

Article

In Vitro Evaluation of Mediterranean *Lamiaceae* Essential Oils as Natural Herbicides for Sustainable Weed Management

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Abstract

Mediterranean aromatic plants are valuable sources of essential oils (EOs) with biological properties that hold promise for sustainable weed management. This study evaluated the phytotoxic activity of EOs from *Thymus vulgaris* L., *Lavandula angustifolia* Mill., and *Salvia officinalis* L. in vitro against key weed species common to Mediterranean agroecosystems. The EOs were tested at concentrations ranging from 0.125 to 1.50 $\mu\text{L mL}^{-1}$, assessing their impact on seed germination and early seedling growth of *Avena fatua* L., *Lolium rigidum* Gaudin, *Sonchus oleraceus* L., and *Amaranthus retroflexus* L. Thyme EO, primarily composed of thymol and γ -terpinene, exhibited the strongest phytotoxic activity, completely inhibiting the germination of *A. fatua*, *L. rigidum*, and *S. oleraceus* at all concentrations tested. In contrast, *A. retroflexus* was the most resistant weed species, with germination fully inhibited only at concentrations $\geq 0.50 \mu\text{L mL}^{-1}$. Lavender EO, rich in linalool and linalyl anthranilate, demonstrated a more species-specific effect. It almost entirely suppressed the germination of *S. oleraceus* at all doses and inhibited germination of *A. fatua* and *L. rigidum* at concentrations $\geq 0.50 \mu\text{L mL}^{-1}$. Again, *A. retroflexus* was the most tolerant species, with complete inhibition occurring only at the highest concentrations applied. Sage EO, containing eucalyptol and thujone as its main constituents, was the least effective, fully inhibiting *S. oleraceus* germination at concentrations $\geq 1.00 \mu\text{L mL}^{-1}$, and *L. rigidum* at 1.50 $\mu\text{L mL}^{-1}$. Seedling growth was highly sensitive to EO treatments, even when germination was not entirely suppressed. These results highlight the potential of Mediterranean *Lamiaceae* EOs as plant-derived bioherbicides for integrated weed management.

Keywords: essential oils; weed control; phytotoxicity; agroecology; seed germination



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1. Introduction

Weed management continues to pose a significant challenge in many agroecosystems, as weeds can considerably reduce crop yield, compromise product quality, and increase production costs [1]. For decades, synthetic herbicides have been the primary method of weed control due to their effectiveness, ease of application, and ability to lower weed

management expenses [2]. However, their repeated and widespread use has raised serious concerns, including environmental pollution, impacts on non-target organisms, and the growing prevalence of herbicide-resistant weed species [2]. Moreover, it has been documented that many synthetic herbicide components are linked to human diseases, being recognized as carcinogenic and neurotoxic substances [3]. Concerns over weed management have led to growing interest in alternative, more sustainable strategies, particularly within organic and low-input agricultural systems [4,5]. Concurrently, current European policies, including Directive 2009/128/EC on the sustainable use of pesticides and the European Green Deal, are encouraging a reduction in reliance on synthetic plant protection products and promoting the development of sustainable agricultural practices and nature-based crop protection solutions. Within this context, plant-derived compounds are attracting attention as potential tools for crop protection [2,6]. Allelopathy is especially pertinent in this area, as it provides a framework for identifying natural compounds with phytotoxic activity that could be harnessed for sustainable weed control [7]. Although their modes of action may vary, phytotoxic effects are commonly assessed through seed germination and early seedling growth parameters, including root and coleoptile elongation [5,8]. Among allelopathic metabolites, essential oils (EOs) are particularly noteworthy as they are complex mixtures of volatile compounds, mainly terpenes, terpenoids, and other low-molecular-weight substances, known for their biological and phytotoxic properties [2,9]. EOs derived from *Thymbra capitata* (L.) Cav., *Eucalyptus camaldulensis* Dehnh., *Santolina chamaecyparissus* L., and peppermint (*Mentha × piperita* L.) have been found to significantly inhibit the germination of *Erigeron bonariensis* L., with *T. capitata* exhibiting the strongest effect [10]. Similarly, isolated EO terpenes, including carvacrol, carvone, thymol, trans-anethole, and linalool, strongly inhibited both the germination and root growth of *Lolium rigidum* Gaudin at appropriate concentrations, with some mixtures also exhibiting synergistic effects [11]. Inhibitory effects have also been reported for aqueous extracts: extracts from peppermint, rosemary (*Salvia rosmarinus* Spenn.), coriander (*Coriandrum sativum* L.), sage (*Salvia officinalis* L.), and thyme (*Thymus vulgaris* L.) reduced the germination and seedling growth of lambsquarter (*Chenopodium album* L.), with thyme being one of the most effective treatments [12]. More recently, hydrolates and residual waters produced during rosemary EO extraction have demonstrated bioherbicidal activity, significantly reducing the germination and root growth of *Amaranthus retroflexus* L. [13]. These studies suggest that aromatic plants may provide various phytotoxic matrices, ranging from isolated EO constituents to whole extracts and distillation by-products, which could be relevant for weed management. Since EOs are among the primary products of medicinal and aromatic plants (MAPs), recognized as major natural sources of aromatic and bioactive compounds, these findings also emphasize the broader value of MAPs as promising resources for the development of EO-based bioherbicides [14,15]. Nevertheless, given the considerable variability in their composition and biological activities, further experimental research is required to better define their true potential and practical applicability [6,16]. Mediterranean MAPs warrant particular attention due to their high diversity, wide distribution, and richness in EOs and other secondary metabolites of interest. The Mediterranean region is one of the world's major biodiversity hotspots, hosting a wide range of MAPs closely linked to local environments, traditional uses, and regional production systems [17]. Its characteristic climate, with mild, rainy winters and hot, dry summers, combined with the marked heterogeneity of soils and landscapes, strongly influences plant performance and chemical expression [18]. This is especially important for MAPs, as their value depends not only on biomass yield but also on EOs' yield and composition, as well as on other bioactive compounds [18]. Environmental conditions, geographic origin, and soil characteristics can all lead to significant variation in volatile profiles, often resulting in distinct chemotypes

within the same species [19]. In Mediterranean environments, recurring abiotic constraints, such as drought, intense sunlight, and summer heat, can influence secondary metabolism, thereby affecting the qualitative profile of plant extracts [20]. Consequently, Mediterranean MAPs cultivated in central Mediterranean regions, such as Sicily, may serve as valuable sources of biologically active compounds for high-value agricultural uses [20]. Their EOs provide a promising foundation for developing plant-based weed management strategies, especially when locally adapted species are chosen. In this regard, thyme (*Thymus vulgaris* L.), lavender (*Lavandula angustifolia* Mill.), and sage (*Salvia officinalis* L.) are especially significant due to their adaptation to Mediterranean environments, rich volatile profiles, and well-documented biological activity [21]. These species are extensively incorporated into Mediterranean agricultural and traditional-use systems and are prized for their culinary, pharmaceutical, and cosmetic applications [18]. Beyond their conventional uses, EOs may also provide additional benefits for crop protection, particularly due to their volatile compounds, which are known for antimicrobial, antioxidant, allelopathic, and phytotoxic properties. EOs from *Lamiaceae* species, such as *Thymus fontanesii* Boiss. et Reut. and *Satureja calamintha* subsp. *nepeta* Briq., have demonstrated inhibitory effects on significant weeds, including *Avena fatua* L. and *Sonchus oleraceus* L. [22]. However, the phytotoxicity of EOs is heavily influenced by their chemical composition, dosage, the target species, and their phenological stage [23,24]. Consequently, further controlled bioassays are required to assess the herbicidal potential of locally produced EOs and to identify promising candidates for subsequent validation in greenhouse and field trials.

