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Phytotoxic Effects of Essential Oils from Six Lamiaceae Species

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Abstract: Essential oils produced by plants, and their components, could be sources of new natural herbicidal compounds. Thirteen oils extracted from six wild Lamiaceae species (namely *Clinopodium suaveolens* (Sm.) Kuntze, *Satureja montana* L. subsp. *montana*, *Thymbra capitata* (L.) Cav., *Salvia fruticosa* Mill. subsp. *thomasii* (Lacaita) Brullo, Guglielmo, Pavone & Terrasi, *Satureja cuneifolia* Ten., and *Thymus spinulosus* Ten.) from South Italy were tested in vitro for the phytotoxic activity to cress and branched broomrape seeds, tomato radicles, and lambsquarters leaf disks. Moreover, the possible correlation between oil composition and biological activity was evaluated. One of the oils from *T. capitata* inhibited cress germination by 96.4% at the lowest tested concentration (100 ppm) and reduced both chlorophyll and carotenoid content in lambsquarters leaf disks by around 50%. Some oils, particularly those from *T. spinulosus*, inhibited tomato radicle elongation by 85% at 1000 ppm. Many oils inhibited broomrape seed germination up to 100% when tested in solution at 1000 ppm or released as vapors. Among the oil components, α-terpinene, p-cymene, β-*cis*-ocimene, *cis*-sabinene hydrate, carvacrol methyl ether, and thymol were mostly correlated to the inhibition of cress seeds germination and tomato radicle elongation. The presence of thymol and p-cymene was also correlated to the inhibition of broomrape seed germination. Some of the tested essential oils or their components could have potential as pre-emergence herbicides and could be useful in the development of new weed control strategies.

Keywords: germination inhibitors; parasitic weeds; broomrape; natural herbicides; natural products; bioherbicides; weed control

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1. Introduction

The prolonged and excessive use of synthetic herbicides has often caused the emergence of environmental problems, such as the appearance of weed resistant biotypes [1], or the accumulation in soils and ground water with adverse effects in living organisms and human health [2,3]. Herbicides based on natural products, especially plant secondary metabolites, are increasing in importance [4], and the study of natural products produced by rare plants could offer the chance to identify new herbicidal compounds. In this regard, essential oils (EOs) and their components, generally having low persistence in the field as well as low incidence of resistance in weeds [5], could be particularly attractive. EOs are natural complexes of semi-volatile and volatile aromatic compounds [6]. They are produced by plants to attract pollinators and defend against predator attacks, and their composition can vary within the same species according to environmental conditions and harvest site characteristics [7]. Those compounds are present in small amounts in different plant organs (leaves, stems, flowers, roots, or fruits) and their use has expanded in the last decades mainly for perfume, cosmetics, and food industries [8]. They have also already been studied for their phytotoxic activities [9–11]. In the European market, no commercial herbicides are available based on EOs, although European patent EP2684457A1 has been registered, based on oregano essential oils [12].

One of the main botanical family producing EOs is Lamiaceae (formerly Labiatae), including 7886 species, thus being the largest family of the Lamiales order (http://www.theplantlist.org/, accessed on 1 December 2022). Most of the species belonging to this family are aromatic. Lamiaceae species are largely present in the whole Mediterranean area, including Italy. EOs extracted from Lamiaceae have many biological applications and activities such as antioxidant, anti-inflammatory, antimicrobial, fungicidal, insecticidal [7], and proved to be effective in inhibiting seed germination [13–15].

Many Lamiaceae species were never or little considered for the herbicidal activity of their EOs, and neither the origin of the plants has been correlated with the biological activities. Hence, we tested the EOs extracted from some Lamiaceae species from the Apulia region (South Italy) having scarce or not exhaustive scientific literature.

EOs' phytotoxic effects were often related to the inhibition of seed germination and to the decrease of the chlorophyll content [9,11].

In this study, we aimed to find EOs with good herbicidal activity to be employed for weed management in organic farming systems. As the main phytotoxic effects occur mostly by leaf damages, germination, and radicle inhibition, we tested, in in vitro conditions, the herbicidal activity of 13 EOs extracted from plants of six Lamiaceae species, evaluating the effects against cress and branched broomrape seed germination, tomato radicle growth, lambsquarters leaf disk phytotoxicity symptoms, and chlorophyll content. We also evaluated if the oils extracted from plants of the same species but originating from different sites were distinguishable by their biological activity and chemical composition.

2. Materials and Methods

2.1. Essential Oils

A total of 13 EO samples, derived from wild plants of six species collected in the Apulia region (South Italy) in different locations (Table 1), were extracted by hydro-distillation and identified by gas chromatography coupled with mass spectrometry in a previous study [16,17]. These EOs were used in this study. Details on locations and environmental characteristics of the sites (climatic, geological, ecological, vegetational, and pedological) are given in the references recalled in the table. The chemical compositions of EOs are included in Table 2. Oils were stored at −20 °C before testing their biological activity.

Table 1. Aromatic plant species, harvest location, and extracted oil acronym.

EO Code	Species	Harvest Site	Reference
Cs1	*Clinopodium suaveolens*	Altamura	[17]
Cs2	*Clinopodium suaveolens*	Poggiorsini	
Sf1	*Salvia fruticosa* subsp. *thomasii*	Mottola	[17]
Sf2	*Salvia fruticosa* subsp. *thomasii*	Laterza	
Sc1	*Satureja cuneifolia*	Fasano	[16]
Sc2	*Satureja cuneifolia*	Mottola	
Sm1	*Satureja montana* subsp. *montana*	Fasano	[17]
Sm2	*Satureja montana* subsp. *montana*	Altamura	
Tc1	*Thymbra capitata*	Mottola	[17]
Tc2	*Thymbra capitata*	Fasano	
Ts1	*Thymus spinolosus*	Altamura	[16]
Ts2	*Thymus spinolosus*	Mottola	
Ts3	*Thymus spinolosus*	Carovigno	

Table 2. EOs chemical composition percentage of *Clinopodium suaveolens* (Cs), *Salvia fruticosa* subsp. *thomasii* (Sf), *Satureja cuneifolia* (Sc) *Satureja montana* subsp. *montana* (Sm), *Thymbra capitata* (Tc), and *T. spinulosus* (Ts) [16,17].

