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Chemical Composition and Biological Activities of Tunisian *Cupressus arizonica* GREENE Essential Oils

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The chemical composition of the essential oils obtained by hydrodistillation of leaves, stems, and female cones of *Cupressus arizonica* GREENE, grown in Tunisia, was studied by GC-FID and GC/MS analyses. Altogether, 62 compounds were identified, 62 in the leaf oil, 19 in the cone oil, and 24 in the stem oil. The cone and stem oils were mainly composed by monoterpene hydrocarbons (96.6 and 85.2%, resp.). In the leaf oil, the total sesquiterpene fraction constituted 36.1% and that of the monoterpene hydrocarbons 33.8% of the total oil composition. The three oils were evaluated for their *in vitro* herbicidal activity by determining their influence on the germination and the shoot and root growth of the four weed species *Sinapis arvensis* L., *Lolium rigidum* GAUDIN, *Trifolium campestre* SCHREB., and *Phalaris canariensis* L. At the highest doses tested (0.8 and 1.0 mg/ml), the leaf essential oil inhibited either totally or almost completely the seed germination and the shoot and root growth of *S. arvensis* and *T. campestre*. The oils were also tested for their antifungal activity; however, their effects on the fungal growth were statistically not significant.

Introduction. – Plants produce a wide and different selection of products, among these, essential oils. In the Mediterranean region, essential oils from aromatic plants play important roles in the protection of plants, having antimicrobial, antiviral, insecticidal, and antiherbivore properties [1] and being implicated in plant interactions with the environment [2]. The phytotoxic activity of essential oils and their main compounds has been studied, showing that these secondary metabolites may influence seed germination and seedling growth of many weeds and cultivated crops [3]. *Cupressus arizonica* GREENE (Cupressaceae), the Arizona cypress, is a coniferous tree native to the southwest of North America, Arizona, and the southwest of New Mexico. It is cultivated as an ornamental tree, but it can also be used for timber, as a privacy screen, and protection against wind. Moreover, this tree has proved to be very suitable as a pioneer species for reforestation, as it can tolerate poor, barren, and superficial

soils. For all these reasons, the Arizona cypress has been introduced in geographic areas that extend far beyond its natural distribution [4]. It has also been introduced in Tunisia as an ornamental and windbreak species and for reforestation [5].

The available literature reports some studies on the possible biological effects of *C. arizonica.* An aqueous extract of the dried leaves of *C. arizonica* inhibited the germination and seedling growth of *Lolium perenne* L. and *Poa pratensis* L., provoking anatomical changes on seedlings and altering the plant structure [6]. *Arouiee et al.* [7] reported the allelopathic effect of aqueous and hydroalcoholic extracts of *C. arizonica* leaves on the seed germination of *L. perenne* L. and *Festuca arundinacea* SCHREB.

Moreover, several studies on the composition of the essential oils obtained from leaves, female cones, and stems of *C. arizonica* have been reported [8–14]. However, only little information could be found in the literature about the possible biological activities of the essential oil of *C. arizonica* [9][13][15][16], and no reports are available on its herbicidal activity.

In this study, the chemical composition of the essential oils obtained from leaves, cones, and stems of *C. arizonica* was investigated, and their possible *in vitro* effects on the germination and the shoot and root growth of four common weeds (*Sinapis arvensis* L., *Lolium rigidum* GAUDIN, *Trifolium campestre* SCHREB., and *Phalaris canariensis* L.) and against ten phytopathogenic fungal strains were evaluated.

Results and Discussion. – *Essential-Oil Yield.* Hydrodistillation of the leaves, cones, and stems of *C. arizonica* afforded pale yellow oils with yields of 0.4, 1.3, and 0.5% (*v*/*w*, on the fresh weight basis), respectively.

Essential-Oil Composition. The GC-FID and GC/MS analyses of the essential oils obtained from *C. arizonica* leaves, cones, and stems collected in winter 2011 allowed the identification of 62 compounds, representing 95.2–98.6% of the total oil compositions. *Table 1* shows the chemical composition of the three oils, with the compounds being listed according to their elution order on a *HP-5 MS* capillary column.

