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Comparative study of two coniferous species (*Pinus pinaster* Aiton and *Cupressus sempervirens* L. var. *dupreziana* [A. Camus] Silba) essential oils: chemical composition and biological activity

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Maritime pine (*Pinus pinaster* Aiton) and Saharan cypress (*Cupressus sempervirens* L. var. *dupreziana* [A. Camus] Silba) are two cone-bearing seed coniferous woody plants. The chemical composition of their essential oils, isolated from needles and leaves by hydrodistillation, was analyzed with gas chromatography (GC) and gas chromatography mass spectrometry (GC/MS). A total of 66 and 28 compounds were identified, which represented 99.5% and 98.9% of total pine and cypress oils, respectively. *Pinus pinaster* oil was found to be rich in α-pinene (31.4%), (*Z*)-caryophyllene (28%), and α-humulene (6.7%); it was characterized by relatively high amounts of monoterpene and sesquiterpene hydrocarbons (44.5% and 46.3%, respectively). The major components identified in cypress oil were manoyl oxide (34.7%), α-pinene (31.8%), α-humulene (9%), and δ-3-carene (8.7%). Results of *in vitro* antifungal test assays showed that both oils significantly inhibit the growth of 10 plant pathogenic fungi. Herbicidal effects of the oils on seed germination, seed vigor, and seedling growth of three common crop weeds *Sinapis arvensis* L., *Phalaris paradoxa* L., and *Raphanus raphanistrum* L. were also determined; the oils completely inhibited seed germination and seedling growth of all the weeds.

Key words: Antifungal activity, *Cupressaceae*, essential oils, phytotoxicity, *Pinaceae*.

INTRODUCTION

Essential oils and plant extracts have attracted considerable attention in the discovery of biologically active compounds. Aromatic plant essential oils are examples of compounds with pest control potential.

Weeds and pathogens are the major competitors of agricultural crops and severely reduce crop production by 25% to 50% (Pimentel et al., 1991; Oerke 2006). Enormous amounts of synthetic pesticides are used to protect agricultural crops. The Agrow (2007) report stated that the total value of the world's agrochemical market was between US$31 and US$35 billion; among the products, herbicides accounted for 48% followed by insecticides (25%) and fungicides (22%). Chemical pesticides are becoming more unpopular because many of them are related to unpleasant side effects. In fact, the excessive use of these chemicals or their repeated applications in crop lands, urban environment, and bodies of water to get rid of noxious pests has resulted in an increased risk of pesticide resistance, enhanced pest resurgence, development of resistance/cross-resistance, toxicological implications to human health and non-target organisms, and increased environmental pollution (Lee et al., 2009). Combating environmental pollution and its ill-effects on life and life support systems is one of the most serious challenges today. There is a need to replace these synthetic chemicals with biological pesticides, which are more or less safer and do not cause any toxicological effects on the environment. Natural pest and disease control, either directly or indirectly, use plant-derived secondary metabolites, which play an important role in plant resistance to pests. Therefore, screening plant essential oils and plant extracts for their biological activity could lead to the discovery of new pest control agents (Isman, 2000; Amri et al., 2012a; 2012b). In the literature, several essential oils have shown herbicidal and antifungal activities (Amri et al., 2011a; 2011b; 2012a; 2012b). Furthermore, activities of their individual compounds have been shown (De Martino et al., 2010). Maritime pine (*Pinus pinaster* Aiton) is a highly valuable coniferous species broadly distributed in the western

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Mediterranean Basin (Silba, 1986). This maritime pine is one of the most important forest species in Tunisia. *Cupressus sempervirens* L. var. *dupreziana* (A. Camus) Silba, the Saharan cypress, is a very rare coniferous tree native to the Tassili n'Ajjer mountains in the central Algerian Sahara Desert. This species is distinct from the *Cupressus sempervirens* Mediterranean cypress, which has much bluer foliage with a white resin spot on each leaf and smaller shoots often flattened in a single plane. It also has smaller cones that are only 1.5 to 2.5 cm long (Krüssmann, 1985). The present study is the continuation of our previous studies on the possible herbicidal and antifungal activity of essential oils from species belonging to the Pinaceae and Cupressaceae families (Amri et al., 2011a; 2011b; 2012a; 2012b). The first aim of this study was to determine the chemical composition of *Pinus pinaster* and *Cupressus sempervirens* var. *dupreziana* essential oils to assess their herbicidal activity against germination and seedling growth of *Sinapis arvensis* L., *Raphanus raphanistrum* L., and *Phalaris paradoxa* L.; the second objective was to assess the toxicity of the essential oils against 10 plant pathogenic fungi.