Code	Compound	Cs1	Cs2	Sf1	Sf2	Sc1	Sc2	Sm1	Sm2	Tc1	Tc2	Ts1	Ts2	Ts3
1	Methyl 3(Z)-Hexenyl Ether	0	0	0.03	0	0	0	0	0	0	0	0	0	0
2	cis-Salvene	0	0	0.06	0.09	0	0	0	0	0	0	0	0	0
3	3-Hexen-1-ol, *trans-*	0	0	0.05	0	0	0	0.07	0.08	0.1	0.11	0	0	0
4	trans-Salvene	0	0	0.01	0.01	0	0	0	0	0	0	0	0	0
5	2-Hexen-1-ol, *trans-*	0	0	0.01	0	0	0	0	0	0	0	0	0	0
6	1-Hexanol	0	0	0.02	0.01	0	0	0	0	0	0	0	0	0
7	2-α-Pinene	0	0	0.11	0.16	0	0	0	0	0	0	0	0	0
8	Tricyclene	0	0	0.24	0.01	0	0	0.11	1.15	0.01	0.02	0	0	0
9	α-Thujene	0.03	0.06	0.39	0.25	0	0	0	0	1.89	1.5	1.84	1.59	1.84
10	α-Pinene	0.51	0.63	5.05	3.58	36.8	38.82	26.96	0.69	0.94	0.69	0.95	0.73	0.83
11	2,4(10)-thujadien	0	0	0.03	0.01	1.28	0.36	0.01	0.02	0.02	0.01	0	0	0
12	Camphene	0.08	0.1	6.32	0.51	1.66	0.49	0.51	0.18	0.27	0.3	0.52	0.28	0.28
13	Verbenene	0	0	0.02	0.01	0	0	0.21	0	0	0	0	0	0
14	Sabinene	0.15	0.21	0	0	0.74	1.71	1.02	0.09	0.08	0.1	0.13	0.09	0.08
15	β-Pinene	0.65	0.72	0.37	0.2	0.36	0.38	0.74	0.62	0.39	0.25	0.87	0.72	0.94
16	1-Octen-3-ol	0	0	0.04	0.1	0	0	0.02	0.01	0.01	0.01	0	0	0
17	3-Octanone	0.01	0.01	0	0	0	0	0	0	0	0	0	0	0
18	β-Mircene	0.33	0.39	2.7	3.9	0	0	2.85	1.67	1.92	1.51	0	0	0
19	3-Octanol	0.1	0.08	0	0	0.09	0.07	0.04	0.02	0.04	0.07	0	0	0
20	Pseudolimonene	0.03	0.04	0.03	0.02	0	0	0	0	0	0	0	0	0
21	α-Phellandrene	0	0	0.05	0.04	0.81	0.71	0.88	0.27	0.34	0.29	0.18	0.14	0.18
22	δ-3-Carene	0	0	0	0	0	0	0.01	0.06	0.16	0.08	0	0	0
23	α-Terpinene	0.01	0.02	0.4	0.3	0.14	0.11	0.11	2.82	2.54	2.81	2.24	1.84	2.9
24	p-Cymene	0.04	0.06	1.17	0.87	1.55	0.63	0.41	10.43	9.25	9.67	17.87	17.51	17.5
25	dl-Limonene	3.35	2.37	1.41	0.84	5.08	6.39	7.05	0.61	0.72	0.59	0.51	0.41	1.43
26	Eucalyptol	0.07	0.08	40.22	60.94	0	0	0	0	0.01	0.01	0	0	0
27	β-Ocimene, *trans-*	0.02	0.02	0.01	0.02	2.8	3.62	11.45	1.98	0.01	0.01	2.48	1.14	0.9
28	β-Ocimene, *cis-*	0.02	0.03	0.67	0.53	1.17	1.52	5.05	0.58	0.07	0.06	15.4	11.68	10.97
29	ϒ-Terpinene	0	0	0	0	0.25	0.19	0.18	14.57	14.95	17.71	0	0	0
30	Sabinene hydrate, *cis-*	0	0	0	0	0.12	0.1	0.08	0.09	0.06	0.06	0.45	0.49	0.52
31	Terpinolene	0.02	0.03	0.16	0.11	0.61	0.3	0.26	0.1	0.14	0.1	0.14	0.14	0.14
32	p-Cymenene	0	0	0.04	0.03	0	0	0.11	0.03	0.03	0.04	0	0	0
33	Linalool	0.48	0.25	0.13	0.15	6.35	6.36	7.37	1.19	1.25	2.38	1.83	2.41	2.9
34	Thujone, *cis-*	0.02	0.01	4.26	4.89	0.1	0.05	0.11	0	0.03	0	0.06	0.49	0.05
35	Thujone, *trans-*	0	0	1.71	1.29	0	0	0	0	0.02	0.02	0	0	0
36	Chrysanthenone	0	0	0	0	0.31	0.1	0.03	0.03	0.06	0.05	0	0	0
37	α-Campholenal	0	0	0	0	1.05	0.33	0.19	0.01	0	0	0.05	0.05	0.19
38	trans-Pinocarveol	0	0	0.21	0.12	1.78	0.78	0	0	0	0	0	0	0
39	Sabinol, *cis-*	0	0	0	0	0	0	0.35	0.05	0	0	0	0	0
40	Verbenol, *cis-*	0	0	0	0	0	0	0.76	0.03	0	0	0	0	0
41	Camphor	0	0	14.88	1.89	0	0	0	0	0	0	0	0	0
42	trans-3-Caren-2-ol	0	0	0	0	4.1	1.9	0.16	0.01	0	0	0	0	0
43	Menthone	0.38	0.56	0	0	0	0	0	0	0	0	0	0	0
44	Pinocarvone	0	0	0	0	0.33	0.12	0.1	0.01	0	0	0	0	0
45	δ-Terpineol	12.22	17.2	0.76	0.81	0	0	0	0	0	0	0	0	0
46	Borneol	0	0	1.59	0	6.9	1.4	1.2	0.35	0.6	0.74	1	0.5	0.5
47	Isopulegone	1.51	1.65	0	0	0	0	0	0	0	0	0	0	0
48	Terpinene-4-ol	0.08	0.08	1.33	0.73	0	0	0.37	0.86	1.17	0.95	0	0	0
49	p-Cymen-8-ol	0	0	0	0	0	0	0.17	0.1	0.03	0.06	0	0	0
50	Hexyl butanoate	0	0	0	0	0	0	0	0	0.04	0.03	0	0	0
51	α-Terpineol	0.08	0.06	2.61	2.71	11.03	17.11	14.92	0.29	0.14	0.11	0.29	0.22	0.19
52	Decanal	0.03	0.03	0	0	0	0	0	0	0.02	0.01	0	0	0
53	Verbenone	0.04	0.03	0	0	0	0	0.15	0.01	0.01	0.01	0	0	0
54	Carveol, *trans-*	0	0	0	0	0.92	0.35	0.11	0.01	0	0	0	0	0
55	Nerol	0	0	0.05	0.01	0	0	0	0	0.04	0.03	0	0	0
56	Thymol, methyl ether	0	0	0	0	0	0	0.01	1.65	0	0	0	0	0
57	Carvacrol, methyl ether	0	0	0	0	0	0	0.01	3.87	0	0	2.05	3.19	0.54
58	Pulegone	79.48	75.1	0	0	0	0	0	0	0	0	0	0	0
59	Cumin aldehyde	0	0	0	0	0	0	0.08	0.02	0	0	0	0	0
60	Z-Citral	0	0	0.04	0	0	0	0	0	0	0	0	0	0
61	Bornyl acetate	0	0	0.06	0	0.21	0.07	0.09	0	0	0	0	0	0
62	Thymol	0	0	0.02	0.52	0	0	0.11	46.1	31.12	35.66	42.87	48.77	45.88
63	Carvacrol	0	0	0.02	0.24	0	0	0.1	2.17	26.01	17.44	0.94	0.62	2
64	Piperitenone	0.13	0.11	0	0	0	0	0	0	0	0	0	0	0
65	Thymol acetate	0	0	0.45	0.13	0	0	0	0.55	0.35	0.77	0	0	0
66	Carvacrol acetate	0	0	0	0	0	0	0	0	0.26	0.41	0	0	0
67	α-Copaene	0	0	0	0	0	0	0.69	0.04	0	0	0	0	0
68	β-Elemene	0	0	0	0	0	0	0.04	0.02	0	0	0	0	0
69	α-Gurjunene	0	0	0	0	0	0	0.06	0.1	0.04	0.02	0	0	0
70	Caryophyllene, *trans-*	0.06	0.04	1.78	1.72	0.07	0.08	0.31	0.9	2.42	2.29	2.07	1.22	2.22
71	α-Bergamotene, *trans-*	0	0	0	0	0	0	0.11	0.04	0.02	0.02	0	0	0
72	Aromadendrene	0	0	0.25	0.22	0	0	0.58	0.03	0.04	0.03	0	0	0
73	α-Humulene	0	0	0.57	0.66	0	0	0.1	0.03	0.03	0.01	0	0	0
74	β-Santalene	0	0	0	0	0	0	0.02	0.03	0.08	0.07	0	0	0
75	Alloaromadendrene	0	0	0	0	0	0	0.04	0.01	0.04	0.02	0	0	0
76	Germacrene D	0.08	0.06	0	0	0	0	0.31	0.59	0	0	0	0	0

Table 2. *Cont.*

Code	Compound	Cs1	Cs2	Sf1	Sf2	Sc1	Sc2	Sm1	Sm2	Tc1	Tc2	Ts1	Ts2	Ts3
77	Guaia-1(10),11-diene	0	0	0.08	0.06	0	0	0.07	0.02	0.04	0.03	0	0	0
78	β-Guaiene, *trans*-	0	0	0.1	0.04	0	0	1.85	0.09	0.19	0.29	0	0	0
79	β-Bisabolene	0	0	0	0	0	0	0	3.53	0.29	0.28	0	0	0
80	β-Curcumene	0	0	0	0	0	0	0.63	0	0	0	0	0	0
81	α-Muurolene	0	0	0.05	0.04	0	0	0.07	0.04	0	0	0	0	0
82	Calamenene, *cis*-	0	0	0.11	0.1	0	0	0.08	0.04	0.03	0.04	0	0	0
83	Sesquiphellandrene	0	0	0	0	0	0	0.03	0.04	0	0	0	0	0
84	α-Bisabolene, (E)-	0	0	0	0	0	0	0	0.1	0.22	0.24	0	0	0
85	Sesquisabinene hydrate, *cis*-	0	0	0	0	0	0	2.79	0.06	0	0	0	0	0
86	trans-Sesquisabinene hydrate	0	0	0	0	0	0	0.15	0.01	0	0	0	0	0
87	(−)-Spathulenol	0	0	0	0	0	0	0.61	0.03	0	0	0	0	0
88	α-Myrcene	0	0	0	0	0.84	1.43	0	0	0	0	1.33	1.1	0.83
89	2,3-Dehydro-1,8-cineole	0	0	0	0	1.78	0.99	0	0	0	0	0	0	0
90	Hotrienol	0	0	0	0	0.12	0.13	0	0	0	0	0.11	0	0

2.2. Bioassays

2.2.1. Cress Germination Bioassay

Lepidium sativum L. subsp. *sativum* (cress) seeds (Larosa Seeds, Andria, Italy) were used to test the inhibitory effect of the EOs on germination. Dry seeds were rinsed repeatedly with sterile distilled water, and then placed in Petri dishes (6 cm diameter) on two layers of filter paper wetted with the solutions (1.5 mL with 100 or 1000 ppm of EO). Control was prepared without adding oil. Each amount was tested in three replicates. Petri dishes containing 15 seeds were sealed with parafilm and incubated in the dark at 25 °C. Two and seven days after incubation (DAT), the number of germinated seeds were counted and the germination percentage was calculated in comparison with the control. Seeds with a radicle at least 3 mm long were considered germinated. The test was repeated twice.

