, in general, more efficient (lowest values of ED_{50}) than the corresponding volatile oil (SFE) in inhibiting seed germination of both weeds and crops (**Table 2**). Although both extracts (HD and SFE) were dominated by carvacrol and thymol in similar amounts (52.2−52.7 and 10.9−11.0%, respectively, **Table 1**), the ED_{50} values were significantly different (except for durum wheat). This highlights the role that minor components can play in bioactive extracts, acting either as antagonists or as agonists of the major compounds. In this case, the major difference in the composition of both extracts is the amount of thymoquinone, which is 15-fold higher in the SFE volatile than in the HD essential oil (**Table 1**). A previous study has also reported the lack of selectivity of winter savory essential oil (HD), containing carvacrol as the main component (56.8%), in inhibiting seed germination of three crops (radish, pepper, and lettuce) and three weeds (lambsquarters, common purslane, and barnyardgrass) (*17*).

Vokou et al. (*26*) tested the effect of 47 monoterpenoids on the germination and seedling growth of lettuce. Using 2.5 μL of linalool, carvacrol, thymol, or 1,8-cineole, four main components of the oils tested in our study, they concluded that lettuce seed germination was inhibited as follows: 1,8-cineole (81.1%) > linalool (60.5%) > carvacrol (54.0%) ≈ thymol (50.0%). However, the authors pointed out that the isolated component may not behave in the same way as in pair or in a mixture. This exception is strongly supported by our results. Actually, lettuce seed germination was markedly affected by essential (HD) and volatile (SFE) oils from winter savory (rich in carvacrol, 52.2−52.7%), coriander (rich in linalool, 67.6−75.9%), and thyme (rich in thymol, 35.4−36.8%). In contrast, the extract from cotton lavender volatiles (rich in 1,8-cineole, 24.8−38.3%) was much lesser injurious. Moreover, in our study, the ED_{50} value of the cotton lavender volatile oil (SFE), containing 38.3% of 1,8-cineole (**Table 1**), was higher (less effective) than that of the correspondent essential oil, with a low percentage of 1,8-cineole (24.8%; **Table 1**). Our results show that lettuce seed germination was less susceptible to 1,8-cineole-rich oils than to volatiles with large amounts of carvacrol, linalool, or thymol. This is in agreement with the results published by Angelini et al. (*17*) that tested thymol, carvacrol, and 1,8-cineole and found that the two phenolic compounds were much more injurious to lettuce and common purslane (100% germination inhibition using an aqueous solutions of 250 mg/L) than 1,8-cineole at the same concentration (no inhibitory effect could be recorded). Azirak and Karaman (*13*) have also shown that these two phenolic monoterpenes were quite active over seven weeds [hollyhock, redroot amaranth, yellow starthistle, wild radish, sorrel, Rumex nepalensis, charlock mustard, and common sowthistle]. They found that the essential oil from coriander leaves (rich in linalool) was ineffective, at low amounts (3 and 6 μL/Petri dish), against the germination of these seven weed species.

The effect of the isolated essential and volatile oils was also assessed on the root and shoot growth of the surviving seedlings

Table 3. Effect of Essential Oils, Volatile Oils, and Synthetic Herbicides on Shoot and Root Lengths of Crop and Weed Species, at the End of 7 Days[a]