In the present study, EOs from *T. vulgaris*, *L. angustifolia*, and *S. officinalis* were tested in vitro on seed germination and early seedling growth of four weed species commonly found in Mediterranean agroecosystems: *L. rigidum*, *A. retroflexus*, *S. oleraceus*, and *A. fatua*.

2. Materials and Methods

2.1. Extraction of Essential Oils

Essential oils from sage (*Salvia officinalis* L.), lavender (*Lavandula angustifolia* Mill.), and common thyme (*Thymus vulgaris* L.) were obtained by hydrodistillation using a 20 L industrial distillation apparatus (Albrigi Luigi S.r.l., Grezzana, Verona, Italy). Plants were organically grown in Piazza Armerina, Sicily, Italy (37.387° N, 14.368° E), and collected fresh at the balsamic stage, between April and May 2024. Lavender flowers, sage, and thyme flowers and leaves were hydrodistilled for 2 h. The EOs were collected in amber glass vials and stored at 4 °C until January 2025.

2.2. Chemical Analysis of Essential Oils

EOs were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The analysis was performed with an Agilent 6850 gas chromatograph coupled to an Agilent 5975c mass spectrometer, an Agilent GC Sampler 80, and an Agilent DB-WAX-UI_MS 20M column. Chromatographic separation conditions involved injecting 0.2 µL of sample into a split/spitless injector at 250 °C with a split ratio of 500:1. Helium 5.6 IP (1 mL min⁻¹) was used as the carrier gas. The oven temperature program was set to 40 °C for 5 min, then increased at 5 °C min⁻¹ up to 240 °C, which was held for 15 min. The transfer line and ion source temperatures were both maintained at 250 °C, with a total runtime of 60 min. Following GC separation, analytes were ionized by positive Electron Impact (EI). Data processing was performed using MassHunter Qualitative Analysis software (version B.04.00; Agilent Technologies, Santa Clara, CA, USA). Spectral identification was carried out by matching spectra against the NIST/EPA/NIH Mass Spectral Library (NIST 11; National Institute of Standards and Technology, Gaithersburg, MD, USA), our in-house library, and the Kovats retention index (RI). The RI was calculated by injecting 100 mg L⁻¹ of a mixture

of normal paraffins in hexane, ranging from C8 to C30 carbon atoms, ensuring spectral and RI similarity greater than 90%. The relative percentage of each identified compound was calculated by dividing its peak area by the total peak area of the identified compounds and multiplying by 100. Therefore, the resulting values represent a semi-quantitative chemical profile rather than absolute compound concentrations.

2.3. Weed Species Tested

The herbicidal activity of the EOs was assessed against four weed species commonly found in Mediterranean agroecosystems: *Sonchus oleraceus* L., *Lolium rigidum* Gaudin, *Avena fatua* L., and *Amaranthus retroflexus* L. Seeds of *L. rigidum* and *A. fatua* were purchased from Cantueso Natural Seeds (Villarrubia, Córdoba, Spain) in 2022. Seeds of *S. oleraceus* were collected from horticultural fields in Meliana, Valencia, Spain (39.529° N, 0.3488° W) in January 2025, while seeds of *A. retroflexus* were collected from citrus orchards in Puçol, Valencia, Spain (39.6167° N, 0.3000° W) in May 2019.

2.4. In Vitro Bioassays

The in vitro assays were conducted in 9 cm-diameter Petri dishes under controlled conditions in a growth chamber. Each dish contained four filter paper disks (75 g m⁻²): two placed at the base as substrate and two covering the seeds. The papers were moistened with 5 mL of distilled water per replicate to ensure consistent and adequate moisture levels. The number of seeds per dish was adjusted according to the size and characteristics of each species, based on prior assays. Five replicates of 20 seeds were prepared for *S. oleraceus* and *A. retroflexus*; ten replicates of 10 seeds for *L. rigidum*, and ten replicates of 5 seeds for *A. fatua*. A total of 100 seeds were used for all species except *A. fatua*, for which 50 seeds were used. Seeds were incubated in an EGCHS series plant growth chamber (Equitec, Madrid, Spain). For the winter species, *A. fatua* and *L. rigidum*, conditions were set at 25 ± 0.1 °C with a 12 h light photoperiod and 18 ± 0.1 °C during 12 h of darkness. In contrast, *S. oleraceus* (a species present year-round) and *A. retroflexus* (a summer species) were incubated at 30 ± 0.1 °C with a 16 h light photoperiod and 20 ± 0.1 °C during 8 h of darkness.

Each EO was tested at the following concentrations: 0 (water control), 0.125, 0.25, 0.50, 0.75, 1.0, and 1.5 µL mL⁻¹. The EOs were applied to the inner filter paper disk placed above the seeds, as reported in previous studies [9–11]. Thyme and lavender EOs were evaluated against all four weed species, whereas sage EO was tested only against *S. oleraceus* and *L. rigidum*. These two species were chosen as representative dicotyledonous and monocotyledonous weeds, respectively, to provide a preliminary assessment of sage EO phytotoxicity across two functionally different weed groups. Compared with thyme and lavender EOs, sage EO has been less extensively studied in the literature for bioherbicidal applications; therefore, it was included in the present study as an initial screening treatment. This approach aligns with previous preliminary screenings for EO phytotoxicity conducted on selected weed species [25].

2.5. Germination and Seedling Growth Measurements

Seed germination was monitored over time, and the number of germinated seeds was recorded. A seed was considered germinated when the emerging radicle was clearly visible. Observations were made at 3, 5, 7, and 10 days after treatment (DAT) for the monocotyledonous species (*L. rigidum* and *A. fatua*), and at 4, 7, 10, and 14 DAT for the dicotyledonous species (*S. oleraceus* and *A. retroflexus*). Photographs of each Petri dish were taken to track seedling development and growth over time. Subsequently, seedling length was measured using Fiji software (version 2.14.0/1.54f, National Institutes of Health, Bethesda, MD, USA) to analyze the images.

2.6. Statistical Analysis

Data were subjected to one-way ANOVA separately for each EO, weed species, and observation time, with EO concentration as the main factor, using Statgraphics® Centurion XVII software (StatPoint Technologies Inc., Warrenton, VA, USA, version 18) and IBM SPSS Statistics (version 31.0.0.0). Percentage values were arcsine-transformed prior to analysis. Means were compared using Fisher's least significant difference (LSD) test at $p < 0.05$.

3. Results and Discussion

3.1. Essential Oil Composition

The differences observed among the analyzed EOs highlight how the quality of the aromatic profile can be influenced by the plant's origin, environmental conditions, phenological stage, and harvest period [20].

A total of 20 compounds were identified in *T. vulgaris* EO, comprising 100% of its total composition (Table 1).

Table 1. *Thymus vulgaris* essential oil composition.

RT	RI	Compound	Area (%)
7.85	1015	Methyl 2-methylbutanoate	0.5
8.15	1024	α -Pinene	1.1
8.35	1030	β -Thujene	2.3
9.67	1069	Camphene	0.3
11.09	1111	β -Pinene	0.3
13.03	1170	β -Myrcene	2.7
13.53	1185	α -Terpinene	2.7
14.15	1204	(+)-Limonene	0.4
14.55	1217	Eucalyptol	0.7
15.66	1254	γ-Terpinene	27.1
16.17	1270	4-Penten-1-ol propanoate	0.3
16.40	1268	<i>p</i>-Cymene	15.7
21.97	1477	4-Thujanol	0.4
23.94	1555	Linalool	4.2
24.14	1563	Linalyl anthranilate	0.2
25.42	1614	($\pm$)-Lavandulyl acetate	2.5
27.68	1711	α -Terpineol	0.6
30.32	1829	Geranyl propanoate	0.2
37.58	2191	Thymol	36.5
38.15	2222	Carvacrol	1.4
		Total identified	100.0

RT: retention time; RI: retention index. Compounds are listed in elution order. Relative peak area percentages were normalized to the total peak area of the identified compounds and represent a semi-quantitative chemical profile. Values in bold indicate the three compounds with the highest relative peak area percentages in the EO.