Leaf-Oil Composition. Altogether, 62 compounds were identified in the leaf oil, accounting for 98.3% of the total oil composition (*Table 1*). In this oil, the monoterpene and the sesquiterpene fractions accounted for 58.5 and 36.4% of the total oil, respectively. The main compounds identified were umbellulone (17.9%), α-pinene (10.3%), and β-cubebene (10.1%). Other compounds represented at considerable amounts were limonene (8.8%) and α-cedrene (8.2%). *Marchuk et al.* [11] reported umbellulone (12.0%) and *cis*-muurola-4(14),5-diene (12.0%) as the major compounds in the leaf oil of *C. arizonica*. Umbellone has been reported by other authors as the principal component of the leaf oil [17][18], while the leaf essential oil of an Iranian sample of *C. arizonica* was dominated by limonene (14.4%) and umbellulone (13.2%) [12].

Cone-Oil Composition. In the cone oil, 19 compounds were identified, accounting for 98.6% of the total oil composition (*Table 1*). Monoterpene hydrocarbons largely dominated the composition of this oil with a content of 96.6%, while the sesquiterpene hydrocarbons represented only 1.1%. The major compound in this essential oil was the monoterpene hydrocarbon α-pinene (79.7%), which is in good agreement with the available literature [9–11][13][14][17].

Table 1. *Composition of the Essential Oils Isolated from Leaves, Cones, and Stems of Tunisian* Cupressus arizonica

Compound name and class	RI_a[a])	RI_p[b])	Content [%]			Identification[c])
			Leaf oil	Cone oil	Stem oil	
Tricyclene	926	1013	0.1	0.1	0.1	*RI*, MS
α-Thujene	930	1014	0.7	–[d])	0.1	*RI*, MS
α-Pinene	939	1075	10.3	79.7	76.6	*RI*, MS, Co-I
α-Fenchene	952		0.1	–	–	*RI*, MS
Camphene	954	1076	2.4	–	0.1	*RI*, MS
Sabinene	975	1132	1.6	0.1	–	*RI*, MS
β-Pinene	979	1118	0.2	0.4	1.7	*RI*, MS, Co-I
β-Myrcene	990	1112	1.3	0.2	–	*RI*, MS, Co-I
δ-Car-2-ene	1002	1146	1.5	–	–	*RI*, MS
α-Phellandrene	1002	1176	0.2	–	–	*RI*, MS, Co-I
δ-Car-3-ene	1011	1160	1.5	10.9	2.3	*RI*, MS
p-Cymene	1024	1269	1.6	–	0.1	*RI*, MS
Limonene	1029	1203	8.8	3.9	2.6	*RI*, MS
δ-Terpinene	1059	1266	2.0	0.1	–	*RI*, MS, Co-I
Terpinolene	1088	1290	1.4	1.0	1.6	*RI*, MS
p-Mentha-1,4,8-triene	1088		0.1	0.2	–	*RI*, MS
Nonan-2-one	1090	1392	0.2	–	0.3	*RI*, MS
Linalool	1096	1553	0.1	–	0.1	*RI*, MS
β-Fenchol	1121	1098	0.4	–	–	*RI*, MS
Alloocimene	1132		0.1	–	–	*RI*, MS
Terpine-1-ol	1133		0.1	0.2	–	*RI*, MS
Camphor	1146	1532	0.2	–	0.1	*RI*, MS
Umbellulone	1171	1656	17.9	–	–	*RI*, MS
Terpinen-4-ol	1177	1611	3.0	0.1	2.3	*RI*, MS
Borneol	1184	1719	0.4	–	–	*RI*, MS
α-Terpineol	1188	1706	0.1	0.2	–	*RI*, MS
(*E*)-Piperitol	1196	1757	0.1	–	–	*RI*, MS
β-Citronellol	1225	1772	0.1	–	–	*RI*, MS
Thymyl ethyl ether	1235	1607	0.2	–	0.1	*RI*, MS
Geraniol	1258	1857	0.1	–	–	*RI*, MS
Isobornyl acetate	1285		0.3	–	0.1	*RI*, MS
Thymol	1290	2198	0.1	–	–	*RI*, MS, Co-I
(*E*)-α-Ionol	1377		1.5	–	–	*RI*, MS
β-Cubebene	1388	1547	10.1	–	–	*RI*, MS
β-Bourbonene	1388	1535	0.2	–	–	*RI*, MS
β-Elemene	1390	1600	0.6	–	0.1	*RI*, MS
α-Cedrene	1411	1568	8.2	0.9	1.3	*RI*, MS, Co-I
Aromadendrene	1441	1628	3.6	–	0.2	*RI*, MS
α-Humulene	1454	1689	0.4	0.1	–	*RI*, MS
Dehydroaromadendrene	1462		1.1	–	–	*RI*, MS
δ-Muurolene	1479	1684	2.6	–	0.1	*RI*, MS
ar-Curcumene	1480	1784	0.2	–	0.2	*RI*, MS
Germacrene D	1485	1726	0.5	0.1	–	*RI*, MS, Co-I
β-Selinene	1490	1717	0.5	–	–	*RI*, MS
α-Farnesene	1505	1740	0.3	–	–	*RI*, MS
(*E*)-Calamanene	1529	1839	2.3	–	3.4	*RI*, MS
α-Calacorene	1545	1918	0.5	–	–	*RI*, MS