2.2.2. Tomato Radicle Elongation Bioassay

Solanum lycopersicum L. var. Marmande (tomato) seeds (Royal Sluis Garden, Mirandola, Italy) were used to test the effect on radicle growth. Dry seeds were sterilized gently shaking them for 10 min in 1% sodium hypochlorite (NaOCl) and then rinsed repeatedly with sterile distilled water. Seeds were allowed to germinate for two days in the dark at 25 °C in Petri dishes (15 cm diameter) on two layers of filter paper wetted with 7 mL of distilled water. Ten healthy seedlings (radicle length around 3 mm) were placed in 6 cm Petri dishes on two layers of filter paper wetted with the test solution (1.5 mL with 100 or 1000 ppm EOs), sealed with parafilm and then incubated in the dark at 25 °C. Three replicates were prepared for each treatment, including control (water without oil). After 3 days, radicle length was measured and expressed as growth inhibition percentage in comparison with the control. The test was repeated twice.

2.2.3. Leaf Disk Bioassay

Leaf discs of *Chenopodium album* L. subsp. *album* (lambsquarters, growing wild in the University Campus of Bari) were used to evaluate the phytotoxicity symptoms caused by the studied EOs and the relevant chlorophyll content. Discs (15 mm diameter) were punched out from healthy leaves of uniform size and rinsed repeatedly with sterile distilled water. Ten discs (≈0.3 g) were placed in Petri dishes (9 cm diameter) on two layers of filter paper wetted with 2.4 mL of distilled water. One drop (30 µL) of a solution (0.2% of each essential oil supplemented with 1:400 v/v of Biopower–Bayer) was applied to each leaf disk. Three replicates were prepared for each treatment, including control (only water or water with Biopower). Dishes were incubated at room temperature (25 + 2 °C) in 12 h alternate dark/white light. Effects of the treatments on leaf discs were assessed 2 DAT by visually estimating phenotypic changes. Injury rating included 3 levels (− no injury, + little injury or necrosis, ++ large necrosis).

Furthermore, 3 DAT, leaf discs were subjected to chlorophyll extraction and spectrophotometric estimation. Disks were homogenized in 80% acetone and incubated for 24 h with shaking at 145 rpm in the dark. The absorbance of the extract was measured at

470, 645 and 662 nm, using a UV–visible spectrophotometer (Varian-Cary 50 Scan). The amount of total chlorophyll and carotenoids was calculated using the equation reported by Dere et al. [18] and expressed as per gram fresh weight of tissue. The whole experiment was repeated twice.

2.2.4. Branched Broomrape Germination Bioassays

Contact bioassay. *Phelipanche ramosa* (L.) Pomel seeds (harvested in naturally infested tomato fields in Gravina in Puglia-Apulia region) were sterilized for 10 min in 1% sodium hypochlorite, supplemented with 0.02% (*v*/*v*) of the wetting agent Tween 20 (Sigma-Aldrich, Saint Louis, MO, USA), and then rinsed with sterile tap water. Seeds were placed in Petri dishes on two layers of glass microfiber filter (GF/A Whatman) wetted with tap water and kept at 26 °C in the dark for 2 weeks. Small pieces of filters, each containing around 100 seeds, were then cut and were moved to 6 cm Petri dishes on two layers of glass microfiber filter wetted with 1 mL of distilled water containing 1 ppm of a synthetic germination stimulant (rac-GR24, StrigoLab Torino) [19], and 100 or 1000 ppm of EOs. Three replicates were prepared for each treatment, including control (water with rac-GR24, without oil). Dishes were wrapped with a thin cellophane film to reduce evaporation. After 5 days of incubation at 25 °C in the dark, the number of germinated seeds was counted and expressed as germination percentage compared with the control. Broomrape seeds showing radicle emergence (at stereoscope observation) were recorded as germinated. The test was repeated twice.

Vapors bioassay. The experiment was carried out as described in the previous paragraph, but EOs (an aliquot of 3 µL) was loaded in a small plastic cap placed inside each *Petri* dish. Broomrape seeds were thus exposed only to EOs vapors. The control did not include the oils. The test was repeated twice.

2.3. Experimental Design and Data Analyses

In all bioassays, Petri dishes were arranged in a completely randomized design with three replicates for each treatment. Data were subjected to statistical analyses of variance (ANOVA) with R statistical software (R Core Team https://www.r-project.org/ (accessed on 24 October 2022). A one-factor linear model was built by using the "lm" function of the stats R package. The normality distribution of the model residual was checked by performing the Shapiro–Wilk normality test. The homoscedasticity was checked using the Levene test. The last ANOVA assumption was satisfied by the experimental design. When the ANOVA showed a significant difference (p-value < 0.05), the estimated marginal means post hoc analysis was performed by using the "emmeans" function with the Bonferroni adjustment of the emmeans R package (https://cran.r-project.org/package=emmeans (accessed on 24 October 2022).

To discriminate EOs basing on chemical composition and biological inhibitory activity, data including EO composition, cress germination with EOs at 100 and 1000 ppm, tomato radicle growth with EOs at 100 and 1000 ppm, chlorophyll and carotenoid content, broomrape germination with EOs at 100 ppm, 1000 ppm, and vapors (expressed as % of inhibition for each parameter) were analyzed by Principal Component Analysis (PCA). The relationships between variables (EOs chemical compounds and biological activities) were tested by Pearson correlation analyses. Multivariate analysis (PCA) was performed by the Unscrambler (version 10.1, CAMO, Oslo, Norway). All statistical analyses were performed by Statistica 13 software (Dell Statistica, version 13 https://software.dell.com (accessed on 4 November 2022).

3. Results

3.1. Cress Germination Bioassay

The effects on cress seed germination are shown in Table 3. Tc2 was significantly active at the lowest tested dose (100 ppm), completely inhibiting seed germination, while many of the tested EOs gave a high inhibitory effect on germination only at the highest dose

(1000 ppm). Cs1 and Cs2 at 100 ppm initially slowed down germination (2 DAT), which was however complete 7 DAT. High germination rate was obtained with 100 ppm of the other EOs. At 1000 ppm, different levels of activity were observed: EOs Sm2, Tc2, Ts1, Ts2, and Ts3 totally blocked germination since the start of the incubation (2 DAT), and 7 DAT; Cs1 and Cs2 highly affected seeds while Sf1 had a low but significant inhibition; the initial inhibitory effect of Sf1, Sf2, Sm1, Sc1, and Sc2 recorded (2 DAT) disappeared in a longer period (7 DAT).

Table 3. Herbicidal effect of two doses of EOs on *Lepidium sativum* germination after 2 and 7 days of incubation (2 DAT and 7 DAT). Data are expressed as mean of three replicates in comparison with the control (100%).

Germination Rate (%)				
	100 ppm		1000 ppm	
Essential Oil	**2 DAT**	**7 DAT**	**2 DAT**	**7 DAT**
Cs1	25.0 bc	82.1 a	0 f	25.0 cd
Cs2	17.8 bc	92.9 a	0 f	39.3 c
Sf1	78.5 a	89.3 a	14.3 ef	71.4 b
Sf2	57.1 ab	100 a	35.7 d	96.4 ab
Sc1	85.7 a	92.9 a	21.4 de	89.3 ab
Sc2	50.0 abc	82.1 a	71.4 bc	89.3 ab
Sm1	78.5 a	96.4 a	60.7 c	100 a
Sm2	60.7 ab	92.9 a	0 f	0 d
Tc1	85.7 a	96.4 a	82.1 ab	96.4 ab
Tc2	0 c	3.6 b	0 f	0 d
Ts1	57.1 ab	96.4 a	0 f	3.6 d
Ts2	60.7 ab	82.1 a	0 f	0 d
Ts3	53.5 ab	89.3 a	0 f	0 d
Control (water)	100 a	100 a	100 a	100 a
p-value	*	***	***	***

Means within each column followed by the same letter are not significantly different. The values followed by * are statistically different according to ANOVA (* $p < 0.05$; *** $p < 0.001$).