MATERIALS AND METHODS

Plant material

Pinus pinaster needles and *C. sempervirens* var. *dupreziana* leaves were collected from the National Research Institute of Rural Engineering, Water, and Forests (INRGREF) arboretum (Tunisia) in August 2009. Five samples were collected from more than five different trees; these were mixed for homogenization, air-dried, finely ground, and employed in three replicates to extract essential oils. Identification was performed in the INRGREF Laboratory of Forest Ecology. A voucher specimen is deposited in the Herbarium of this laboratory.

Essential oil extraction

The essential oils were extracted by hydrodistillation of fresh plant material (100 g each sample in 500 mL distilled water) with a Clevenger-type apparatus for 3 h in accordance with the standard procedure described in the European Pharmacopoeia (2004). After extraction, oils were dried over anhydrous sodium sulfate (a pinch per 10 mL^{1}) and stored in sealed glass vials at 4 °C prior to analysis. Yield was calculated based on sample dry weight (w/w %, weight/dry weight, mean of three replicates).

Chemical analysis of essential oils

Oil composition was studied with gas chromatography (GC) and gas chromatography mass spectrometry (GC/MS). Gas chromatography analysis was carried out with an HP5890-series II gas chromatograph (Agilent Technologies, Santa Clara, California, USA) equipped with Flame Ionization Detectors (FID) under the following conditions: fused silica capillary column, apolar HP-5 and polar HP Innowax (30 m-0.25 mm ID, film thickness 0.25 mm). The oven temperature was kept at 50 °C for 1 min then programmed at a rate of 5 °C min^{-1} to 240 °C and maintained isothermal for 4 min. The carrier gas was N at a flow rate of 1.2 mL min^{-1}; injector temperature at 250 °C and detector at 280 °C; and volume injected, 0.1 mL of 1% solution (diluted in hexane) in splitless mode. The percentages of the constituents were calculated by electronic integration of FID peak areas without the use of response factor correction. GC/MS was performed in a Hewlett Packard 5972 A MSD System (HP Agilent, Hazlet, New Jersey, USA). An HP-5 MS capillary column (30 m-0.25 mm ID, film thickness 0.25 mm) was directly coupled to the mass spectrometry. The carrier gas was He, with a flow rate of 1.2 mL min^{-1}. Oven temperature was programmed (50 °C for 1 min, then 50 to 240 °C at 5 °C min^{-1}) and subsequently maintained isothermal for 4 min, the injector port was at 250 °C, the detector at 280 °C, and the volume injected was 0.1 mL of 1% solution (diluted in hexane) in splitless mode; the mass spectrometer (HP5972) recorded at 70 eV; scan time, 1.5 s; and mass range, 40-300 amu. The ChemStation (Agilent Technologies, Santa Clara, California, USA) software was adopted to handle mass spectra and chromatograms. The oil components were identified by comparing their mass spectra with those in the Wiley 275 GC-MS library (John Wiley and Sons, 1996) and those in the literature. Retention index data generated from a series of alkane retention indices (related to C9 to C28 on the HP-5 column) further confirmed this (Adams, 2001).

Seed germination and seedling growth experiments

Sinapis arvensis, *Phalaris paradoxa*, and *Raphanus raphanistrum* seeds were collected from parent plants growing in Tunisia in July 2009. Seeds were sterilized before treatment with 15% sodium hypochlorite for 20 min and then rinsed with abundant distilled water. Empty and undeveloped seeds floating in tap water were discarded and the remaining seeds were air-dried. Germination was carried out on Petri dishes where 30 seeds were placed on double-layered Whatman nr 1 filter paper moistened with different concentrations (0.5, 1.0, and 2.0 μL mL^{-1}) of essential oil in a 1% Tween 20 solution (Tworkoski, 2002). A similar set-up with no essential oil served as control and the commercial herbicide 2,4-D isooctyl ester was the reference. The Petri dishes were closed and sealed with adhesive tape to prevent oil volatilization. Cultures were incubated under controlled conditions of 25 °C, 70% relative humidity, and 16:8 photoperiod of 1500 lux light (Tworkoski, 2002). The number of germinated seeds was counted daily and seedling length was measured. The assays were arranged in a completely randomized design with three replicates, including controls. Seed vigor was calculated by the following formula (Agrawal, 1980; AOSA, 1996):

$$\text{Seed vigor} = \sum \text{Daily counts of number of seeds germinated/Number of days.}$$