Table 1 (cont.)

Compound name and class	RI_a[a])	RI_p[b])	Content [%]			Identification[c])
			Leaf oil	Cone oil	Stem oil	
Spathulenol	1578	2150	0.2	–	–	*RI*, MS
Alloaromadendrene epoxide	1641	1897	0.1	–	–	*RI*, MS
α-Cedrol	1600	2120	2.1	0.1	1.5	*RI*, MS
β-Oplopenone	1607	2100	0.1	–	–	*RI*, MS
τ-Cadinol	1640	2187	0.7	–	–	*RI*, MS
α-Cadinol	1654	2255	0.1	–	0.1	*RI*, MS, Co-I
β-Acorenone	1692	1988	0.3	0.1	–	*RI*, MS
14-Norcadin-5-en-4-ol	1697		1.4	–	–	*RI*, MS
Hydrocinnamaldehyde	1738		0.4	–	–	*RI*, MS
Guaiazulene	1856		0.3	–	–	*RI*, MS
Kaur-15-ene	1997		0.2	–	–	*RI*, MS
Manoyl oxide	2010	2393	1.8	0.2	–	*RI*, MS
Abietatriene	2056	2625	0.2	–	–	*RI*, MS
Nezukol	2133		0.3	–	–	*RI*, MS
(*Z*)-Totarol	2314		0.2	–	0.1	*RI*, MS
Total identified			**98.2**	**98.6**	**95.2**	
Monoterpene hydrocarbons			23.1	96.6	85.2	
Oxygenated monoterpenes			35.4	0.5	2.7	
Sesquiterpene hydrocarbons			31.4	1.1	5.3	
Oxygenated sesquiterpenes			5.0	0.2	1.6	
Diterpene hydrocarbons			0.4	–	–	
Oxygenated diterpenes			2.3	0.2	0.1	
Others			0.6	–	0.3	

[a])[b]) RI_a and RI_p are the *Kovats* retention indices determined relative to a series of *n*-alkanes (C_{10}–C_{35}) on the apolar *HP-5 MS* and the polar *HP Innowax* capillary columns, respectively. [c]) Identification method: *RI*, comparison of *Kovats* retention indices with published data; MS, comparison of mass spectra with those listed in the *NIST 02* and *Wiley 275* libraries and with published data; Co-I, coinjection with authentic compound. [d]) – : Not detected.

Stem-Oil Composition. In the stem oil, 24 compounds were identified, accounting for 95.2% of the total oil composition (*Table 1*). The monoterpene hydrocarbons composed 85.2% of the oil, while the total sesquiterpene content was 6.9%. The major compound in this oil was α-pinene (76.6%), as in the cone oil. The present data conform well to the literature [5][8][17].

Multivariate Statistical Analysis. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were carried out to evaluate whether the identified essential oil components may be useful in reflecting the variability relationships between the leaf, cone, and stem essential oils of *C. arizonica*. As variables for the PCA and the HCA, 13 compounds presenting a content of at least 1.5% in at least one of the oils extracted from the three plant organs were selected (*Fig.*). The first principal component (horizontal axis) of the PCA explained 75.6% of the total variance and the vertical axis a further 24.3% (*Fig.*). According to both the HCA (results not shown) and the PCA (*Fig.*), the three oil samples were divided into two principal groups with a dissimilarity >20. The leaf oil constituted a separate group, *i.e.*, *Group A*, while the

stem and cone oils composed *Group B*. The latter was further divided into two subgroups (stem and cone oils) with a dissimilarity <2. Since the essential oil components within the same group were significantly correlated and tended to vary in the same way, each group can be considered as a chemotype (*Fig.*). In fact, *Group A*, composed of the leaf oil, was highly positively correlated with the vertical axis, with an essential oil distinguished by high contents of umbellulone. *Subgroup B1*, comprising the stem oil, was highly positively correlated with the horizontal axis, as *Subgroup B2*, constituted of the cone oil, with both oils showing a high content of α-pinene.