The oil was predominantly composed by thymol (36.5%), γ -terpinene (27.1%), and *p*-cymene (15.7%), which together accounted for 79.3% of the total identified constituents. Other constituents included linalool (4.2%), β -myrcene (2.7%), α -terpinene (2.7%), carvacrol (1.4%), and α -pinene (1.1%). This composition reflects a thymol-rich chemical profile, consistent with previous studies on *T. vulgaris* EO [26–28]. Several minor compounds were also detected, such as β -myrcene (2.7%), α -caryophyllene (2.7%), lavandulyl acetate (2.5%), β -thujene (2.3%), isothymol (1.4%), and α -pinene (1.1%). A similar profile was reported by Dong et al. [29] for *T. vulgaris* EO from Beijing, China, which was mainly characterized by thymol (42.4%), γ -terpinene (17.8%), and *p*-cymene (17.2%), along with minor constituents including linalool (2.3%) and β -myrcene (2.5%). Compared with the Sicilian EO analyzed in the present study, this profile exhibited the same dominant constituents but in different

relative proportions. These differences may be attributed to the geographical origin of the plant material, as well as factors such as genotype/chemotype, environmental conditions, phenological stage, harvest period, and extraction conditions. Chemical analysis of lavender essential oil identified a total of 31 compounds. The oil was primarily characterized by linalool (28.1%), linalyl anthranilate (anthranilic acid, linalyl ester) (14.6%), eucalyptol (1,8-cineole) (14.3%), and camphor (9.1%) (Table 2), which together accounted for 66.1% of the identified fraction.

Table 2. *Lavandula angustifolia* essential oil composition.

RT	RI	Compound	Area (%)
8.16	1024	α -Pinene	1.3
9.67	1069	Camphene	0.6
11.09	1111	β -Pinene	1.2
11.58	1126	Sabinene	0.4
12.52	1154	δ -3-Carene	0.3
13.04	1170	β -Myrcene	1.4
14.16	1204	(+)-Limonene	1.8
14.47	1215	β -Phellandrene	1.5
14.56	1218	Eucalyptol	14.3
15.30	1242	trans- β -Ocimene	2.8
15.66	1254	γ -Terpinene	0.3
15.82	1259	cis- β -Ocimene	2.9
16.81	1291	Terpinolene	0.6
20.57	1425	Hexyl butyrate	0.6
21.33	1453	n-Hexyl isovalerate	0.3
23.43	1532	Camphor	9.1
23.97	1556	Linalool	28.2
24.22	1566	Linalyl anthranilate	14.7
25.43	1615	($\pm$)-Lavandulyl acetate	3.6
25.47	1617	1-Menthen-4-ol	3.9
26.81	1673	trans- β -Farnesene	1.8
27.68	1711	α -Terpineol	1.4
28.07	1728	Germacrene D	2.5
28.24	1735	exo-Borneol	1.2
28.32	1739	endo-Borneol	1.1
28.64	1753	Elixene	0.3
28.93	1766	cis-Geranyl acetate	0.8
29.21	1779	γ -Cadinene	0.2
31.29	1874	Geraniol	0.3
37.57	2191	Thymol	0.3
38.30	2231	α -Bisabolol	0.5
		Total identified	100.0

RT: retention time; RI: retention index. Compounds are listed in elution order. Relative peak area percentages were normalized to the total peak area of the identified compounds and represent a semi-quantitative chemical profile. Values in bold indicate the three compounds with the highest relative peak area percentages in the EO.

Other compounds were detected at lower percentages, including 1-menthen-4-ol (3.9%), lavandulyl acetate (3.6%), cis- β -ocimene (2.9%), trans- β -ocimene (2.8%), germacrene D (2.5%), and (+)-limonene (1.8%). This profile aligns with the considerable chemical variability reported for *Lavandula angustifolia* EO in various studies [30–32]. Notably, Todorova et al. [31] compared the composition of *L. angustifolia* EO from biocultivated plants grown in the Thracian Lowland floristic region of Bulgaria with commercial lavender EO products from the Bulgarian market, identifying linalool and linalyl acetate as the dominant compounds. Similarly, Massoud et al. [32] reported a linalool-rich profile (32.5%) for *L. angustifolia* EO cultivated in Lebanon, although with different relative proportions of

the main constituents, camphor (9.9%), and 1,8-cineole (eucalyptol) (8.8%). The authors also identified minor compounds, including (E)- β -farnesene (8.7%), 4-terpineol (7.6%), linalyl acetate (4.2%), borneol (4.2%), and α -terpinyl acetate (4.4%). Compared with these Bulgarian and Lebanese oils, the Sicilian lavender EO analyzed in the present study shared linalool as a major constituent but exhibited a different relative distribution of secondary compounds, including linalyl anthranilate and 1,8-cineole.

Interestingly, linalyl anthranilate has also been identified in the essential oils of various *L. angustifolia* cultivars. Adaszyńska et al. [33] detected this compound among the principal constituents of five lavender varieties cultivated in Poland, with relative abundances ranging from 1.6% to 12.3%, alongside linalool, terpinen-4-ol, α -terpineol, and linalool oxide.

The sage EO was primarily characterized by eucalyptol (21.1%), thujone (11.5%), camphene (8.9%), α -pinene (6.5%), iso-thujone (5.6%), β -pinene (5.5%), and lavandulyl acetate (4.2%), which together comprised 63.3% of the total profile (Table 3). This composition aligns with findings from other studies on sage EO [34,35]. A significant presence of 1,8-cineole was also reported by Ilić et al. [36] in the EO of wild *S. officinalis* plants collected near Herceg Novi, Montenegro, although at a lower percentage than in the present study (13.8% vs. 21.1%). Conversely, thujone was considerably higher in that EO, reaching 43.3%, compared with 11.5% in the current sample.

Table 3. *Salvia officinalis* essential oil composition.

RT	RI	Compound	Area (%)
5.61	942	Salvene	0.4
7.65	1009	Cyclene	0.4
8.16	1024	α -Pinene	6.5
8.35	1030	β -Thujene	0.4
9.67	1069	Camphene	8.9
11.09	1111	β -Pinene	5.5
11.58	1126	Sabinene	0.3
13.03	1170	β -Myrcene	2.6
13.54	1185	α -Caryophyllene	0.2
14.16	1204	(+)-Limonene	2.2
14.57	1218	Eucalyptol	21.1
15.66	1254	γ -Terpinene	0.5
16.40	1278	<i>p</i> -Cymene	0.4
16.81	1291	Terpinolene	0.3
20.86	1436	Thujone	11.5
21.38	1455	iso-Thujone	5.6
23.43	1534	d-Camphor	1.9
23.73	1546	(-)- α -Gurjunene	0.8
25.43	1615	($\pm$)-Lavandulyl acetate	4.2
25.56	1621	Selina-3,7(11)-diene	0.3
25.69	1626	Aromadendrene	1.4
27.17	1689	α -Caryophyllene	2.4
27.56	1705	γ -Muurolene	0.3
27.68	1711	α -Terpineol	0.3
27.74	1713	Ledene	0.4
28.08	1728	Germacrene D	1.0
28.64	1753	Elixene	0.8
29.10	1774	δ -Cadinene	0.3
32.91	1952	Palustrol	0.6
34.11	2011	Caryophyllene oxide	0.2

Table 3. *Cont.*

RT	RI	Compound	Area (%)
34.97	2055	Ledol	0.3
36.02	2109	Viridiflorol	0.6
37.58	2191	Thymol	0.3
		Total identified	100.0

RT: retention time; RI: retention index. Compounds are listed in elution order. Relative peak area percentages were normalized to the total peak area of the identified compounds and represent a semi-quantitative chemical profile. Values in bold indicate the three compounds with the highest relative peak area percentages in the EO.