3.2. Tomato Radicle Elongation Bioassay

The effects of the tested EOs on *S. lycopersicum* seedlings are shown in Table 4. At 1000 ppm, the growth was drastically affected by 6 out of the 13 tested EOs (Cs1, Sm2, Tc2, Ts1, Ts2, Ts3) which completely inhibited the radicle growth. The other 7 tested EOs did not cause any length reduction and radicles grew without any phytotoxicity symptom. Based on this result, only the active EOs were tested at the lower dose (100 ppm): only Ts3 caused a significant reduction in the radicle length (19.4% lower than the control).

Table 4. Effect of two doses of EOs on radicle elongation of *Solanum lycopersicum* var. Marmande after 3 days of incubation. Data are expressed as mean of three replicates of 10 seedlings each, in comparison with the control (100%).

Radicle Elongation (%)		
Essential Oil	**1000 ppm**	**100 ppm**
Cs1	15 b	83.6 ab
Cs2	103.3 a	n.t.
Sf1	97 a	n.t.
Sf2	107.5 a	n.t.
Sc1	99 a	n.t.
Sc2	98.3 a	n.t.
Sm1	97 a	n.t.
Sm2	15 b	89.1 ab
Tc1	97.8 a	n.t.
Tc2	15 b	86.2 ab
Ts1	15 b	83.0 ab
Ts2	15 b	87.7 ab
Ts3	15 b	80.6 b
Control (water)	100 a	100 a
p-value	***	***

Means within each column followed by the same letter are not significantly different. The values followed by * are statistically different according to ANOVA (*** $p < 0.001$). n.t.: not tested.

3.3. Leaf Disk Bioassay

The effects on *C. album* leaf disks are reported in Table 5. Two DAT, Tc2, Ts2, and Ts3 caused large necrosis (4–6 mm diameter). In particular, Tc2 caused the widest necrosis (5–6 mm diam.) on all the leaf disks, while Ts2 and Ts3 caused smaller necrosis. Sm2 and Ts1 slightly injured only some leaf disks causing smaller (2–4 mm) necrosis. Other EOs caused irrelevant or no injury.

Table 5. Total chlorophyll and carotenoids content and injury rating from *Chenopodium album* leaf disks exposed to 0.2% of essential oil supplemented with Biopower. Data are expressed as mean of three replicates of 10 leaf disks each, in comparison with the control.

Essential Oil	Chlorophyll (μg/g FW)		Carotenoids (μg/g FW)		Injury
Cs1	1034.2	a	296.4	a	−
Cs2	1031.4	a	312.6	a	−
Sf1	1070.3	a	302.6	a	−
Sf2	984.7	ab	264.8	ab	−
Sc1	1086.3	a	337.2	a	−
Sc2	1103.6	a	333.2	a	−
Sm1	1141.8	a	354.8	a	−
Sm2	932.5	ab	263.0	ab	+
Tc1	1248.4	a	393.0	a	−
Tc2	601.8	b	148.0	b	++
Ts1	1217.1	a	379.8	a	+
Ts2	1038.0	a	315.6	a	++
Ts3	942.0	ab	272.0	ab	++
Control (water)	1187.6	a	365.7	a	−
Control (Biopower)	1067.1	a	326.6	a	−
p-value	**		**		

Means within each column followed by the same letter are not significantly different. The values followed by * are statistically different according to ANOVA (** $p < 0.01$). Injury ratings were based on visual estimates (− no injury, + little injury or necrosis, ++ large necrosis).

Three DAT, only Tc2 significantly caused a reduction of the total content of chlorophyll and carotenoids (Table 5) (49.3% and 59.5%, and 43.6% and 54.7% in comparison with the water and the Biopower control, respectively). No significant reductions were found for the other tested oils.

3.4. Branched Broomrape Germination Bioassay

Contact bioassay. Five DAT, the EOs application (1000 ppm) differently affected *P. ramosa* seed germination (Table 6): Sm2, Tc2, Ts1, Ts2, and Ts3 totally inhibited germination, whereas with Cs1, Cs2, and Sc1, the inhibition was nearly complete (but with no statistical differences among them); Sc2 and Tc1 reduced germination by 58.5 and 53.6% respectively; Sf1, Sf2, and Sm1 slightly reduced germination (by 25.7 to 36.4%). At 100 ppm, none of the EOs affected broomrape seed germination.

Vapors bioassay. EO vapors affected broomrape seed germination as summarized in Table 6. A total of 8 EOs out of 13 (Cs1, Sm2, Sc1, Sc2, Tc2, Ts1, Ts2, and Ts3) totally inhibited germination, and Cs2 caused a reduction of 68%. The other oils caused not significant reductions of the seed germination.

Table 6. Herbicidal effect on *Phelipanche ramosa* seed germination after 3 days of incubation with EOs applied in direct contact (100 and 1000 ppm) or as vapors (3 µL). Data are expressed as mean of three replicates in comparison with the control (100%).

	Germination Rate (%)					
Essential Oil	**100 ppm**		**1000 ppm**		**Vapors**	
Cs1	75.7	b	3.6	d	0	c
Cs2	103.6	ab	11.4	d	32	bc
Sf1	96.4	ab	74.3	b	100.7	a
Sf2	72.9	b	70.0	b	98.7	a
Sc1	82.1	ab	8.6	d	0	c
Sc2	101.4	ab	41.4	c	0	c
Sm1	95.7	ab	63.6	b	61.4	ab
Sm2	97.9	ab	0	d	0	c
Tc1	80.7	ab	46.4	c	52.9	abc
Tc2	96.4	ab	0	d	0	c
Ts1	112.9	a	0	d	0	c
Ts2	104.3	ab	0	d	0	c
Ts3	113.6	a	0	d	0	c
Control (water + rac-GR24)	100.0	ab	100	a	100	a
p-value	**		***		**	

Means within each column followed by the same letter are not significantly different. The values followed by * are statistically different according to ANOVA (** $p < 0.01$; *** $p < 0.001$).

3.5. Principal Component Analysis

The PCA plot resulting from the analysis of all data is shown in Figure 1. The plot, showing the distribution of scores (oils, Figure 1A) and loadings (compounds and activities, Figure 1B), clearly indicates a discrimination of EO samples based on their composition and biological inhibitory activity. Ts1, Ts2, Ts3, Cs1, and Cs2 are located in the left part of the graph and were characterized by a similar biological activity (mainly C1000, T100, and T1000) and by the presence of specific chemical compounds, namely β-pinene (15), 3-octanone (17), menthone (43), δ-terpineol (45), isopulegone (47), pulegone (58), and piperitenone (64) for Cs1 and Cs2, and β-pinene (15), α-terpinene (23), p-cymene (24), β-*cis*-ocimene (28), *cis*-sabinene hydrate (30), carvacrol methyl ether (57), and thymol (62) for Ts1, Ts2, Ts3. Samples Tc1, Tc2, and Sm2 were located close to the center of the plot and then were not significantly discriminated. They were mostly characterized by α-terpinene (23), p-cymene (24), ϒ-terpinene (29), thymol (62), carvacrol (63), thymol acetate (65), β-bisabolene (79), and α-bisabolene (84). However, Tc2 was characterized by the highest C100, Chl and Car activities. Interestingly, Tc1 and Tc2 contained the same components, among which also hexyl butanoate (50) and carvacrol acetate (66), even showing a different activity. In Sm2, the most distinguishing constituents were thymol-methyl ether (56), carvacrol methyl ether (57), and β-bisabolene (79). Samples Sc1 and Sc2 were distinguished from the other samples, and located in the lower part of the plot since characterized only by considerable B1000 and Bvap, while the chemical pattern was sometimes common to other samples and mainly characterized by: 2,4(10)-thujadien (11), chrysanthenone (36), α-campholenal (37), *trans*-pinocarveol (38), *trans*-3-caren-2-ol (42), borneol (46), 2,3-dehydro-1,8-cineole (89), and hotrienol (90). Sm1 was located in the right part of the plot, opposite to the biological activities, since it was characterized by the almost complete absence of inhibitory activity even if the metabolite pattern composition was similar to other samples. Finally, samples Sf1 and Sf2 were more clearly discriminated in the upper part of the plot and were not distinguished for the biological activity (almost missing), but by the presence or abundance of some phytochemicals: *cis*-salvene (2), *trans*-salvene (4), 1-hexanol (6), 2-α-pinene (7), eucalyptol (26), cis-thujone (34), *trans*-thujone (35), camphor (41), and thymol acetate (65).