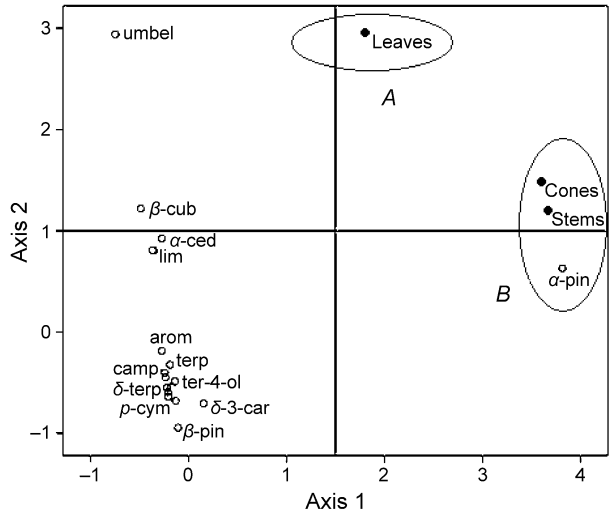

Figure. *Principal component analysis based on 13 compounds identified in the essential oils isolated from the aerial parts* (leaves, cones, and stems) *of* Cupressus arizonica. Abbreviations of the compounds: α-pin, α-pinene; camp, camphene; β-pin, β-pinene; δ-3-car, δ-3-carene; *p*-cym, *p*-cymene; lim, limonene; δ-terp, δ-terpinene; terp, terpinolene; umbel, umbellulone; ter-4-ol, terpinen-4-ol; β-cub, β-cubebene; α-ced, α-cedrene; arom, aromadendrene.

Herbicidal Activity of the Essential Oils. The herbicidal activity of the three essential oils was evaluated by determining their influence on the germination and the shoot and root growth of four weed species (*Tables 2–4*). The oils affected the germination and the shoot and root growth in different ways. The leaf essential oil showed to be more effective than the other two essential oils. Indeed, the seed germination of *S. arvensis* and *T. campestre* were either totally or almost completely inhibited by the leaf essential oil at the highest doses tested (0.8 and 1.0 mg/ml; *Table 2*). At both highest doses, the leaf essential oil also totally inhibited the shoot and the root growth of *S. arvensis*, and, at 1.0 mg/ml, as well the shoot and root growth of *T. campestre* were totally inhibited (*Tables 3* and *4*). At the same doses, the leaf essential oil of *C. arizonica* partially reduced the germination and the shoot and root growth of *P. canariensis* and *L. rigidum*. On the contrary, the stem and cone essential oils of *C. arizonica* were less active on all four weeds. The difference in biological activity of the oils seems to be related to their different chemical composition. The leaf oil was much richer in oxygenated monoterpenes (23.2%) than the cone and stem oils (0.5 and 2.7%, resp.).

Graña et al. [19] reported the mode of action of oxygenated monoterpenes and monoterpene hydrocarbons in plant–plant interactions (germination, seedling and shoot growth, and radical elongation). Moreover, 1,8-cineole and camphor were shown to have strong phytotoxic effects against various plants [20][21]. In a previous paper, we showed that, among monoterpenes, alcohols, phenols, and ketones were more active than the compounds possessing other chemical groups [22].