The presence of compounds such as thymol, linalool, p-cymene, 1,8-cineole, camphene, and thujone is significant, as some of these have been identified as responsible for the biological and phytotoxic properties of EOs. For instance, thymol, carvacrol, linalool, 1,8-cineole, camphor, α -pinene, and limonene have been reported as components of EOs exhibiting strong phytotoxicity [37–40]. The differences observed in the analyzed EOs underscore how the quality of the aromatic profile can be influenced by the plant's origin, environmental conditions, phenological stage, and harvest period [20,41].

3.2. Phytotoxic Effects of Essential Oils on Weed Germination

Thyme EO exhibited the strongest phytotoxic effect, completely inhibiting the germination of three of the four species tested, *L. rigidum*, *A. fatua*, and *S. oleraceus*, at all concentrations examined (Table 4). In contrast, *A. retroflexus* was the most resistant species to this EO. At the lowest concentrations (0.125 and 0.25 $\mu\text{L mL}^{-1}$), germination was inhibited by 69.6% and 65.7%, respectively, compared with the control, while doses above 0.25 $\mu\text{L mL}^{-1}$ entirely prevented germination.

Table 4. Effect of *Thymus vulgaris* essential oil on seed germination (%) of the tested weed species.

Concentration ($\mu\text{L mL}^{-1}$)	Seed Germination (%)			
	<i>Avena fatua</i>	<i>Lolium rigidum</i>	<i>Sonchus oleraceus</i>	<i>Amaranthus retroflexus</i>
0 (CONTROL)	92.0 $\pm$ 4.4 a	88.0 $\pm$ 5.8 a	34.0 $\pm$ 5.3 a	70.0 $\pm$ 8.3 a
0.125	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	22.0 $\pm$ 8.1 b
0.25	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	24.0 $\pm$ 6.4 b
0.50	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 c
0.75	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 c
1.00	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 c
1.50	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 c

Values represent mean seed germination (%) $\pm$ SE. Essential oil treatments were applied at 0.125, 0.25, 0.50, 0.75, 1.00, and 1.50 $\mu\text{L mL}^{-1}$, with distilled water as the control. For the dicotyledonous species *S. oleraceus* and *A. retroflexus*, data were obtained from five replicates of 20 seeds, evaluated after 14 days of incubation. For *L. rigidum* and *A. fatua*, data were obtained from 10 replicates of 10 and 5 seeds, respectively, evaluated after 10 days of incubation. Within each species, different letters indicate significant differences among EO concentrations according to Fisher's LSD test ($p < 0.05$).

The germination dynamics of weed seeds following treatment with thyme EO are illustrated in Figure 1. No germination was observed in the seeds of *L. rigidum*, *A. fatua*, and *S. oleraceus* throughout the entire experiment. In *A. retroflexus*, the two lowest concentrations of thyme EO tested (0.125 and 0.25 $\mu\text{L mL}^{-1}$) delayed and reduced germination, while the higher concentrations completely inhibited it.

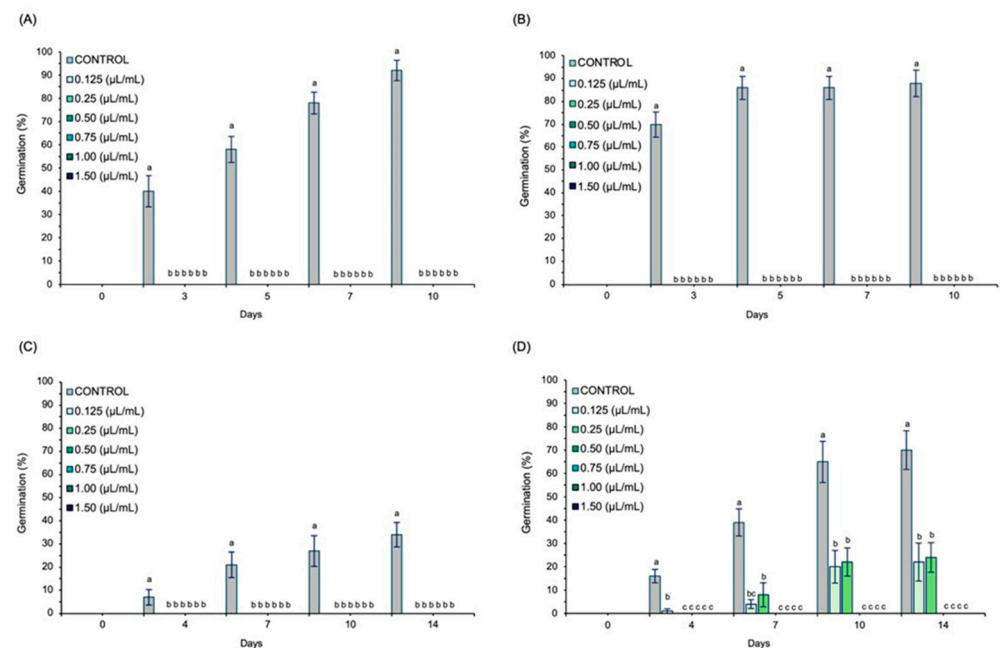


Figure 1. Germination evolution of *Avena fatua* (A), *Lolium rigidum* (B), *Sonchus oleraceus* (C), and *Amaranthus retroflexus* (D) seeds treated with *Thymus vulgaris* essential oil. Essential oil treatments were applied at 0.125, 0.25, 0.50, 0.75, 1.00, and 1.50 $\mu\text{L mL}^{-1}$, with distilled water as the control. Values represent mean germination (%) $\pm$ SE. Within each observation date, different letters indicate significant differences among concentrations according to Fisher's LSD test ($p < 0.05$).