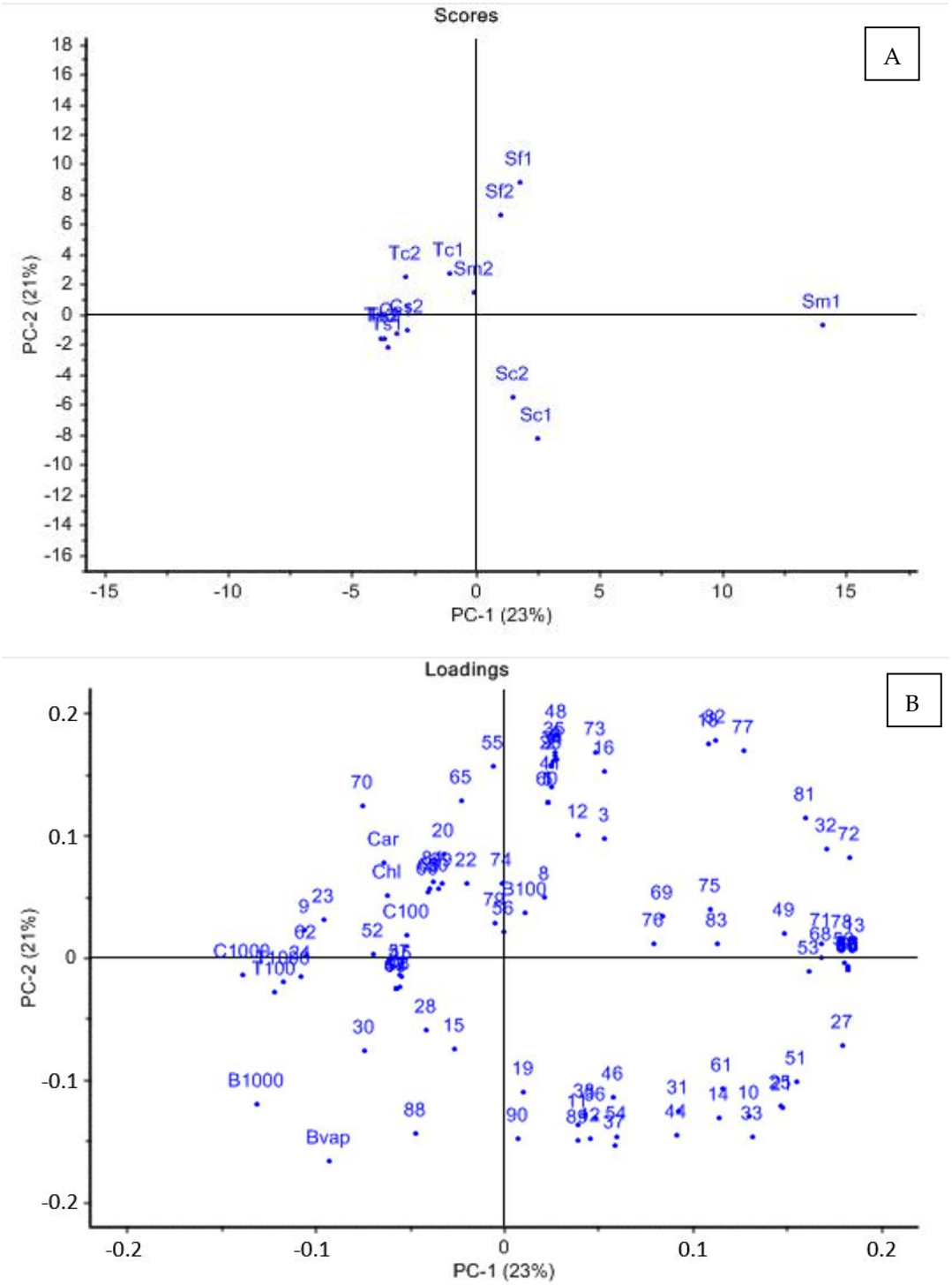

Figure 1. Principal component analysis (PCA) of data from the biological activity of EOs (expressed as % of inhibition) and their chemical composition. The score plot (**A**) indicates the sample distribution (EOs) based on the biological activity and metabolite composition. The loading plot (**B**) defines the correlation among variables: cress germination with EOs at 100 and 1000 ppm (C100 and C1000), tomato radicle growth with EOs at 100 and 1000 ppm (T100 and T1000), chlorophyll and carotenoid content (Chl and Car), broomrape germination with EOs at 100 ppm, 1000 ppm, and vapors (B100, B1000, and Bvap), concentration of the single compounds (numbered from 1 to 90, see Table 2) for each EO. Relationships between variables were further determined by Pearson correlation analysis (Table 7).

Table 7. Pearson correlation matrix between the chemical composition (%) of EO and the inhibitory biological activity: cress germination with EOs at 100 and 1000 ppm (C100 and C1000), tomato radicle growth with EOs at 100 and 1000 ppm (T100 and T1000), chlorophyll and carotenoid content, broomrape germination with EOs at 100 ppm, 1000 ppm, and vapors (B100, B1000, Bvap).