Antifungal activity assays

The phytopathogenic fungi used in the experiments were *Gibberella avenacea* R.J. Cook, 1967, *Fusarium culmorum* (W.G. Sm.) Sacc., *Fusarium oxysporum* (Schltdl.) (1824), *Fusarium subglutinans* (Wollenw. & Reinking) (1983), *Fusarium verticillioides* (Sacc.) Nirenberg, (1976), *Fusarium nygamai* L.W. Burgess & Trimboli, *Rhizoctonia solani* Kuhn, 1858, *Microdochium nivale* var. *nivale* (Fr.) Samuels & Hallett, *Alternaria alternata* (Fr.) Keissl., 1912, and *Bipolaris sorokiniana* (Sacc.) Shoemake. All the strains were obtained from the culture collection of the Tunisian National Institute of Agronomic Research. Cultures of each of the fungi were maintained on potato dextrose agar (PDA) medium and stored at 4 °C in 1 mL 25% glycerol at -20 °C. Antifungal activity was studied by an *in vitro* contact assay which produces hyphal growth inhibition (Cakir et al., 2004). Essential oil was dissolved in 1 mL Tween 20 (0.1% v/v) and then added to 20 mL PDA at 50 °C to obtain different final concentrations. A mycelial disk of approximately 5 mm in diameter was cut from the periphery of a 7-d-old culture; it was inoculated in the center of each PDA plate (90 mm diameter) and then incubated in the dark at 24 °C for 7 d. PDA plates treated with Tween 20 (0.1%) without essential oil were the negative control. Tests were triplicated. Growth inhibition was calculated as the percentage of radial growth inhibition related to the control by the following formula:

$$\% \text{ Inhibition} = (C\text{-}T)/C \times 100$$

where *C* is the mean of three replicates of hyphal extension (mm) of controls and *T* is the mean of three replicates of hyphal extension (mm) of plates treated with essential oil.

Statistical analysis

Data of seed germination, seedling growth, and antifungal activity assays were subjected to one-way ANOVA with the SPSS (Statistical Package for the Social Sciences) 13.0 software package (IBM Corporation, Armonk, New York, USA, 2005). Differences between means were tested through Student-Newman-Keuls test and values of $P \leq 0.05$ were considered significantly different (Sokal and Rohlf, 1995).

RESULTS AND DISCUSSION

Composition of essential oils

Essential oil yield (weight/dry weight of plant) is 0.4% for *P. pinaster* needles and 0.3% for *C. sempervirens* var. *dupreziana* leaves. Gas chromatography and GC-MS analysis of hydrodistillated *P. pinaster* essential oil allowed identifying 66 different components representing 99.5% of the total compounds. The global chromatographic analysis of *P. pinaster* oil showed a complex mixture consisting mainly of mono- and sesquiterpene hydrocarbons and small amounts of oxygenated mono- and sesquiterpenes. It was dominated by mono- (44.5%) and sesquiterpene hydrocarbons (46.3%), while oxygenated monoterpenes and sesquiterpenes were present only in low percentages (3.1% and 5.6%, respectively). The major components detected in the oil were α-pinene (31.4%) and (*Z*)-caryophyllene (28.1%) followed by α-humulene (6.8%), β-pinene (4.2%), and bicyclogermacrene (4.0%). The chemical composition of *P. pinaster* essential oil was studied in Greece agreed with our result by showing that α-pinene, germacrene D, and (*Z*)-caryophylene were the major oil components at different levels (20.9%, 19.2%, and 14.8%, respectively). Moreover, germacrene D was the most abundant in the oil of *P. pinaster* growing in Greece and absent in the Tunisian sample (Petrakis et al., 2005). In *C. sempervirens* var. *dupreziana* oil, GC and GC/MS analysis identified 29 compounds, which represented 98.9% of the oil (Table 1).

The major oil components were manoyl oxide (34.7%), which usually occurs in the *Cupressaceae* family; α-pinene was dominant among the major components (31.8 %) followed by α-humulene (9%) and δ-3-carene (8.7%). Oil composition is largely dominated by monoterpene hydrocarbon components (45%) followed by oxygen-containing diterpenes (35.7%). The sesquiterpene hydrocarbons and oxygenated monoterpenes make up 11.7% and 0.2%, respectively. In previous studies, essential oils of Algerian cypress were studied by Ramdani et al. (2012). Data from this study show the richness of the oil in manoyl oxide (14.1% to 26%), α-pinene (12.4% to 19.7%), and Δ-3-carene (8% to 17.7%); this agrees with our results. However, the same authors indicated in another report that cypress essential oils were characterized by their richness of α-pinene (11.5% to 44.2%), δ-3-carene (5.7% to 31.7%), and germacrene-D (15.7% to 54.1%) (Ramdani et al., 2011). These differences found between the main oil constituents obtained from *P. pinaster* and *C. sempervirens* var. *dupreziana* growing in Tunisia and from the same species growing in other countries could be especially related to climate, soils, and the genetic background of the tree.