Table 2. *Effect of Increasing Doses of* Cupressus arizonica *Leaf, Cone, and Stem Essential Oils on the Seed Germination of Four Weed Species*

Weed	Dose [mg/ml]	Germination [%][a])		
		Leaf oil	Cone oil	Stem oil
Sinapis arvensis	0.0 (Control)[b])	100.0±0.0 (a)	100.0±0.0 (a)	100.0±0.0 (a)
	0.2	63.3±5.8 (b)	100.0±0.0 (a)	100.0±0.0 (a)
	0.4	46.7±5.8 (c)	93.3±5.8 (a)	93.3±5.8 (a)
	0.6	13.3±5.8 (d)	100.0±0.0 (a)	96.7±5.8 (a)
	0.8	0.0±0.0 (e)	76.7±5.8 (b)	90.0±10.0 (a)
	1.0	0.0±0.0 (e)	73.3±5.8 (b)	83.3±5.8 (b)
Trifolium campestre	0.0 (Control)	96.7±5.8 (a)	96.7±5.8 (a)	96.7±5.8 (a)
	0.2	73.3±11.5 (b)	100.0±0.0 (a)	100.0±0.0 (a)
	0.4	50.0±10.0 (c)	96.7±5.8 (a)	93.3±5.8 (a)
	0.6	23.3±5.8 (d)	96.7±5.8 (a)	96.7±5.8 (a)
	0.8	3.3±5.8 (e)	83.3±5.8 (a)	90.0±10.0 (a)
	1.0	3.3±5.8 (e)	83.3±11.5 (a)	86.7±5.8 (a)
Phalaris canariensis	0.0 (Control)	93.3±5.8 (a)	93.3±5.8 (a)	93.3±5.8 (a)
	0.2	70.0±10.0 (b)	86.7±5.8 (a)	96.7±5.8 (a)
	0.4	63.3±5.8 (b)	86.7±5.8 (a)	90.0±10.0 (a)
	0.6	56.7±5.8 (b)	86.7±5.8 (a)	93.3±5.8 (a)
	0.8	43.3±5.8 (c)	96.7±5.8 (a)	83.3±5.8 (a)
	1.0	26.7±5.8 (d)	83.3±5.8 (a)	96.6±5.8 (a)
Lolium rigidum	0.0 (Control)	86.7±5.8 (a)	86.7±5.8 (a)	86.7±5.8 (a)
	0.2	73.3±3.3 (b)	80.0±0.0 (a)	93.3±5.8 (a)
	0.4	60.0±10.0 (c)	86.7±5.8 (a)	86.7±5.8 (a)
	0.6	43.3±5.8 (d)	96.7±5.8 (a)	90.0±0.0 (a)
	0.8	46.7±5.8 (d)	90.0±10.0 (a)	93.3±5.8 (a)
	1.0	33.3±5.8 (d)	86.7±5.8 (a)	86.7±5.8 (a)

[a]) Values are means±standard deviations ($n=3$); means followed by the same letter in parentheses within the same column are not significantly different by the *Student–Newman–Keuls* test ($p>0.05$). [b]) The control contains dist. H_2O instead of the essential oil.

Antifungal Activity. The percentages of fungal-growth inhibition induced by the essential oils obtained from the leaves, cones, and stems of *C. arizonica* at a dose of 4 mg/ml were similar, and their statistical significance was not relevant.

Conclusions. – The chemical composition of Tunisian *C. arizonica* essential oils obtained from three different plant organs, *i.e.*, leaves, cones, and stems, were characterized. The composition of the leaf oil was clearly distinct form that of the cone and stem oils. Moreover, the *C. arizonica* oils, especially the leaf oil, revealed good *in*

Table 3. *Effect of Increasing Doses of* Cupressus arizonica *Leaf, Cone, and Stem Essential Oils on the Shoot Growth* (percentage of growth) *of Four Weed Species*