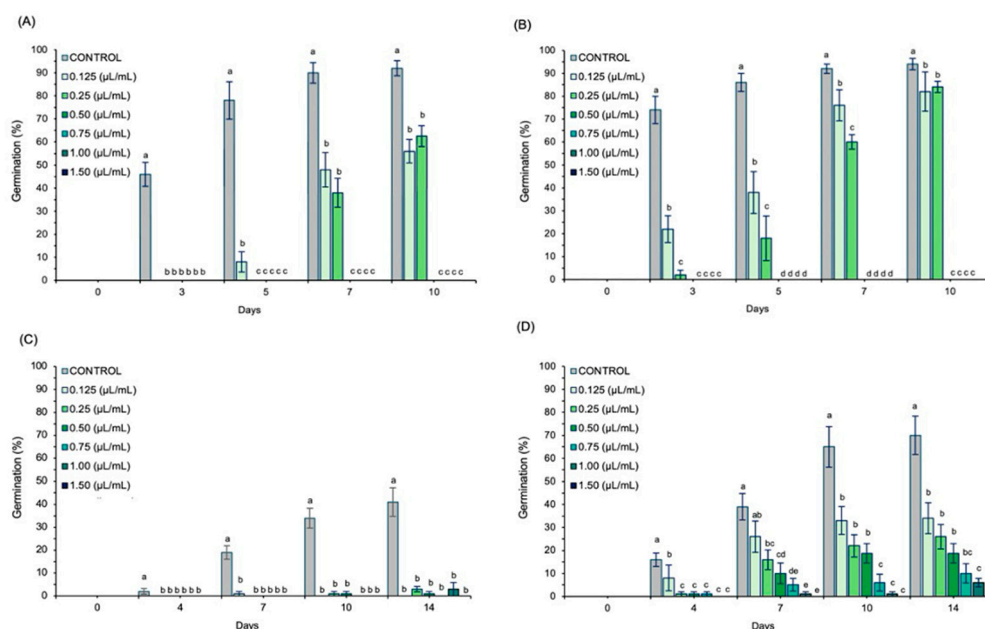
Although EO phytotoxicity cannot be attributed to a single constituent, the pronounced inhibitory effects observed with thyme EO suggest that its major components play a significant role in inhibiting seed germination. Previous studies on Mediterranean *Lamiaceae* EOs have similarly linked phytotoxic activity to compounds such as thymol, carvacrol, and p-cymene. Consistent with the results presented here, Verdeguer et al. [10] reported strong herbicidal activity for the carvacrol-rich EO of *T. capitata*, while Boukhalfa et al. [25] observed marked germination inhibition in *L. perenne* and *A. retroflexus* following treatment with various *T. vulgaris* EOs rich in p-cymene, thymol, and carvacrol. Likewise, thyme EO in the present study completely suppressed the germination of *L. rigidum*, *A. fatua*, and *S. oleraceus* at the lowest concentration tested and fully inhibited *A. retroflexus* at concentrations of 0.50 $\mu\text{L mL}^{-1}$ or higher. Lavender EO exhibited lower inhibitory activity than thyme EO. Among the tested species, *S. oleraceus* was the most sensitive to lavender EO, with seed germination almost entirely inhibited at all concentrations tested. The two monocotyledonous species also showed significant sensitivity to lavender EO, with germination completely inhibited at concentrations $\geq 0.50 \mu\text{L mL}^{-1}$, although *A. fatua* exhibited greater reductions in germination at lower concentrations than *L. rigidum*. *A. retroflexus* was the most tolerant species, displaying lower sensitivity to the concentrations tested. The phytotoxic effect of lavender EO on seed germination was dependent on both weed species and the EO concentration (Table 5).

Figure 2 illustrates the temporal evolution of seed germination (%) in weed species treated with lavender EO. The results reveal a distinctly species-specific response to the lavender EO. In both *A. fatua* and *L. rigidum*, partial germination occurred at the lowest concentrations, while germination was entirely inhibited at concentrations of 0.50 $\mu\text{L mL}^{-1}$ or higher. In *S. oleraceus*, germination was significantly suppressed from the early stages of the experiment at nearly all tested concentrations, although complete inhibition was not consistently observed. By contrast, *A. retroflexus* displayed the clearest dose-dependent response, with germination steadily decreasing as the EO concentration increased.

Table 5. Effect of *Lavandula angustifolia* essential oil on seed germination (%) of the tested weed species.

Concentration ($\mu\text{L mL}^{-1}$)	Seed Germination (%)			
	<i>Avena fatua</i>	<i>Lolium rigidum</i>	<i>Sonchus oleraceus</i>	<i>Amaranthus retroflexus</i>
0 (CONTROL)	92.0 $\pm$ 3.27 a	94.0 $\pm$ 2.4 a	41.0 $\pm$ 6.2 a	70.0 $\pm$ 8.3 a
0.125	56.0 $\pm$ 4.9 b	82.0 $\pm$ 8.6 b	0.0 $\pm$ 0.0 b	34.0 $\pm$ 6.7 b
0.25	62.5 $\pm$ 4.5 b	84.0 $\pm$ 2.4 b	3.0 $\pm$ 1.2 b	26.0 $\pm$ 5.3 b
0.50	0.0 $\pm$ 0.0 c	0.0 $\pm$ 0.0 c	1.0 $\pm$ 1.0 b	18.7 $\pm$ 4.2 b
0.75	0.0 $\pm$ 0.0 c	0.0 $\pm$ 0.0 c	0.0 $\pm$ 0.0 b	10.0 $\pm$ 4.1 bc
1.00	0.0 $\pm$ 0.0 c	0.0 $\pm$ 0.0 c	3.0 $\pm$ 3.0 b	6.0 $\pm$ 1.8 c
1.50	0.0 $\pm$ 0.0 c	0.0 $\pm$ 0.0 c	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 d

Values represent mean seed germination (%) $\pm$ SE. Essential oil treatments were applied at 0.125, 0.25, 0.50, 0.75, 1.00, and 1.50 $\mu\text{L mL}^{-1}$, with distilled water as the control. For dicotyledonous species *S. oleraceus* and *A. retroflexus*, data were obtained from 5 replicates of 20 seeds, evaluated after 14 days of incubation. For *L. rigidum* and *A. fatua*, data were obtained from 10 replicates of 10 and 5 seeds, respectively, evaluated after 10 days of incubation. Within each species, different letters indicate significant differences among concentrations according to Fisher's LSD test ($p < 0.05$).

**Figure 2.** Germination evolution of *Avena fatua* (A), *Lolium rigidum* (B), *Sonchus oleraceus* (C), and *Amaranthus retroflexus* (D) seeds treated with *Lavandula angustifolia* essential oil. Essential oil treatments were applied at 0.125, 0.25, 0.50, 0.75, 1.00, and 1.50 $\mu\text{L mL}^{-1}$, with distilled water as the control. Values represent mean germination (%) $\pm$ SE. Within each observation date, different letters indicate significant differences among concentrations according to Fisher's LSD test ($p < 0.05$).

The phytotoxic activity observed in lavender EO aligns with previous studies reporting herbicidal effects of several constituents commonly found in *Lavandula angustifolia* EOs. Koïou et al. [30] evaluated the phytotoxic effects of selected components of *L. angustifolia* EO against *Setaria verticillata* (L.) P. Beauv. and found that terpinen-4-ol completely inhibited seed germination. Strong inhibitory effects were also noted for linalyl acetate and lavalindulyl acetate, which reduced germination by 84.54% and 81.91%, respectively, and root growth by 74.01% and 70.26%, respectively. However, the EO analyzed in the present study exhibited a different chemical profile, being primarily characterized by linalool, linalyl anthranilate, and 1,8-cineole. Therefore, the findings of Koïou et al. [30] cannot be directly attributed to the same major constituents, but they support the broader evidence that the phytotoxicity of lavender EOs is strongly influenced by their specific chemical composition.

Similarly, Haig et al. [42] reported that the phytotoxic properties of lavender extracts vary among *Lavandula* species and plant organs, further emphasizing the importance of chemical composition in determining biological activity. Our results concur with these findings, as lavender EO exhibited a clear dose-dependent phytotoxic effect that was generally less pronounced than that of thyme EO. This behavior may be related to its chemical profile, which is dominated by linalool, linalyl anthranilate, and 1,8-cineole. In comparison with thyme and lavender EOs, sage EO showed the weakest inhibitory activity and a species-dependent response. Germination of *S. oleraceus* was suppressed at concentrations of 1.00 $\mu\text{L mL}^{-1}$ or higher, whereas *L. rigidum* was more tolerant, with germination completely inhibited only at the highest concentration tested (1.50 $\mu\text{L mL}^{-1}$) (Table 6).

Table 6. Effect of *Salvia officinalis* essential oil on seed germination (%) of the tested weed species.