Herbicidal effects of oil on weed germination and seedling growth

The phytotoxic effects of *P. pinaster* and *C. sempervirens* var. *dupreziana* oils were tested on seed germination, seed vigor, and seedling growth of *S. arvensis*, *R. raphanistrum,* and *P. paradoxa*. These are very aggressive weeds in Tunisia that reduce crop production and in most cases are considered as host plants for pests. Data show that essential oil strongly inhibited germination, speed of germination, and seedling growth of tested weeds in a rate-dependent way and they were significantly more effective on *S. arvensis* than *R. raphanistrum* and *P. paradoxa*. At lower concentrations, from 0.5 to 1 μL mL^{-1} for *S. arvensis* and from 0.5 to 2 μL mL^{-1} for *Trifolium campestre* and *Phalaris canariensis*, weed germination and seedling growth were partially reduced. However, at

Table 1. Chemical composition of *Pinus pinaster* and *Cupressus sempervirens* var. *dupreziana* essential oils.

Peaks	IR[a]	IR[b]	Compounds (%)	Pine	Cypress	Methods of identification
1	926	1015	Tricyclene	0.8	0.1	MS, RI
2	931	1020	α-Thujene	0.3	0.1	MS, RI
3*	939	1026	α-Pinene	31.4	31.8	MS, RI, Co-GLC
4	953	1044	Fenchene	-	0.2	
5*	953	1052	Camphene	tr	0.1	MS, RI
6	958	1121	Thuja-2,4(10)-diene	0.3	-	MS, RI
7	976	1125	Sabinene	0.2	0.2	MS, RI
8*	980	1129	β-Pinene	4.2	0.8	MS, RI, Co-GLC
9*	988	1152	β-Myrcene	0.5	1	MS, RI, Co-GLC
10	991	1156	3-Octanone	tr	-	MS, RI
11*	1005	1160	α-Phellandrene	0.9	-	MS, RI
12*	1011	1148	δ-3-Carene	tr	8.7	MS, RI, Co-GLC
13*	1018	1187	α-Terpinene	0.1	-	MS, RI, Co-GLC
14*	1026	1258	p-Cymene	0.8	-	MS, RI
15*	1031	1218	Limonene	0.6	1.1	MS, RI, Co-GLC
16	1041	1221	Phenyl acetaldehyde	tr	-	MS, RI
17	1050	1251	(E)-β-Ocimene	tr	-	MS, RI
18*	1062	1236	δ-Terpinene	2.1	-	MS, RI
19	1068	1560	(Z)-Sabinene hydrate	tr	-	MS, RI
20	1088	1287	α-Terpinolene	1.3	0.9	MS, RI, Co-GLC
21	1094	1311	α-Pinene oxide	0.1	-	MS, RI
22*	1115	1450	β-Thujone	0.9	-	MS, RI
23	1121	1644	(Z)-p-Menth-2-en-1-ol	tr	-	MS, RI
24	1125	1508	α-Campholenal	0.2	-	MS, RI
25	1140	1650	(E)-p-Menth-2-en-1-ol	0.1	-	MS, RI
26*	1143	1473	Camphor	0.2	-	MS, RI
27	1161	1412	Pinocarvone	0.1	-	MS, RI
28	1168	1646	Umbellelol	0.2	-	MS, RI