		Pearson Correlation Coefficients								
Code	**Compound**	**C100**	**C1000**	**T1000**	**T100**	**Chl**	**Car**	**B100**	**B1000**	**Bvap**
1	Methyl 3(Z)-Hexenyl Ether	−0.06	−0.17	−0.26	−0.26	−0.09	0.00	−0.12	−0.50	−0.57
2	cis-Salvene	−0.20	−0.39	−0.39	−0.37	0.02	0.17	0.42	−0.68	−0.81
3	3-Hexen-1-ol, *trans*-	0.45	−0.04	0.01	−0.10	0.35	0.33	−0.05	−0.17	−0.18
4	trans-Salvene	−0.18	−0.37	−0.40	−0.38	0.00	0.14	0.32	−0.71	−0.83
5	2-Hexen-1-ol, *trans*-	−0.06	−0.17	−0.26	−0.26	−0.09	0.00	−0.12	−0.50	−0.57
6	1-Hexanol	−0.14	−0.31	−0.37	−0.36	−0.04	0.09	0.15	−0.67	−0.78
7	2-α-Pinene	−0.20	−0.39	−0.39	−0.37	0.02	0.17	0.41	−0.69	−0.81
8	Tricyclene	−0.12	0.25	0.24	0.07	0.16	0.19	−0.20	0.10	0.06
9	α-Thujene	0.25	0.44	0.47	0.53	0.11	0.07	−0.22	0.32	0.21
10	α-Pinene	−0.14	−0.62	−0.54	−0.53	−0.31	−0.36	0.00	−0.27	0.08
11	2,4(10)-thujadien	−0.09	−0.38	−0.35	−0.34	−0.16	−0.22	0.23	0.10	0.24
12	Camphene	−0.09	−0.27	−0.34	−0.34	−0.14	−0.06	−0.06	−0.50	−0.54
13	Verbenene	−0.16	−0.40	−0.30	−0.30	−0.23	−0.26	−0.09	−0.47	−0.35
14	Sabinene	−0.08	−0.50	−0.43	−0.42	−0.29	−0.35	−0.18	−0.22	0.18
15	β-Pinene	−0.33	0.53	0.48	0.56	−0.28	−0.33	−0.50	0.47	0.40
16	1-Octen-3-ol	−0.17	−0.44	−0.39	−0.39	0.07	0.20	0.49	−0.70	−0.80
17	3-Octanone	−0.06	0.15	0.02	0.07	−0.02	−0.02	0.19	0.25	0.12
18	β-Mircene	−0.07	−0.49	−0.42	−0.47	0.08	0.18	0.37	−0.78	−0.86
19	3-Octanol	0.30	−0.19	−0.19	−0.19	0.12	0.05	0.27	0.23	0.36
20	Pseudolimonene	−0.16	−0.10	−0.28	−0.23	−0.03	0.05	0.24	−0.23	−0.44
21	α-Phellandrene	−0.03	−0.51	−0.38	−0.39	−0.23	−0.32	−0.08	−0.17	0.16
22	δ-3-Carene	0.27	−0.07	−0.01	−0.08	0.14	0.12	0.19	−0.03	−0.04
23	α-Terpinene	0.33	0.63	0.65	0.61	0.38	0.34	−0.32	0.48	0.37
24	p-Cymene	0.13	0.71	0.72	0.72	0.13	0.08	−0.43	0.57	0.46
25	dl-Limonene	−0.18	−0.57	−0.49	−0.45	−0.34	−0.39	0.02	−0.28	0.04
26	Eucalyptol	−0.20	−0.39	−0.39	−0.37	0.02	0.17	0.42	−0.68	−0.81
27	β-Ocimene, *trans*-	−0.22	−0.35	−0.23	−0.24	−0.34	−0.40	−0.23	−0.27	−0.04
28	β-Ocimene, *cis*-	−0.18	0.47	0.50	0.57	−0.25	−0.29	−0.47	0.36	0.33
29	ϒ-Terpinene	0.54	0.21	0.24	0.11	0.51	0.48	0.01	0.19	0.14
30	Sabinene hydrate, *cis*-	−0.09	0.56	0.58	0.63	−0.10	−0.16	−0.47	0.50	0.48
31	Terpinolene	−0.15	−0.49	−0.42	−0.40	−0.27	−0.34	0.09	−0.10	0.12
32	p-Cymenene	0.07	−0.39	−0.29	−0.35	0.03	0.04	0.00	−0.58	−0.53
33	Linalool	0.00	−0.37	−0.25	−0.23	−0.19	−0.29	−0.20	−0.06	0.27
34	Thujone, *cis*-	−0.20	−0.37	−0.38	−0.37	−0.01	0.14	0.34	−0.70	−0.82
35	Thujone, *trans*-	−0.16	−0.35	−0.39	−0.38	−0.02	0.13	0.25	−0.70	−0.82
36	Chrysanthenone	0.01	−0.40	−0.35	−0.36	−0.10	−0.17	0.24	0.09	0.26
37	α-Campholenal	−0.14	−0.36	−0.31	−0.27	−0.19	−0.27	0.14	0.11	0.28
38	trans-Pinocarveol	−0.11	−0.44	−0.42	−0.40	−0.18	−0.23	0.21	0.00	0.18
39	Sabinol, *cis*-	−0.16	−0.32	−0.21	−0.24	−0.20	−0.24	−0.13	−0.36	−0.24
40	Verbenol, *cis*-	−0.15	−0.35	−0.25	−0.25	−0.22	−0.26	−0.11	−0.38	−0.26
41	Camphor	−0.08	−0.21	−0.29	−0.29	−0.08	0.02	−0.05	−0.56	−0.64
42	trans-3-Caren-2-ol	−0.09	−0.41	−0.38	−0.36	−0.19	−0.25	0.17	0.06	0.27
43	Menthone	−0.07	0.13	−0.06	−0.02	−0.02	−0.03	0.08	0.24	0.08
44	Pinocarvone	−0.13	−0.47	−0.42	−0.41	−0.23	−0.30	0.17	−0.01	0.20
45	δ-Terpineol	−0.08	0.12	−0.07	−0.03	−0.02	−0.02	0.11	0.20	0.04
46	Borneol	−0.08	−0.36	−0.33	−0.32	−0.19	−0.24	0.15	0.04	0.15
47	Isopulegone	−0.06	0.15	0.01	0.05	−0.02	−0.02	0.16	0.25	0.11
48	Terpinene-4-ol	0.21	−0.22	−0.21	−0.30	0.24	0.32	0.19	−0.51	−0.61
49	p-Cymen-8-ol	0.10	−0.11	−0.01	−0.11	0.11	0.07	−0.17	−0.18	−0.10
50	Hexyl butanoate	0.49	−0.08	−0.02	−0.05	0.26	0.22	0.20	−0.03	−0.04
51	α-Terpineol	−0.16	−0.65	−0.56	−0.55	−0.33	−0.36	−0.05	−0.41	−0.05
52	Decanal	0.11	0.06	−0.03	0.01	0.03	0.02	0.30	0.20	0.07
53	Verbenone	−0.11	−0.29	−0.21	−0.21	−0.19	−0.23	−0.02	−0.29	−0.21
54	Carveol, *trans*-	−0.11	−0.43	−0.39	−0.37	−0.20	−0.26	0.19	0.05	0.24
55	Nerol	0.29	−0.24	−0.26	−0.28	0.14	0.20	0.14	−0.48	−0.56
56	Thymol, methyl ether	−0.10	0.32	0.31	0.15	0.19	0.20	−0.17	0.24	0.20
57	Carvacrol, methyl ether	−0.13	0.62	0.61	0.45	0.02	0.00	−0.40	0.48	0.40
58	Pulegone	−0.05	0.15	0.04	0.08	−0.02	−0.01	0.20	0.26	0.13
59	Cumin aldehyde	−0.17	−0.28	−0.18	−0.22	−0.18	−0.22	−0.14	−0.33	−0.22
60	Z-Citral	−0.06	−0.17	−0.26	−0.26	−0.09	0.00	−0.12	−0.50	−0.57
61	Bornyl acetate	−0.16	−0.55	−0.51	−0.50	−0.28	−0.32	0.13	−0.19	0.00
62	Thymol	0.22	0.74	0.75	0.70	0.27	0.22	−0.42	0.59	0.48
63	Carvacrol	0.43	−0.05	0.01	−0.02	0.23	0.20	0.19	−0.01	−0.02
64	Piperitenone	−0.05	0.16	0.06	0.11	−0.02	−0.01	0.23	0.26	0.13
65	Thymol acetate	0.61	0.19	0.17	0.04	0.64	0.66	−0.06	−0.04	−0.13
66	Carvacrol acetate	0.77	0.09	0.13	0.08	0.56	0.52	0.08	0.09	0.07
67	α-Copaene	−0.15	−0.35	−0.24	−0.25	−0.22	−0.26	−0.11	−0.38	−0.26
68	β-Elemene	−0.18	−0.19	−0.09	−0.17	−0.13	−0.16	−0.17	−0.25	−0.15
69	α-Gurjunene	−0.05	0.03	0.10	−0.05	0.08	0.06	−0.11	−0.02	0.00
70	Caryophyllene, *trans*-	0.26	0.26	0.30	0.33	0.26	0.30	−0.05	−0.05	−0.21
71	α-Bergamotene, *trans*-	−0.03	−0.24	−0.13	−0.20	−0.06	−0.11	−0.12	−0.29	−0.19
72	Aromadendrene	−0.20	−0.52	−0.43	−0.44	−0.19	−0.16	0.06	−0.73	−0.68
73	α-Humulene	−0.21	−0.43	−0.43	−0.42	−0.02	0.13	0.36	−0.76	−0.87

Table 7. *Cont.*

		Pearson Correlation Coefficients								
Code	**Compound**	**C100**	**C1000**	**T1000**	**T100**	**Chl**	**Car**	**B100**	**B1000**	**Bvap**
74	β-Santalene	0.50	−0.02	0.05	−0.03	0.34	0.30	0.11	−0.01	−0.01
75	Alloaromadendrene	0.12	−0.34	−0.21	−0.26	−0.06	−0.11	0.09	−0.31	−0.23
76	Germacrene D	−0.17	0.15	0.18	0.03	0.06	0.06	−0.17	0.08	0.08
77	Guaia-1(10),11-diene	−0.05	−0.52	−0.46	−0.50	−0.02	0.07	0.21	−0.84	−0.88
78	β-Guaiene, *trans*-	−0.02	−0.35	−0.24	−0.26	−0.13	−0.17	−0.09	−0.41	−0.30
79	β-Bisabolene	−0.04	0.32	0.32	0.15	0.23	0.24	−0.15	0.25	0.21
80	β-Curcumene	−0.15	−0.36	−0.26	−0.26	−0.23	−0.27	−0.10	−0.39	−0.27
81	α-Muurolene	−0.27	−0.38	−0.33	−0.39	−0.11	−0.03	0.02	−0.67	−0.68
82	Calamenene, *cis*-	−0.04	−0.44	−0.40	−0.45	0.08	0.19	0.22	−0.80	−0.87
83	Sesquiphellandrene	−0.18	0.04	0.10	−0.04	0.01	0.00	−0.21	−0.04	0.00
84	α-Bisabolene, (E)-	0.61	0.11	0.15	0.06	0.48	0.44	0.09	0.11	0.08
85	Sesquisabinene hydrate, *cis*-	−0.15	−0.36	−0.25	−0.26	−0.22	−0.27	−0.11	−0.39	−0.27
86	trans-Sesquisabinene hydrate	−0.15	−0.34	−0.24	−0.25	−0.22	−0.26	−0.12	−0.38	−0.26
87	(−)-Spathulenol	−0.15	−0.35	−0.24	−0.25	−0.22	−0.26	−0.11	−0.38	−0.26
88	α-Myrcene	−0.12	0.17	0.19	0.25	−0.30	−0.36	−0.40	0.33	0.54
89	2,3-Dehydro-1,8-cineole	−0.08	−0.41	−0.38	−0.36	−0.19	−0.24	0.15	0.06	0.29
90	Hotrienol	−0.13	−0.21	−0.17	−0.12	−0.37	−0.41	−0.10	0.13	0.39

Red-marked correlations are significant at $p < 0.05$.