Weed	Dose [mg/ml]	Shoot Growth [%][a])		
		Leaf oil	Cone oil	Stem oil
Sinapis arvensis	0.0 (Control)[b])	13.3±1.6 (a)	13.3±1.6 (a)	13.3±1.6 (a)
	0.2	11.3±1.3 (b)	15.0±1.9 (ab)	12.7±2.3 (a)
	0.4	7.3±1.1 (c)	13.7±2.8 (ab)	14.0±1.3 (a)
	0.6	5.6±1.0 (c)	11.5±0.6 (ab)	11.4±1.2 (ab)
	0.8	0.0±0.0 (d)	11.6±2.7 (ab)	9.1±0.9 (ab)
	1.0	0.0±0.0 (d)	9.3±1.57 (b)	11.7±0.9 (b)
Trifolium campestre	0.0 (Control)	13.4±0.9 (a)	13.4±0.9 (a)	13.4±0.9 (a)
	0.2	7.3±1.1 (b)	13.8±2.2 (a)	12.0±1.9 (a)
	0.4	6.4±1.2 (b)	11.0±2.3 (a)	13.2±1.3 (ab)
	0.6	6.2±0.6 (b)	11.8±2.2 (a)	8.6±0.7 (b)
	0.8	1.4±0.4 (c)	11.3±1.1 (a)	9.7±2.1 (b)
	1.0	0.0±0.0 (c)	10.4±0.8 (a)	8.8±1.3 (b)
Phalaris canariensis	0.0 (Control)	15.7±1.0 (a)	15.8±1.0 (a)	15.8±1.0 (a)
	0.2	11.2±2.3 (b)	13.6±1.0 (ab)	12.9±1.9 (a)
	0.4	8.4±0.8 (c)	13.2±2.3 (ab)	12.5±2.4 (a)
	0.6	6.1±0.9 (cd)	9.7±1.5 (bc)	11.7±1.4 (a)
	0.8	4.7±1.21 (de)	10.7±0.9 (c)	12.2±1.8 (a)
	1.0	3.9±0.9 (e)	9.1±1 (c)	11.0±1.6 (a)
Lolium rigidum	0.0 (Control)	15.1±2.1 (a)	15.1±2.1 (a)	15.1±2.0 (a)
	0.2	12.6±1.1 (ab)	15.5±1.4 (a)	14.7±1.5 (a)
	0.4	13.4±2.2 (ab)	13.8±2.8 (a)	12.4±3.5 (a)
	0.6	11.1±1.5 (b)	11.0±3.6 (a)	10.7±0.9 (a)
	0.8	8.6±0.7 (c)	10.0±1.8 (a)	10.4±2.2 (a)
	1.0	5.2±1.1 (d)	10.9±2.0 (a)	10.7±0.8 (a)

[a]) Values are means±standard deviations ($n=3$); means followed by the same letter in parentheses within the same column are not significantly different by the *Student–Newman–Keuls* test ($p>0.05$). [b]) The control contains dist. H_2O instead of essential oil.

vitro herbicidal activity and might be useful as natural pesticides. On the other hand, the oils showed no significant antifungal activity. Further studies are needed to explore the Mediterranean flora and to identify biologically active secondary metabolites.

Experimental Part

Plant Material. The leaves, cones, and stems of *Cupressus arizonica* Greene were collected from the arboretum of the National Institute of Researches on Rural Engineering, Water and Forests (INRGREF), in the region of Ain Draham (Tunisia), in December 2011. Five samples were collected from more than five different trees, mixed for homogenization, and used in three replicates for essential oil extractions. The plant material was identified by *L. H.* (INRGREF), and a voucher specimen was deposited with the Herbarium of the Institute.

Isolation of the Essential Oils. To isolate the essential oils of the different plant parts, 100 g each of fresh leaves, cones, and stems of *C. arizonica* were separately ground in a *Waring* blender and then subjected to hydrodistillation in a *Clevenger*-type apparatus for 3 h, according to the standard procedure

Table 4. *Effect of Increasing Doses of* Cupressus arizonica *Leaf, Cone, and Stem Essential Oils on the Root Growth* (percentage of growth) *of Four Weed Species*