29*	1177	1571	α-Terpinen-4-ol	0.4	0.1	MS, RI
30*	1189	1673	α-Terpeneol	0.2	-	MS, RI
31	1196	1668	(Z)-Piperetol	tr	-	MS, RI
32	1203	1733	Verbenone	tr	-	MS, RI
33	1213	-	*iso*-Dihydrocarveol	tr	-	MS, RI
34	1219	1564	(Z)-Sabinene hydrate acetate	0.1	-	MS, RI
35*	1229	1764	Citronellol	tr	-	MS, RI
36	1235	1597	Thymol methyl ether	0.3	0.1	MS, RI
37	1244	1586	Carvacrol methyl ether	tr	-	MS, RI
38	1252	1812	Piperetine	0.1	-	MS, RI
39	1279	1562	iso-Bornyl acetate	tr	-	MS, RI
40*	1305	-	Carvacrol	tr	-	MS, RI
41	1332	-	δ-Elemene	0.2	-	MS, RI
42	1349	1677	α-Terpenyl acetate	0.1	-	MS, RI
43	1363	1520	β-Bourbounene	0.4	-	MS, RI
44	1376	1515	α-Copaene	tr	0.4	MS, RI
45	1383	1566	β-Cububene	1.2	-	MS, RI
46	1397	1573	Langifolene	1.5	-	MS, RI
47	1418	1608	(Z)-Caryophyllene	28.1	0.4	MS, RI, Co-GLC
48	1439	-	allo-Aromadendiene	0.7	-	MS, RI
49	1444	1834	(Z)-Muurrola-4-(14), 5-diene	0.1	-	MS, RI
50	1454	1670	α-Humulene	6.8	9	MS, RI, Co-GLC
51	1459	-	(Z)-β-Farnasene	0.9	-	MS, RI
52	1480	1721	Germacrene D	-	1.3	
53	1484		α-Amorphene	-	0.4	
54	1490	-	(E)-Eudesma-6.1.1-diene	0.5	-	MS, RI
55	1493	-	Zingibirene	-	0.2	
56	1495	1755	Bicyclo gernacrene	4	-	MS, RI
57	1499	-	α-Murrolene	-	0.4	
58	1501	-	epi-Zonarene	tr	-	MS, RI
59	1509	1929	Cubebol	0.3	-	MS, RI
60	1511	1741	β-Bisabolene	0.7	-	MS, RI
61	1523	1524	δ-Cadinene	1.3	0.1	MS, IR
62	1536	1735	Itacilene ether	1.4	-	MS, RI
63	1546	1913	β-Calacorene	0.1	-	MS, RI
64	1559	2215	(Z)-Muurola-5-en-4-ol	0 .2	-	MS, RI
65	1581	2008	Caryophyllene oxide	1.6	0.1	MS, RI, Co-GLC
66	1596	2093	Cedrol	0.2	-	MS, RI, Co-GLC
67	1606	2002	Humulene oxide II	0.1	-	MS, RI
68	1630	2163	α-Acorenol	0.3	-	MS, RI
69	1634	2212	β-Acorenol	tr	-	MS, RI
70	1654	2170	α-Cadinol	1.2	0.6	MS, RI, Co-GLC
71	1987	-	Manoyl oxide	-	34.7	
72			Ledene oxide	-	0.6	
73	1969	-	Sandaracopimara-8(14), 16-diene	-	2.2	
74	2054	2530	Abietatriene	0.4	-	MS, RI
75	2080	-	Abietadiene	-	1.9	
76	2288	2265	Tartarol	-	0.3	
Yields, % (W/W)				0.4	0.3	
Total identification				99.5	98.9	
Monoterpene hydrocarbons				44.5	45	
Oxygenated monoterpenes				3.1	0.2	
Sesquiterpene hydrocarbons				45.9	11.7	
Oxygenated sesquiterpenes				5.6	1.1	
Diterpene hydrocarbons				0.4	35.7	
Oxygenated diterpenes				-	5.1	