The biological activities were mostly positively correlated to compounds 15, 23, 24, 28, 30, 57, 62, 65, 66, and 84 (correlation Table 7). Those compounds were contained in the most active oils (Cs1, Cs2, Tc2, Sm2, Ts1, Ts2, Ts3) and in Tc1 (less active), while they were absent or scarcely present in the less active oils (Sc1, Sc2, Sm1, Sf1, and Sf2). Interestingly, compounds 23, 24, 30, 57, 62 (contained together in Ts1, Ts2, Ts3) showed a significant positive correlation with both C1000 and T1000. C100 was correlated to 65, 66, 84, contained in Tc2 (responsible for a high inhibition), Tc1 and Sm2. Regarding T100, although the activity was limited, a positive significant correlation was found with 15, 23, 24, 28, 30, and 62, which were identified in Ts1, Ts2, and Ts3, showing the activity. Chl was positively correlated to 66 and 65, the latter also correlated to Car. The two compounds were more abundant in Tc2, which had the highest Car and Chl activities. Finally, B1000, recorded for all samples at different percentage, was mainly associated to the compounds 24 and 62 which were found in almost all samples, but were more abundant in the most active samples (Ts1, Ts2, Ts3, Tc2, and Sm2, Table 2). For Bvap and B100, no significant positive correlations were found with any compounds, and they did not significantly contribute to discriminate samples.

4. Discussion

The results of our study in vitro confirm the potential biological activity of essential oils from aromatic Lamiaceae wild plants of Italian origin.

We tested 13 essential oils extracted each from plants of six Lamiacee species (Table 1): in particular, two oils extracted from *Clinopodium suaveolens* (Sm.) Kuntze, *Salvia fruticosa* Mill. subsp. *thomasii* (Lacaita) Brullo, Guglielmo, Pavone & Terrasi, *Satureja cuneifolia* Ten., *Satureja montana* L. subsp. *montana*, *Thymbra capitata* (L.) Cav., and three oils from *Thymus spinulosus* Ten. Each oil is characterized by two or three major phytochemical constituents, all monoterpenes, and many others present in low amount (Table 2). As overall observation, the tested essential oils have herbicidal activity against germination and early radicle growth of two common test species (*L. sativum* subsp. *sativum* and *S. lycopersicum*) and one parasitic species (*P. ramosa*).

Within the same species, oils from *T. spinulosus*, *C. suaveolens*, and *S. cuneifolia* gave consistent results among the different experiments and showed the same strong herbicidal activities. Oils from *S. fruticosa* subsp. *thomasii* gave consistent results too but proved to be the less active tested ones. Indeed, all oils from *T. spinulosus* (Ts1, Ts2, Ts3) at 1000 ppm totally inhibited the initial growth of tomato radicles and the germination of cress and broomrape seeds. Even vapors from the three oils totally inhibited broomrape seed germination (Table 6). In addition, Ts3 had a light activity on tomato radicle at the lowest tested dose of 100 ppm (Table 4). Both essential oils from *C. suaveolens* (Cs1, Cs2)

at 1000 ppm showed a strong herbicidal activity (total or near total inhibition of cress and broomrape germination, by direct contact and vapors) with the only exception of the effect on the tomato radicle elongation (total inhibition from Cs1 and no activity from Cs2). Essential oils from *S. cuneifolia* (Sc1, Sc2), both in direct contact and as vapors, caused great injury to broomrape seeds hampering germination, while they did not show any activity in the other tests. At the tested concentrations, both *S. fruticosa* subsp. *thomasii* oils (Sf1 and Sf2) proved to be the less effective oils, having a very light inhibitory effect on broomrape, but no effect in the other experiments.

Within the same species, oils from *T. capitata* (Tc1 and Tc2) and *S. montana* subsp. *montana* (Sm1 and Sm2) gave inconsistent results and showed different herbicidal activities. In particular, Tc2 was the most powerful oil of the 13 tested, as it was strongly active in all experiments at 1000 ppm, totally injuring cress and broomrape seeds (both by direct contact and by vapors), completely inhibiting the growth of tomato radicles, and additionally, it was the only oil out of 13 active at the lowest tested concentration, causing a nearly complete inhibition of cress seed germination at 100 ppm (Table 3) and reducing the chlorophyll and carotenoids content in lambsquarters leaf disks (Table 5). In contrast, Tc1 proved to be among the less active tested oils, being only partially active against broomrape seed germination at 1000 ppm. Similar observations can be shared for *S. montana* subsp. *montana* EOs, where Sm2 was strongly active on tomato radicles, cress and broomrape seed germination (both by direct contact and by vapors), while on the other hand Sm1 was nearly inactive in all experiments.

The good or scarce activity of the tested oils could be related to the presence or absence of those compounds which were found to be positively correlated to the biological activities (Table 7). Indeed, compounds 15, 23, 24, 28, 30, 57, 62, 65, 66, and 84 were contained in the most active oils (Cs1, Cs2, Tc2, Sm2, Ts1, Ts2, and Ts3) and in Tc1 (less active), while they were absent or scarcely present in the less active oils (Sc1, Sc2, Sm1, Sf1, and Sf2) (Table 2).

In the case of *T. spinulosus*, the explanation of the consistent results among the three extracted oils could be that the extracted oils were stable in composition, regardless of environmental, climatic, and vegetational differences of the harvest sites [16]. For three *T. spinulosus* harvest sites having the same macroclimate features, differences concerning bioclimatic, geological, pedological characteristics, and plant communities were found. EOs Ts1, Ts2, and Ts3 composition consists of a total of 27 compounds for all three sites (Table 2). The environmental differences very lightly influenced the chemical composition of EOs. Indeed, in the previous research, low differences were observed on the abundance and patterns among the three sites, since they share 25 compounds; only one compound (hotrienol) was exclusive to Ts1, and one to Ts2 (caryophyllene-oxide). The phytochemicals having the highest abundance in all of the 3 samples were thymol (62), p-cymene (24) and β-*cis*-ocimene (28). Few differences in percentages were observed among the three oils for thymol (42.9, 48.8, and 45.9%, respectively), and β-ocimene (15.4, 11.7, and 10.1%, respectively). All the other compounds had very slight quantitative differences. The three most abundant constituents (24, 28, 62) were correlated to the biological activities C1000, T100, T1000, B1000 (Table 7) and that could explain the good phytotoxic effects of those oils.

The EOs Cs1 and Cs2 extracted from *C. suaveolens* had the very same composition, but there were some differences in terms of composition percentage between them. In particular, pulegone (58), δ-terpineol (45), and isopulegone-trans were found to be the most abundant constituents in both oils (Table 2), the first being more abundant in Cs1 and the latter more abundant in Cs2 [17,20]. None of the phytochemicals correlated to the biological activities were found as abundant in the two oils, while β-pinene (15), α-terpinene (23), p-cymene (24), and β-*cis*-ocimene (28), as a whole positively correlated with C1000, T100, T1000, and B1000 (Table 7), were present in small amount with negligible quantitative differences, and that could explain the good phytotoxic effects of those oils.

Also for *S. cuneifolia*, the explanation of the consistent results between Sc1 and Sc2 could be that the extracted oils were stable in composition, regardless of environmental, climatic, and vegetational differences of the harvest sites [16]. For *S. cuneifolia* at both

investigated sites, with the same Mediterranean macroclimate, there were environmental, bioclimatic, geological, lithological and ecological, pedological, and vegetational differences. Even so, those different environmental conditions very little affected the phytochemical properties of the two oils. In total, for both oils, 36 compounds were identified with very few quantitative differences (Table 2). In particular, the most abundant compounds in Sc1 and Sc2 were α-pinene (10) and α-terpineol (51), the latter showing a small abundance difference (11 and 17.1%, respectively). All the other minor compounds had small to irrelevant abundance differences. None of the phytochemicals positively correlated to the biological activities were found as abundant in the two oils, and that could explain the limited phytotoxic effects of those oils. Moreover, p-cymene (24), which was found to be positively correlated with B1000 (Table 7), was present in different small amount in Sc1 and Sc2 (1.55 and 0.63%, respectively), and that could explain the broomrape seeds injury occurred (higher in Sc1 than in Sc2).

Despite the aligned (not promising) results obtained from *S. fruticosa* subsp. *thomasii* extracted oils, Sf1 and Sf2 were different in composition due to environmental differences of the harvest sites, being Sf1 collected from a less disturbed site than Sf2. That was reflected in a higher number of compounds (48) identified in Sf1 than in Sf2 (42 compounds), with six exclusive to Sf1, and 42 common (Table 2). The most abundant components of *S. fruticosa* subsp. *thomasii* oils were eucalyptol (26) (40.2 to 60.9%), camphor (41) (1.9 to 14.9%), and α-pinene (10) (3.6 to 5%). Those data confirm the ones available in literature on the genus *Salvia*, which show eucalyptol to be usually the most abundant compound [21,22]. Like for *S. cuneifolia*, none of the phytochemicals positively correlated to the biological activities were found as abundant in the two oils, and that could explain the limited phytotoxic effects of those oils. P-cymene (24), which was found to be positively correlated with B1000 (Table 7), was present in a small amount, and that could explain the broomrape seeds injury occurred.