Weed	Dose [mg/ml]	Root Growth [%][a])		
		Leaf oil	Cone oil	Stem oil
Sinapis arvensis	0.0 (Control)[b])	13.2±1.8 (a)	13.2±1.8 (ab)	13.2±1.8 (a)
	0.2	7.4±1.3 (b)	15.0±1.2 (a)	13.3±1.2 (a)
	0.4	5.6±1.0 (b)	12.8±1.5 (ab)	11.0±0.7 (a)
	0.6	2.3±0.7 (c)	13.6±2.6 (ab)	11.2±1.8 (a)
	0.8	0.0±0.0 (d)	11.9±1.3 (ab)	10.4±1.3 (a)
	1.0	0.0±0.0 (d)	10.1±1.9 (b)	10.0±1.4 (a)
Trifolium campestre	0.0 (Control)	14.4±1.4 (a)	14.4±1.4 (a)	14.4±1.4 (ab)
	0.2	5.5±1.2 (b)	13.7±0.6 (a)	15.7±1.3 (a)
	0.4	3.3±0.9 (c)	12.9±1.7 (a)	12.4±1.8 (bc)
	0.6	2.0±1.2 (cd)	13.7±2.6 (a)	10.4±0.8 (c)
	0.8	0.4±0.7 (d)	11.6±1.3 (a)	9.8±1.5 (c)
	1.0	0.0±0.0 (d)	9.4±0.7 (a)	9.5±1.1 (c)
Phalaris canariensis	0.0 (Control)	11.3±0.9 (a)	11.3±0.9 (ab)	11.3±0.9 (a)
	0.2	9.7±1.2 (a)	12.4±1.1 (ab)	12.1±1.8 (a)
	0.4	5.5±1.5 (b)	10.5±1.4 (ab)	11.1±1.6 (a)
	0.6	3.8±0.9 (bc)	12.7±1.6 (a)	9.4±0.8 (ab)
	0.8	2.6±0.7 (c)	12.0±1.2 (ab)	9.8±1.7 (ab)
	1.0	1.8±0.7 (c)	9.4±0.8 (b)	7.7±0.7 (b)
Lolium rigidum	0.0 (Control)	12.6±1.1 (a)	12.6±1.1 (a)	12.6±1.1 (a)
	0.2	8.3±1.1 (b)	10.2±0.7 (a)	9.5±1.3 (b)
	0.4	5.5±0.7 (c)	11.7±1.2 (a)	8.9±1.0 (b)
	0.6	5.1±1.9 (c)	9.3±2.3 (a)	8.7±0.5 (b)
	0.8	2.5±0.7 (d)	12.0±3.0 (a)	9.4±0.7 (b)
	1.0	1.5±0.7 (d)	13.0±1.8 (a)	8.9±0.7 (b)

[a]) Values are means±standard deviations ($n=3$); means followed by the same letter in parentheses within the same column are not significantly different by the *Student–Newman–Keuls* test ($p>0.05$). [b]) The control contains dist. H_2O instead of essential oil.

described in the *European Pharmacopoeia* [23]. The oils were dried (Na_2SO_4) and stored at 4° in the dark until tested and analyzed. The calculated essential-oil yield was expressed in % (*v*/*w*), based on the weight of the fresh plant material. All extractions were done in triplicate.

GC-FID Analysis. The GC-FID analyses were carried out with a *Hewlett-Packard 5890 series II* apparatus (*Agilent Technologies*, CA, USA) equipped with a flame ionization detector (FID) and two fused-silica cap. columns, an apolar *HP-5 MS* (30 m × 0.25 mm i.d., film thickness 0.25 μm) and a polar *HP Innowax* (50 m × 0.20 mm i.d., film thickness, 0.25 μm) column. The oven temp. was programmed isothermal at 50° for 1 min, then rising from 50 to 240° at 5°/min, and finally held isothermal at 240° for 4 min; injector temp., 250°; detector temp., 280°; carrier gas, N_2 (1.2 ml/min); injection volume, 0.1 ml (1% essential-oil soln. in hexane). Quantitative data, *i.e.*, compound contents in the essential oils, expressed as percentages, were obtained electronically from the FID-area data (peak area normalization), without using correction factors. The *Kovats* retention indices (*RI*s) were determined rel. to the retention times (t_R) of a series of *n*-alkanes (C_{10}–C_{35}) analyzed under the same operating conditions.

GC/MS Analysis. The GC/MS analyses were performed using a *Hewlett-Packard 5890* series *II* apparatus equipped with a *HP 5972* mass selective detector and a *HP-5 MS* cap. column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temp. was programmed as described above (*cf. GC-FID Analysis*);

injector temp., 250°; detector temp., 280°; carrier gas, He (1.2 ml/min); injection volume, 0.1 ml (1% essential-oil soln. in hexane); split ratio, 1:50; ionization energy, 70 eV, mass range, 40–300 amu; scan time, 1.5 s. The software adopted to handle mass spectra and chromatograms was ChemStation.

Identification of Essential Oil Components. The identification of the essential-oil constituents was based on the comparison of their *RI*s, determined rel. to the t_R of *n*-alkanes (C_{10}–C_{35}) on both cap. columns, with those of the literature [24–27] and their mass spectra with those of authentic compounds available in our laboratories or those listed in the *NIST 02* and *Wiley 275* mass spectral libraries [28]. For some compounds, the identification was confirmed by coinjection with an authentic sample (*cf. Table 1*)

Multivariate Statistical Analyses of the Essential Oils Extracted from the Different Aerial Parts. To evaluate whether the identified components in the leaf, cone, and stem essential oils of *C. arizonica* are useful in reflecting the chemical relationships between plant organs, 13 compounds presenting a content of at least 1.5% in at least one of the oils extracted from the three plant organs were subjected to principal component analysis (PCA) and hierarchical cluster analysis (HCA) using SPSS 17.0 software [29].