RI: Retention index; MS: mass spectrometry; Co-GLC: co-injection; tr: trace (< 0.1%); -: not detected.
[a]Apolar HP-5MS column.
[b]Polar HP Innowax column.
*Compounds cited in the literature that have herbicidal effects.

high concentrations (2 μL mL^{-1}) germination and seedling growth of *S. arvensis* were totally inhibited and only reduced for the other two weeds (Tables 2 and 3). These results agree with recent reports, which have shown the herbicidal effects of some species belonging to different *Pinaceae*, *Cupressaceae*, *Myrtaceae,* and *Anacardiaceae* families (Amri et al., 2011a; 2011b; 2012a; 2012b). According to these studies, *Pinus* species exhibited a potent herbicidal activity. We have recently demonstrated that *P. pinea* L. and *P. patula* Schltdl. & Cham. displayed inhibitory effects against germination and seedling growth of *S. arvensis*, *Lolium rigidum* Gaudin, and *R. raphanistrum* (Amri et al., 2011b; 2012a). This agrees with our results which confirm the herbicidal potential of these species. Similarly, recent reports for herbicidal effects of *C. sempervirens* var. *dupreziana* have shown the phytotoxic potential of many essential oils belonging to the *Cupressaceae* family.

Juniperus oxycedrus L. and (Amri et al., 2013) *J. phoenicea* L. essential oils have been reported as having herbicidal effects against weed germination and seedling growth (Amri et al., 2011a; 2012b). Our data mostly agree with the literature on the inhibitory activity exerted by essential oils against germination and seedling growth of weeds and cultivated crops; their phytotoxicity was generally attributed to the allelopathic potential of some terpenes (Tworkoski, 2002; Abrahim et al., 2003; Scrivanti et al., 2003; Singh et al., 2006). It has been shown that the herbicidal effects of essential oils resulted from the combined reactions of additive, synergetic, and antagonistic effects among several compounds (Vokou et

Table 2. Contact inhibitory effects of *Pinus pinaster* essential oils on germination, seedling growth, and seed vigor of three weed species.

Weeds	Samples	Doses	Germination	Seed vigor	Seedling growth	
					Aerial parts	Roots
		μL mL^{-1}	%		mm	
Sinapis arvensis	Control	0.0	100.0 ± 0.0d	19.7	72.0 ± 4.3e	62.7 ± 1.8e
	Essential oil	0.5	66.7 ± 1.9c	13.2	51.7 ± 0.9d	50.0 ± 0.6d
		1.0	18.9 ± 2.9b	4.5	32.3 ± 1.2c	44.3 ± 0.9c
		2.0	0.0 ± 0.0a	0.3	0.0 ± 0.0a	0.0 ± 0.0a
	2,4-d	2.0	14.4 ± 2.9b	4.0	10.7 ± 0.9b	12.0 ± 0.6b
Raphanus raphanistrum	Control	0.0	82.2 ± 2.2d	5.1	59.7 ± 3.4e	50.3 ± 1.8d
	Essential oil	0.5	37.8 ± 2.9c	1.9	43.0 ± 0.6d	39.0 ± 0.6c
		1.0	18.9 ± 2.9b	1.1	33.0 ± 0.6c	24.3 ± 2.8b
		2.0	1.1 ± 1.1a	0.1	17.7 ± 2.2b	8.7 ± 1.8a
	2,4-d	2.0	7.8 ± 1.1a	0.8	9.3 ± 1.8a	6.7 ± 0.3a
Phalaris paradoxa	Control	0.0	86.7 ± 1.9e	3.9	55.3 ± 1.8e	66.0 ± 2.3 d
	Essential oil	0.5	46.7 ± 1.9d	1.7	44.7 ± 2.6d	49.0 ± 0.6 c
		1.0	22.2 ± 2.9c	0.9	30.0 ± 0.6c	25.3 ± 2.4b
		2.0	2.2 ± 2.2a	0.2	19.0 ± 2.1b	10.3 ± 0.3a
	2,4-d	2.0	14.4 ± 2.9b	0.8	9.3 ± 0.7a	6.7 ± 0.3a

Means in the same column with the same letter are not significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$). Values are expressed as means ± standard error mean.

Table 3. Contact inhibitory effects of *Cupressus sempervirens* var. *dupreziana* essential oils on germination, seedling growth, and seed vigor of three weed species.

Weeds	Samples	Doses	Germination	Seed vigor	Seedling growth	
					Aerial parts	Roots
		μL mL^{-1}	%		mm	
Sinapis arvensis	Control	0.0	100.0 ± 0.0e	50.0	36.0 ± 1.0e	43.3 ± 0.3e
	Essential oil	0.5	22.0 ± 0.3d	8.8	29.3 ± 0.3d	30.7 ± 0.3d
		1.0	14.0 ± 0.3b	6.0	24.0 ± 1.0c	25.7 ± 0.3c
		2.0	0.0 ± 0.0a	0.0	0.0 ± 0.0a	0.0 ± 0.0a
	2,4-d	2.0	12.0 ± 3.0b	4.5	19.3 ± 2.3b	22.7 ± 0.3b
Raphanus raphanistrum	Control	0.0	92.0 ± 3.0e	18.0	33.3 ± 0.3e	25.7 ± 0.3e
	Essential oil	0.5	30.0 ± 5.0d	9.5	27.7 ± 0.3d	20.7 ± 0.3d
		1.0	22.0 ± 1.0c	7.6	23.0 ± 0.0c	23.0 ± 0.0c
		2.0	0.0 ± 0.0a	0.0	0.0 ± 0.0a	0.0 ± 0.0a
	2,4-d	2.0	6.0 ± 1.0b	1.5	14.0 ± 2.3b	14.0 ± 2.3b
Phalaris paradoxa	Control	0.0	66.0 ± 4.0e	7.2	35.7 ± 1.3e	33.7 ± 0.3d
	Essential oil	0.5	30.0 ± 1.0d	7.7	27.3 ± 0.3d	26.0 ± 0.8c
		1.0	16.0 ± 0.4c	4.3	18.7 ± 1.0c	20.0 ± 1.1b
		2.0	0.0 ± 0.0a	0.0	0.0 ± 0.0a	0.0 ± 0.0a
	2,4-d	2.0	10.0 ± 3.0b	1.5	15.7 ± 0.3b	21.3 ± 0.3b