	sweet corn		durum wheat		dwarf pea		lettuce		common purslane		common vetch	
	shoot length	root length	shoot length	root length	shoot length	root length	shoot length	root length	shoot length	root length	shoot length	root length
Coriander HD	4.7^{a}	7.9^{a,c}	3.5^{a}	<2	3.9^{a}	8.3^{a}	7.3^{a}	7.6^{a}	4.8^{a}	3.0^{a}	6.7	<2
Coriander SFE	8.0^{a}	5.7^{a}	5.0^{b}	<2	3.9^{a}	<2	7.1^{a}	2.0^{b}	2.8^{b}	<2	8.0	<2
Winter savoury HD	6.9^{a}	7.2^{a,c}	6.8^{c}	<2	<2	6.3^{b}	2.1^{a}	<2	<2	5.4^{b}	6.9	3.8
Winter savoury SFE	8.7^{a}	12.7^{b}	7.5^{c}	5.2	12.1^{b}	15.2^{c}	5.8^{a,c}	2.1^{b}	2.8^{b}	3.0^{a}	6.5	3.5
Cotton lavender HD	8.3^{a}	9.2^{b,c}	8.2^{c,d}	3.9	>20	>20	7.3^{a,c}	6.8^{a}	<2	<2	7.7	4.8
Cotton lavender SFE	<2	9.7^{b,c}	8.8^{d}	3.9	<2	9.0^{a}	16.7^{b,c}	9.5^{a}	<2	<2	<2	<2
Thyme HD	<2	10.2^{b,c}	<2	<2	14.0^{b}	18.9^{d}	2.0^{a}	<2	<2	<2	<2	<2
Thyme SFE	14.1^{b}	>20	<2	3.7	>20	>20	10.7^{c}	6.0^{a,b}	<2	<2	8.8	<2
Agrocide	n.i.	n.i.	7.6^{c,d}	<2	6.7^{c}	<2	4.7^{a,c}	<2	<2	<2	<2	<2
Prowl®	<2	<2	<2	<2	7.7^{c}	>20	20.0^{b}	<2	12.5^{c}	15.7^{c}	10.0	n.i.
p (ANOVA)	***	***	***	ns	***	***	***	**	***	***	ns	ns

[a] ED_{50} values (μL oil or herbicide/ Petri dish) are expressed as mean of three independent assays. Within the same column, different superscript lowercase letters denote statistically significant differences between the oils (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$); ns − differences not statistically significant.

(**Table 3**). As stated above, four oils (winter savory SFE, thyme SFE, cotton lavender HD, and thyme HD) caused lower injuries on sweet corn and dwarf pea seed germination when compared with the synthetic herbicides. From these extracts, only the volatile oil (SFE) isolated from thyme did not compromise the development of the surviving seedlings of sweet corn (ED_{50} = 14.1 μL/Petri dish for shoot and ED_{50} > 20.0 μL/Petri dish for root development, **Table 3**), although no deleterious effect could be recorded in the assays with the synthetic herbicide Agrocide (**Table 3**). In addition to the volatile oil (SFE) of thyme, the essential oil (HD) of cotton lavender also did not halt the growth of dwarf pea seedlings (all with ED_{50} > 20.0 μL/Petri dish, **Table 3**). All of the other oils affected, to some degree, the development of sweet corn and dwarf pea seedlings (lower values of ED_{50}).

Durum wheat and lettuce target species showed higher sensitivity to all oil extracts tested than sweet corn and dwarf pea. Nevertheless, cotton lavender volatile oil (SFE) showed to be the less active oil in inhibiting root and shoot growth of lettuce (ED_{50} = 16.7 μL/Petri dish for shoot length and ED_{50} = 9.5 μL/Petri dish for root length, **Table 3**). Durum wheat seedling growth was remarkably impaired by either oil extracts or synthetic herbicides. Coriander essential (HD) and volatile (SFE) oils and Prowl were the most injurious to this crop, whereas all of the other oils showed an inhibitory capacity similar to or slightly lower than that of Agrocide (ED_{50} = 7.6 μL/Petri dish for shoot length and ED_{50} < 2 μL/Petri dish for root length, **Table 3**). On the other hand, the weed growth inhibition recorded for almost all of the tested oils was, in general, higher than that recorded for Prowl.

Taking together our results on seed germination and root and shoot growth, the essential oil (HD) isolated from winter savory appears to be a promising new natural herbicide for uncultivated fields, whereas the volatile oils (SFE) from thyme and cotton lavender seem to be more suitable for cultivated ones. The advantage of using volatile herbicides is obvious due to their low persistence in the field when compared with nonvolatile herbicides such as, for instance, Agrocide and Prowl. However, there is still a long way to go until a suitable formulation containing volatile oils is available to deliver natural herbicides into the field.

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