Seed Germination (%)		
Concentration ($\mu\text{L mL}^{-1}$)	<i>Sonchus oleraceus</i>	<i>Lolium rigidum</i>
0 (CONTROL)	43.0 $\pm$ 2.5 a	92.0 $\pm$ 3.7 a
0.125	41.0 $\pm$ 4.0 a	92.0 $\pm$ 3.7 a
0.25	31.2 $\pm$ 4.2 b	88.0 $\pm$ 8.0 a
0.50	33.7 $\pm$ 4.2 b	88.0 $\pm$ 5.8 a
0.75	33.7 $\pm$ 2.3 b	60.0 $\pm$ 11.8 b
1.00	0.0 $\pm$ 0.0 c	12.0 $\pm$ 7.3 c
1.50	0.0 $\pm$ 0.0 c	0.0 $\pm$ 0.0 c

Values represent mean seed germination (%) $\pm$ SE. Essential oil treatments were applied at 0.125, 0.25, 0.50, 0.75, 1.00, and 1.50 $\mu\text{L mL}^{-1}$, with distilled water as the control. For *S. oleraceus*, data were obtained from 5 replicates of 20 seeds, evaluated after 14 days of incubation. For *L. rigidum*, data were obtained from 10 replicates of 10 seeds, evaluated after 10 days of incubation. Within each species, different letters indicate significant differences among concentrations according to Fisher's LSD test ($p < 0.05$).

Regarding the germination progress of weed seeds treated with sage EO (Figure 3), it was observed that lower doses delayed germination in both species tested.

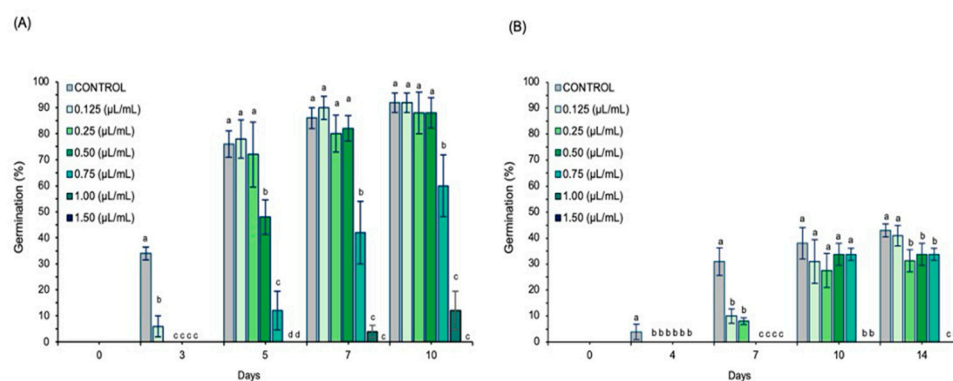


Figure 3. Germination evolution of *Lolium rigidum* (A) and *Sonchus oleraceus* (B) seeds treated with *Salvia officinalis* essential oil. Essential oil treatments were applied at 0.125, 0.25, 0.50, 0.75, 1.00, and 1.50 $\mu\text{L mL}^{-1}$, with distilled water as the control. Values represent mean germination (%) $\pm$ SE. Within each observation date, different letters indicate significant differences among concentrations according to Fisher's LSD test ($p < 0.05$).

A similar species- and dose-dependent response was reported by Alexa et al. [43], who assessed the allelopathic activity of *S. officinalis* and *T. vulgaris* EOs against *A. retroflexus*, *C. album*, and *Echinochloa crus-galli*. In their study, sage EO exhibited a strong inhibitory effect on *A. retroflexus*, achieving 92.1% germination inhibition at 0.3% and complete inhibition at 0.6% and 1.0%. However, its efficacy was less consistent across the other weed species: complete inhibition of *C. album* occurred only at 1.0%, whereas *E. crus-galli* germination

was reduced but not entirely suppressed, even at the highest concentrations. In contrast, thyme EO demonstrated a more potent overall phytotoxic activity, completely inhibiting the germination of *C. album* and *E. crus-galli* at 0.6% and 1.0%. Therefore, our findings align with those of Alexa et al. [43], indicating that sage EO can effectively reduce seed germination, although its activity tends to increase progressively with concentration. In our study, sage EO inhibited the germination of both *S. oleraceus* and *L. rigidum*, but complete inhibition was only achieved at higher concentrations than those required for thyme and lavender EOs. These differences may be attributed to variations in EO composition. Indeed, the sage EO analyzed by Alexa et al. [43] comprised caryophyllene (26.4%), camphene (10.9%), eucalyptol (10.8%), and β -pinene (10.1%), whereas the thyme EO they tested was characterized by high levels of γ -terpinene (68.4%) and thymol (8.9%). De Mastro et al. [16] highlighted the significance of *Lamiaceae* EOs, including those from oregano, thyme, rosemary, sage, and mint, as promising sources for the development of natural herbicides. Nevertheless, sage EO has been less extensively studied than thyme, oregano, and rosemary EOs.

3.3. Phytotoxic Effects of Essential Oils on Seedling Growth

In addition to assessing seed germination, seedling growth was evaluated to provide a more comprehensive assessment of the phytotoxicity of EOs. This approach is especially pertinent as EOs may allow partial germination, particularly at low concentrations, while still significantly inhibiting early seedling development [44]. This distinction is important because small or poorly developed seedlings are likely to have reduced establishment potential and diminished competitive ability against crops [5]. Therefore, seedling growth serves as a valuable indicator of phytotoxic activity, complementing germination assessments and offering insights particularly relevant to the potential application of EOs in post-emergence weed control strategies [45].

Thymus vulgaris EO strongly inhibited both seed germination and seedling growth in all the weed species tested. Germination responses are presented in Table 4 and Figure 1, while seedling growth responses are shown in Table 7 and Figure 4. In *S. oleraceus*, *L. rigidum*, and *A. fatua*, germination and subsequent seedling development were completely suppressed (Table 7). *A. retroflexus* exhibited slightly greater tolerance at low concentrations of *T. vulgaris* EO. The length of seedlings treated with $0.125 \mu\text{L mL}^{-1}$ did not differ significantly from the controls, whereas treatment with $0.25 \mu\text{L mL}^{-1}$ reduced seedling growth by 37%. At concentrations of $0.50 \mu\text{L mL}^{-1}$ or higher, both germination and seedling growth of *A. retroflexus* were entirely inhibited throughout the assay (Table 7 and Figure 4).

Table 7. Effect of *Thymus vulgaris* essential oil on seedling length (mm) of the tested weed species.

Concentration ($\mu\text{L mL}^{-1}$)	Seedling Length (mm)			
	<i>Avena fatua</i>	<i>Lolium rigidum</i>	<i>Sonchus oleraceus</i>	<i>Amaranthus retroflexus</i>
0 (CONTROL)	52.5 $\pm$ 5.4 a	39.8 $\pm$ 6.4 a	80.0 $\pm$ 2.6 a	31.4 $\pm$ 0.4 a
0.125	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	22.2 $\pm$ 2.0 a
0.25	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	19.6 $\pm$ 3.5 b
0.50	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 c
0.75	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 c
1.00	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 c
1.50	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 c

Values represent mean seedling length (mm) $\pm$ SE. Essential oil treatments were applied at 0.125, 0.25, 0.50, 0.75, 1.00, and $1.50 \mu\text{L mL}^{-1}$, with distilled water as the control. Seedling length was assessed after 14 days of incubation for the dicotyledonous species (*S. oleraceus* and *A. retroflexus*) and after 10 days for the monocotyledonous species (*A. fatua* and *L. rigidum*). Within each species, different letters indicate significant differences among *T. vulgaris* EO concentrations according to Fisher's LSD test ($p < 0.05$).