Means in the same column with the same letter are not significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$). Values are expressed as means ± standard error mean.

al., 2003). Our results agree with several studies that have tested the activity of pure and combined compounds (De Feo et al., 2002; Vokou et al., 2003; Bulut et al., 2006; Singh et al., 2006; Wang et al., 2009). More than 17 compounds are known to have herbicidal activity in the chemical composition of pine and cypress oils (Vokou et al., 2003; De Martino et al., 2010); these compounds are present in the oils under study with different percentages and they are also known for their potential herbicidal activity. The oils in our study were rich in sesquiterpenes (11.9% and 49% in cypress and pine oils, respectively), that is, (*Z*)-caryophyllene (28.1% in pine oil) and α-humulene (9% in cypress oil), which are known for their phytotoxic effects (Kil et al., 2000; De Feo et al., 2002; Singh et al., 2006; Wang et al., 2009). Singh (2006) demonstrated that exposing seedlings to α-pinene (major component in the oils under study) inhibited seedling growth by causing oxidative damage in the root tissue. Kil et al. (2000) reported that (*Z*)-caryophyllene, present in cypress and pine oil, was an important sesquiterpene of *Artemisia lavandulifolia* DC. essential oil which suppressed seedling growth of *Achyranthes japonica* (Miq.) Nakai. Wang et al. (2009) showed that (*Z*)-caryophyllene at the rate of 3 mg L^{-1} significantly inhibited germination rates and seedling growth of *Brassica rapa* L. subsp. *oleifera* (DC.) Metzg., and *Raphanus sativus*.

Both monoterpenoids and sesquiterpenoids mostly appear to be involved in the herbicidal activity of essential oils, although the exact essential oil mechanisms on germination and seedling growth inhibition remain unclear. Inhibitory effects could be caused by terpenes, which interfere with physiological and biochemical processes in target species (Weir et al., 2004). Previous studies showed that essential oils have phytotoxic effects

that can cause anatomical and physiological changes in plant seedlings, such as lipid globule accumulation in the cytoplasm and reduction in some organelles, such as mitochondria, possibly due to DNA synthesis inhibition or disruption of membranes surrounding mitochondria and nuclei (Koitabashi et al., 1997; Zunino and Zygadlo, 2004; Nishida et al., 2005). Abrahim et al. (2003) have demonstrated that α-pinene acts on *Zea mays* L. seedling growth by two mechanisms, that is, uncoupling oxidative phosphorylation and inhibition of electron transfer, which result in the uncoupling of mitochondrial energy metabolism and inhibition of mitochondrial ATP production. In the same report, it is demonstrated that the actions of α-pinene on isolated mitochondria are consequences of unspecific disturbances in the inner mitochondrial membrane (Abrahim et al., 2003).

Antifungal activity

Essential oils isolated from *P. pinaster* needles and *C. sempervirens* var. *dupreziana* leaves were tested for antifungal activity against 10 agricultural fungal species that attack fruit trees and cereals and whose effects on crop loss were classified as severe to very severe. These results showed that oils significantly reduced the growth of the fungal species over a very broad spectrum. Oils exhibited different degrees of inhibition growth on tested fungi; *B. sorokiniana* was the most resistant to pine oil and *G. avenacea* was the most resistant to cypress oil (Table 4). However, *F. oxysporum* and *A. alternata*, were the most sensitive to pine and cypress oils, respectively, but the effects of the tested oils are considered fungistatic in all cases. Results confirm the antifungal activity of conifer essential oils reported by others authors (Lis-Balchin et al., 1998).

Pine and cypress species essential oils are known to have an antifungal activity and this activity was related to the high level of hydrocarbonated monoterpenes and some sesquiterpenes (Lis-Balchin et al., 1998; Amri et al., 2011b; 2013). A previous study of chemical composition and antifungal properties of pine species needle oil, that is, *P. densiflora* Siebold & Zucc., *P. koraiensis* Siebold & Zucc., *P. ponderosa* P. Lawson & C. Lawson, and *P. resinosa* Aiton reported that α- and β-pinene as predominant constituents and these compounds can be responsible for their antifungal properties (Krauze et al., 2002); this agrees with our results. Several authors have demonstrated the antifungal proprieties of these compounds; Sokovic and Griensven (2006) showed that α-pinene and limonene (MIC 4.0-9.0 μL mL^{-1}) had an effect against *Lecanicillium fungicola* (Preuss) Zare and Gams and *Trichoderma harzianum* Pers. 1794. Similar results were obtained by Lis-Balchin et al. (1998), who related the antifungal activity of essential oils to their high α and β-pinene content. Chang et al. (2008) studied and showed the fungicide activity of (*Z*)-caryophyllene and α- and β-pinene against *Fusarium solani* (Mart.) Sacc. and *Colletotrichum gloeosporioides* (Penz.).