Herbicidal Activity of the Essential Oils: Influence on Seed Germination and Seedling Growth. The herbicidal potential of the essential oils obtained from the leaves, cones, and stems of *C. arizonica* was evaluated by investigating their effect on the seed germination and the shoot and root growth of four weeds. The seeds of *Sinapis arvensis* L., *Lolium rigidum* GAUDIN, *Trifolium campestre* SCHREB., and *Phalaris canariensis* L. were collected in July 2011 from parent plants growing in Tunisia. The seeds were surface sterilized in 15% NaClO for 20 min and then rinsed with abundant dist. H_2O. Empty and undeveloped seeds were discarded by floating in tap water, and the remaining seeds were sown in *Petri* dishes (90 mm diameter) containing two layers of *Whatman* filter paper impregnated with 8 ml of either the negative control soln. (1% (*v*/*v*) soln. of *Tween 20*® in H_2O), the positive control soln. (the herbicide 2,4-D isooctyl ester), or the essential oil solns. at the different assayed doses. The essential oils were dissolved in and diluted to the desired doses (0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml) with a 1% (*v*/*v*) soln. of *Tween 20*® in H_2O. Afterward, 20 seeds of *S. arvensis*, *L. rigidum*, *T. campestre*, and *P. canariensis* were placed on the impregnated filter papers. The *Petri* dishes were closed with an adhesive tape to prevent escaping of volatile compounds and kept at 25° in a growth chamber supplied with 12 h of fluorescent light [30]. The number of germinated seeds and the seedling lengths were measured after 10 d. All tests were arranged in a completely randomized design with three replications for each treatment and control. The experimental data for the germination and seedling-growth inhibition were subjected to one-way analysis of variance (ANOVA) using the SPSS 13.0 software package. The significance of the differences between means was evaluated with the *Student–Newman–Keuls* (SNK) test and values of $p \leq 0.05$ were considered significantly different [29].

Antifungal Activity. The antifungal activity against *Fusarium culmorum*, *F. avenaceum*, *F. oxysporum*, *F. subglutinans*, *F. verticillioides*, *F. nygamai*, *Bipolaris sorikoniana*, *Botrytis cinerea*, *Microdochium nivale*, and *Alternaria* sp. was studied by using the contact assay *in vitro*, which evaluates the hyphal-growth inhibition [31]. All the phytopathogenic fungal strains were obtained from the culture collection of the Tunisian National Institute of Agronomic Research (INRAT). Cultures of each strain were maintained on potato dextrose agar (PDA) at 4° and were stored in 1 ml of glycerol 25% at −20°. The essential oils were dissolved in 1 ml of a 0.1% (*v*/*v*) soln. of *Tween 20*® in H_2O and then added into 20 ml of PDA at 50° to obtain a final concentration of 4 mg/ml. Mycelia disks of 5 mm in diameter, cut from the periphery of 7-day-old cultures, were inoculated in the center of each PDA plate (90 mm diameter) and then incubated at 24° for 7 d. PDA plates treated with *Tween 20*® (0.1%) without essential oil were used as first negative control, while PDA plates treated without *Tween 20*® and without essential oil were used as second negative control. Tests were performed in triplicate. After the incubation period, the antifungal activity of the oils was evaluated by measuring the diameter of the radial growth of the fungal colonies. Growth inhibition was calculated as the percentage of inhibition of radial growth relative to the control according to *Eqn. 1:*

$$\% \text{ Inhibition} = (C - T)/C \times 100 \quad (1)$$

where *C* and *T* are the average of three replicates of hyphal extension (mm) in the control and the essential-oil treated plates, resp. [32].

The data obtained for the fungal inhibition were subjected to one-way analysis of variance (ANOVA) using the SPSS 13.0 software package. The significance of the differences between means was evaluated with the *Student–Newman–Keuls* (SNK) test and values of $p \leq 0.05$ were considered significantly different [29].

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