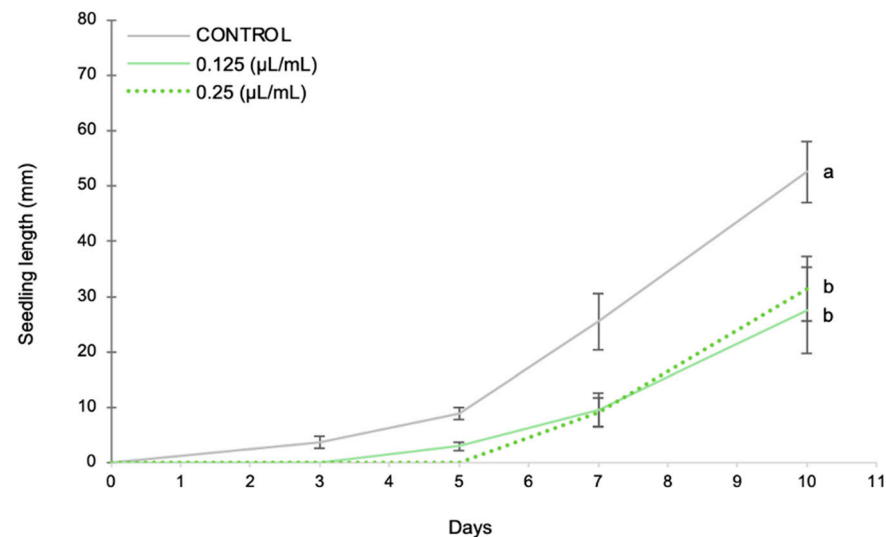


Figure 4. Effect of *Thymus vulgaris* essential oil on seedling growth of *Amaranthus retroflexus* throughout the assay. Essential oil treatments were applied at 0.125, 0.25, 0.50, 0.75, 1.00, and 1.50 $\mu\text{L mL}^{-1}$, with distilled water as the control. Values represent mean seedling length (mm) $\pm$ SE. At 10 and/or 14 days after treatment, different letters indicate significant differences among concentrations according to Fisher's LSD test ($p < 0.05$).

Verdeguer et al. [10] observed almost complete growth inhibition in *A. retroflexus* treated with *T. capitata* EO. This more pronounced effect is attributed to the different composition, as the *T. capitata* EO tested contained a high concentration of carvacrol. In the present study, *A. retroflexus* was the only species in which seedlings developed when treated with the lowest concentrations of *T. vulgaris* EO. Similar findings were reported by Alexa et al. [43], who found that *A. retroflexus* was less sensitive to *T. vulgaris* EO than other weed species. In their study, thyme EO, primarily composed of γ -terpinene and thymol, caused only limited inhibition of germination at 0.3% and approximately 60.6% inhibition at 0.6% and 1.0%. In contrast, complete inhibition was observed in *C. album* and *E. crus-galli* at the higher concentrations. Although their study focused on seed germination rather than seedling growth, these results align with the lower sensitivity of *A. retroflexus* observed in the present work.

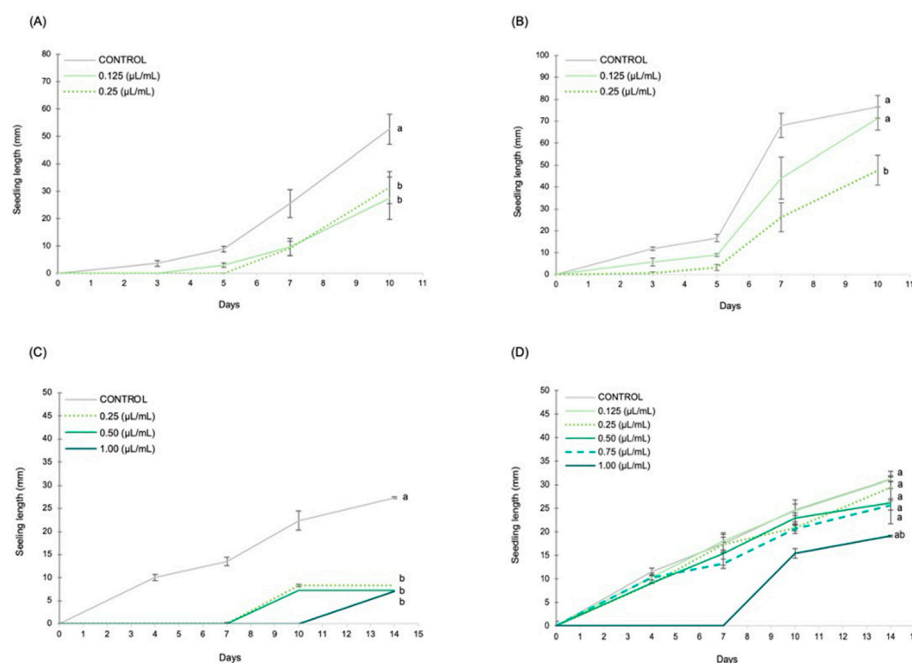
The EO of *L. angustifolia* exhibited an inhibitory effect on the development of seedlings across all the weed species tested (Table 8). In the monocotyledonous species (*A. fatua* and *L. rigidum*), only seedlings treated with the two lowest concentrations of *L. angustifolia* EO were able to develop. The maximum inhibition of seedling growth observed was 43.24% for *A. fatua* (0.125 $\mu\text{L mL}^{-1}$) and 37.77% for *L. rigidum* (0.25 $\mu\text{L mL}^{-1}$). Seedling growth of *S. oleraceus* was completely inhibited at concentrations of 0.25, 0.75, and 1.50 $\mu\text{L mL}^{-1}$. Other tested doses also significantly reduced the seedling length by up to 74% at 1 $\mu\text{L mL}^{-1}$. Growth of *A. retroflexus* seedlings was significantly reduced only when *L. angustifolia* EO was applied at 1 $\mu\text{L mL}^{-1}$.

Seedling growth over time confirmed the species-specific response to *L. angustifolia* EO (Figure 5). In *A. fatua* and *S. oleraceus*, seedling growth was severely restricted from the earliest stages due to their sensitivity. In contrast, *L. rigidum* showed an intermediate growth pattern, with low doses permitting seedling development. *A. retroflexus* maintained a consistent growth trend between the control and the lowest treatment levels, confirming its tolerance.

Table 8. Effect of *Lavandula angustifolia* essential oil on seedling length (mm) of the tested weed species.

Concentration ($\mu\text{L mL}^{-1}$)	Seedling Length (mm)			
	<i>Avena fatua</i>	<i>Lolium rigidum</i>	<i>Sonchus oleraceus</i>	<i>Amaranthus retroflexus</i>
0 (CONTROL)	52.5 $\pm$ 5.4 a	76.5 $\pm$ 5.1 a	27.3 $\pm$ 0.2 a	31.1 $\pm$ 0.4 a
0.125	27.4 $\pm$ 7.7 b	71.2 $\pm$ 5.2 a	0.0 $\pm$ 0.0 b	31.0 $\pm$ 1.7 a
0.25	29.8 $\pm$ 5.8 b	47.6 $\pm$ 6.7 b	8.3 $\pm$ 0.3 b	29.4 $\pm$ 2.3 a
0.50	0.0 $\pm$ 0.0 c	0.0 $\pm$ 0.0 c	7.3 $\pm$ 0.0 b	26.1 $\pm$ 4.4 a
0.75	0.0 $\pm$ 0.0 c	0.0 $\pm$ 0.0 c	0.0 $\pm$ 0.0 b	25.6 $\pm$ 3.0 a
1.00	0.0 $\pm$ 0.0 c	0.0 $\pm$ 0.0 c	7.1 $\pm$ 0.0 b	19.1 $\pm$ 0.2 ab
1.50	0.0 $\pm$ 0.0 c	0.0 $\pm$ 0.0 c	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b

Values represent mean seedling length (mm) $\pm$ SE. Essential oil treatments were applied at 0.125, 0.25, 0.50, 0.75, 1.00, and 1.50 $\mu\text{L mL}^{-1}$, with distilled water as the control. Seedling length was assessed after 14 days of incubation for the dicotyledonous species (*S. oleraceus* and *A. retroflexus*) and after 10 days for the monocotyledonous species (*A. fatua* and *L. rigidum*). Within each species, different letters indicate significant differences among *L. angustifolia* EO concentrations according to Fisher's LSD test ($p < 0.05$).