Essential oils from various sources exhibit a broad spectrum of antimicrobial activity. Their biological activity has been related to their chemical composition. Compounds such as β-pinene, limonene, β-myrcene, and (*Z*)-caryophyllene have been shown to exert various biological activities. These compounds increase fungal cell permeability and membrane fluidity and inhibit medium acidification. Moreover, terpenes (mono- and sesquiterpenes) are thought to produce alterations in cell permeability by inserting themselves between the fatty acyl chains that make up the membrane lipid bilayers, disrupting lipid packing, and causing changes to membrane properties and functions such as interacting with the enzymes and proteins of the membrane, such as the membrane H^+/ATPase pumping. This produces a flux of protons towards the cell exterior which induces changes in the cells and, finally, their death (Sikkema et al., 1995; Christine et al., 2002; Cristani et al., 2007; Viuda-Martos et al., 2008; Tatsadjieu et al., 2009). This theory is strongly supported by data from previous studies that demonstrate changes in permeability and increases in membrane fluidity after treatment with terpenes (Uribe et al., 1985; Bard et al., 1988; Hammer et al., 2004).

Table 4. Antifungal activity of *Pinus pinaster* and *Cupressus sempervirens* var. *dupreziana* needle essential oils on 10 plant pathogens.

		Essential oil (4 μL mL^{-1})			
		Pine		Cypress	
Fungi	Control growth	Growth	Inhibition	Growth	Inhibition
	mm		%	mm	%
Gibberella avenacea	68.3 ± 1.4	32.3 ± 1.2	52.7bc	35.0 ± 1.4	48.8 ± 2.1a
Fusarium culmorum	61.7 ± 2.5	29.3 ± 1.2	52.1bc	24.0 ± 2.8	61.1 ± 4.6b
Fusarium oxysporum	75.0 ± 2.5	33.3 ± 0.3	55.5c	24.5 ± 0.7	67.3 ± 0.9bc
Fusarium subglutinans	61.3 ± 1.1	31.3 ± 1.3	48.9abc	23.5 ± 2.1	61.7 ± 3.4b
Fusarium verticillioides	56.3 ± 0.3	25.6 ± 1.4	54.4c	19.5 ± 2.1	65.4 ± 3.8bc
Fusarium nygamai	48.0 ± 1.5	25.0 ± 2.5	47.9abc	16.5 ± 0.7	65.6 ± 1.5bc
Rhizoctonia solani	66.7 ± 0.3	30.3 ± 2.8	54.45c	22.0 ± 1.4	67.0 ± 2.1bc
Microdochium nivale	78.3 ± 0.9	36.7 ± 1.3	53.2bc	20.0 ± 2.8	65.8 ± 4.4bc
Alternaria alternata	64.3 ± 2.18	35.7 ± 0.88	44.6ab	19.0 ± 1.4	74.5 ± 2.6c
Fusarium culmorum	53.7 ± 1.85	30.7 ± 0.66	42.9a	22.0 ± 2.8	65.8 ± 4.4bc

Means in the same column with the same letter are not significantly different according to the Student-Newman-Keuls test (P ≤ 0.05). Values are expressed as means ± standard error mean.

Regarding pine and cypress oils reported in this study, their richness in monoterpenes, particularly in α-pinene, can significantly contribute to their antifungal activity as mentioned above and emphasized by Bougatsos et al. (2004). In addition, the antifungal activity of our samples might not only be attributable to their major components. There is another option to whole oil action through a synergistic effect of individual compounds on each other.

CONCLUSIONS

Our study could provide a solution focused on the chemical composition of essential oils extracted from Tunisian *Pinus pinaster* and *Cupressus sempervirens* var. *dupreziana* and their effectiveness as antifungal and herbicidal agents. Results of essential oil bioactivities showed that the oils exhibited stronger phytotoxic and antifungal effects. Based on our preliminary results, these essential oils could be proposed as alternative herbicides and fungicides. However, further studies are required to determine the cost, applicability, safety, and phytotoxicity against plants of these agents as potential bio-pesticides.

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