T. capitata oils Tc1 and Tc2 shared 50 compounds, and Tc1 had 51 (Table 2). The most abundant compounds were carvacrol (63), thymol (62), ϒ-terpinene (29), and p-cymene (24), the last three being more abundant in Tc2 than in Tc1 (Table 2). In *S. montana* subsp. *montana*, the phytochemicals with the highest abundance in both samples were α-pinene (10), thymol (62), and ϒ-terpinene (29), the last two being much more abundant in Sm2 than in Sm1. Sm1 and Sm2 shared 55 compounds, whereas 3 were exclusive of Sm1 and 3 of Sm2. Thus, for *T. capitata* and *S. montana* subsp. *montana*, the different biological activities among oils of the same species could be attributed to the different chemical composition of the extracted oils due to environmental and vegetational differences between the harvest sites [17]. The variability among provenances is also confirmed by Angelini et al. who tested EOs from *S. montana* subsp. *montana* and their main compounds on different weeds and crops, finding that carvacrol was the most abundant (57%) and effective [23]. Tc1 and Tc2 contained compounds 15, 23, 24, 28, 30, 62, 65, 66, and 84, positively correlated as a whole to all the tested activities (C100, C1000, T100, T1000, B1000, Chl, and Car) but Bvap (Table 7). Most of those compounds were more abundant in Tc2 than in Tc1. That could explain the phytotoxicity of Tc2 in all experiments including C100, Chl, and Car, positively correlated to thymol acetate (65) and carvacrol acetate (66). Therefore, compounds 65 and 66 seems to be responsible for those activities. Compounds 23, 24, 57, 62, 65, and 84 (positively correlated as a whole to the tested activities C100, C1000, T100, T1000, B1000, C100, Chl, and Car) were also more abundant in Sm2 than in Sm1 and that could explain the good phytotoxic effect of Sm2.

The active compounds abovementioned are monoterpenes and monoterpenoids. Some of them had been studied individually or in combination and proved to be very active compounds. Vasilakoglou et al. found that carvacrol and thymol were very phytotoxic components (the most phytotoxic among 19 they tested), completely inhibiting rigid ryegrass germination and root length at 160 nL/cm^3 [24]. Thymol was found to have high inhibitory effect against seeds of several weeds [25]. That is congruent and could explain the high phytotoxicity of *T. spinulosus*, *T. capitata*, and *S. montana* subsp. *montana* oils,

and even the different activity between oils extracted from the same species. Indeed, all tested *T. spinulosus* oils where highly active and rich in thymol, as well as Tc2 and Sm2 which were much more active than the corresponding Tc1 and Sm1, the former being richer in thymol than the latter. In Sm1, α-pinene was much more abundant than in Sm2, but its phytotoxic activity has been proven to be very light on *Lolium rigidum* Gaudin at all tested doses [24], in accordance with the result of our experiments in which Sm1 was not active. The promising results from our experiments regarding *T. capitata* also confirm previous studies in which extracted oils showed variability among provenances and the species resulted weed-killer against *Sinapis arvensis* [26], *Erigeron bonariensis* in pre- and post-emergence assays in greenhouse conditions [14], *Avena fatua* L., *Echinochloa crus-galli* (L.) *P. Beauv.* subsp. *crus-galli*, *Portulaca oleracea* L., and *Amaranthus retroflexus* L. [15].

As for pulegone, the most abundant monoterpene constituent of oils from *C. suaveolens*, previous results are consistent with ours. Indeed, in the literature pulegone was found to be a very toxic compound for cucumber (*Cucumis sativus* L.), inhibiting root and mitochondrial respiration for concentrations ranging from 50 to 900 ppm [27]. It also showed inhibitory activity in the germination, seedling, and shoot growth of the test plant *Lactuca sativa* [28]. Pulegone was the main constituent (84%) of *Minthostachys mollis* (Benth.) Griseb., which showed inhibitory effects on germination, shoot, and root elongation of the tested species lettuce, tomato, cucumber, and *Bidens pilosa* L. [29].

Alpha-pinene was the main constituent of Sc1 and Sc2 from *Satureja cuneifolia*, and Sm1. In Singh et al., the compound inhibited early root growth and caused oxidative damage in root tissue in five test species, and results were concentration dependent [30]. In Abrahim et al., α-pinene concentrations of 0.05–1.0 mM stimulated respiration while at concentrations higher than 1.0 mM, α-pinene inhibited respiration; moreover, α-pinene had less activity than camphor and eucalyptol in inhibiting seed germination and primary root growth, despite the fact that it had a higher activity on the oxidative metabolism of mitochondria [31]. In Vasilakoglou et al., α-pinene phytotoxic activity has been proved to be very light on *L. rigidum* at dose of 640 nL/cm^3 [24]. At the concentration we tested, both *Satureja cuneifolia* oils and Sm1 were active against broomrape seed germination, but not active in the other experiments. That indicates the need to test further those oils at different doses to better understand their potentiality. The same need for further investigations at different doses is valid for *S. fruticosa* subsp. *thomasii* oils, which were very partially active only on broomrape seeds, although they contained a high amount of eucalyptol, which proved to be active in other studies. Eucalyptol is one of the most studied monoterpenes, but some previous results are in contrast with ours. It severely affected the germination, speed of germination, seedling growth, chlorophyll content, and respiratory activity of *Ageratum conyzoides* (bill goat weed). After two weeks of exposure, plants wilted [32]. Eucalyptol also inhibited germination of *Brassica rapa* L. subsp. *campestris* (L.) A.R. Clapham seeds at high concentrations [33]. Angelini et al. tested in vitro the EO extracted from *Salvia rosmarinus* Schleid. (whose main compound was eucalyptol 47%) on three weeds, and it completely inhibited their germination [23].

P. ramosa is one of the most widespread and troublesome parasitic weeds, mostly present in the Mediterranean area. Seeds proved to be sensitive to all the tested oils at the highest tested dose by direct contact (1000 ppm) and insensitive at the lowest (100 ppm) (Table 6). Concentrations lower than 1000 ppm should be investigated to find the lowest active dose. As for vapors, broomrape seeds were completely injured by Cs1, Tc2, Sm2, Sc1, Sc2, Ts1, Ts2, and Ts3. Injury was very high for *C. suaveolens* Cs2. Those results are promising as those oils could be supplied by micro-irrigation or fumigations to the soil, where broomrape seeds stay. Often EOs face a drop of effectiveness when used row in the field, due to their volatility; that could be overturned in an advantage if vapors are to be used as soil fumigants for broomrape control. Moreover, from our analysis thymol (62) and p-cymene (24) are correlated to the broomrape seed inhibition, so new opportunities should be tested.

As for the leaf disk bioassay, only oil Tc2 from *T. capitata* gave a reduction in the chlorophyll content. It is likely that the leaf disks were not covered for the most part by the essential oils. Therefore, segments without coverage would have increased injury with a more complete exposition.

5. Conclusions

Most of the tested essential oils at the tested doses have herbicidal activity and showed different potential to prevent seed germination, radicle growth and to cause leaf injury. The biological activity is mainly correlated with the presence of monoterpenes (thymol being the most abundant and present in the most active oils) and monoterpenoids.

Based on those results, the highest phytotoxicity was demonstrated by Tc2: it was the only oil which totally inhibited cress seed germination at concentration of 100 ppm and reduced the chlorophyll and carotenoids content in lambsquarters leaf disks. The most effective species was *T. spinulosus*, as all of the three extracted oils proved to be highly active in most of the experiments. Those essential oils or their components have the potential for use in weed seed control and as pre-emergence herbicides in the development of new weed control strategies. New scenarios could open for *P. ramosa* which proved to be highly sensitive to most of the tested oils.

The complexity of the chemical composition of the tested essential oils together with their variability, make the identification of the effects complicated. In the literature, there is no clear evidence reported as to how active compounds of a plant extract reveal their activity; that is to say, it is not clear whether the exhibited toxic effect is due to the joined action of many compounds or to the phytotoxicity of a single one; on the other hand, the mode of action of many single constituents has been observed in controlled conditions, being an easier topic.

Results open opportunities to employ essential oils for weed management. Essential oils are extracted from plants, and thus may be natural alternatives of synthetic herbicides for organic farming systems.

Further studies are needed to evaluate the phytotoxic effect of single constituent compounds tested alone or in combinations, the mode of action, the application techniques, the efficacy under field condition, the extraction yield, and the economic aspects before they could develop as commercial formulations.

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