**Figure 5.** Effect of *Lavandula angustifolia* essential oil on seedling growth of *Avena fatua* (A), *Lolium rigidum* (B), *Sonchus oleraceus* (C), and *Amaranthus retroflexus* (D) throughout the assay. Essential oil treatments were applied at 0.125, 0.25, 0.50, 0.75, 1.00, and 1.50 $\mu\text{L mL}^{-1}$, with distilled water as the control. Values represent mean seedling length (mm) $\pm$ SE. At 10 and/or 14 days after treatment, different letters indicate significant differences among concentrations according to Fisher's LSD test ($p < 0.05$).

The inhibitory effect of lavender EO on seedling growth is consistent with findings from previous studies. Koiou et al. [30] reported that the primary compounds of *L. angustifolia* EO, including terpinen-4-ol, lavandulol, and linalyl acetate, suppressed the germination and root length of *S. verticillata* seeds. Notably, terpinen-4-ol completely inhibited germination and root growth at a concentration of 80 nL cm^{-3} . Further research by Haig et al. [42] examined the phytotoxicity of aqueous extracts from various *Lavandula* taxa and cultivars, such as *L. × intermedia* 'Grosso', *L. × heterophylla*, *L. angustifolia* 'Edgerton Blue', *L. × allardii* 'Mitchum', *L. dentata* 'Candicans', *L. viridis*, and *L. stoechas* 'Fairy Wings', against *L. rigidum*. Among these, the aqueous extract derived from the stems and leaves of *Lavandula × intermedia* 'Grosso' exhibited the strongest phytotoxic effect, significantly inhibiting root growth.

Bioassay-guided fractionation identified coumarin and 7-methoxycoumarin as the principal phytotoxic compounds in the most active fraction. However, since these compounds were found in aqueous extracts rather than essential oils, their activity cannot be directly applied to explain the effects observed for the *L. angustifolia* EO evaluated in the present study. Nevertheless, these findings underscore the importance of investigating not only EOs but also other plant-derived extracts and by-products, as they may offer additional opportunities to optimize the use of the entire production chain. Other plant-derived matrices may also contain compounds of interest for weed management. For example, Rafiq et al. [46] identified several constituents previously associated with herbicidal activity in a methanolic stem extract of *Carthamus oxyantha*. This is particularly pertinent for lavender species EOs, which are predominantly utilized in the cosmetic and perfumery industries, while aqueous extracts, extraction residues, and other processing by-products could serve as valuable sources of bioactive compounds for agricultural applications, including weed management. Vasilakoglou et al. [11] emphasized the strong correlation between the biological activity of an EO and its composition, providing a detailed examination of the herbicidal activity of 19 major EO constituents. They found that thymol, linalool, carvacrol, trans-anethole, and carvone produced the greatest inhibition of both germination and root length in *L. rigidum*. The biological activities reported for thymol and linalool are particularly helpful in interpreting the responses observed in the present study, where thymol was the dominant constituent of *T. vulgaris* EO, and linalool, alongside eucalyptol, was among the main components of *L. angustifolia* EO. However, predicting the activity of an EO based solely on its major compounds is not straightforward, as minor constituents may also affect its phytotoxicity through synergistic, additive, or antagonistic interactions [16].

Sage EO had a marked effect on the final seedling length in both weed species studied, although the response patterns differed between them. In the case of *S. oleraceus*, seedling growth was slightly reduced at the lowest concentration tested. A significant decrease in seedling length was observed at 0.25 $\mu\text{L mL}^{-1}$, with complete growth inhibition occurring at the highest concentrations of 1.00 and 1.50 $\mu\text{L mL}^{-1}$. For *L. rigidum*, the effect of sage EO on seedling length was more gradual and concentration-dependent; as the EO concentration increased, seedling length progressively decreased (Table 9; Figure 6).

Table 9. Effect of *Salvia officinalis* essential oil on seedling length (mm) of the tested weed species.

Concentration ($\mu\text{L mL}^{-1}$)	Seedling Length (mm)	
	<i>Sonchus oleraceus</i>	<i>Lolium rigidum</i>
0 (CONTROL)	31.3 $\pm$ 1.6 a	71.1 $\pm$ 5.5 a
0.125	28.1 $\pm$ 1.5 a	61.3 $\pm$ 3.6 b
0.25	17.8 $\pm$ 1.7 b	41.1 $\pm$ 2.4 c
0.50	18.8 $\pm$ 2.0 b	24.5 $\pm$ 3.3 d
0.75	17.7 $\pm$ 2.3 b	17.5 $\pm$ 1.3 de
1.00	0.0 $\pm$ 0.0 c	7.3 $\pm$ 3.3 ef
1.50	0.0 $\pm$ 0.0 c	0.0 $\pm$ 0.0 f

Values represent mean seedling length (mm) $\pm$ SE. Essential oil treatments were applied at 0.125, 0.25, 0.50, 0.75, 1.00, and 1.50 $\mu\text{L mL}^{-1}$, with distilled water as the control. Seedling length was assessed after 14 days of incubation for *S. oleraceus* and after 10 days for *L. rigidum*. Within each species, different letters indicate significant differences among *S. officinalis* EO concentrations according to Fisher's LSD test ($p < 0.05$).

Sage influenced seedling growth over time in both species studied. In *S. oleraceus*, seedling growth was observed up to 0.75 $\mu\text{L mL}^{-1}$, whereas the two highest concentrations completely inhibited seedling development. In *L. rigidum*, significant reductions were noted from 0.125 $\mu\text{L mL}^{-1}$, although some seedling growth was still evident at 1.00 $\mu\text{L mL}^{-1}$, albeit very limited compared with the control (Figure 6). These findings align with the in vitro study of the herbicidal activity of clary sage (*Salvia sclarea* L.) conducted by Raveau

et al. [47]. Their research revealed that *S. sclarea* EO, unlike other MAP species, was less effective in controlling the growth of *L. perenne*. The EO was primarily composed of linalyl acetate and germacrene D and also contained linalool. The presence of germacrene D, compared to compounds such as thymol or camphor, likely resulted in a milder phytotoxic effect [47]. Another study testing various EOs, including *S. officinalis*, to inhibit *Abutilon theophrasti* Medik confirmed that *S. officinalis* exhibited a milder herbicidal effect than thyme or rosemary when applied at low concentrations. The authors reported that at higher doses, the phytotoxic effect was more pronounced, completely inhibiting seedling growth. Specifically, the *S. officinalis* EO studied was rich in cis-thujone (20.8%) and camphor (20.8%) [48].

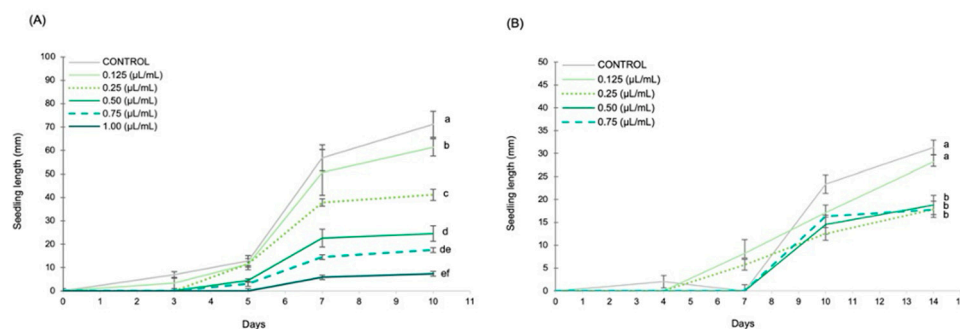


Figure 6. Effect of *Salvia officinalis* essential oil on seedling growth of *Lolium rigidum* (A) and *Sonchus oleraceus* (B) throughout the assay. Essential oil treatments were applied at 0.125, 0.25, 0.50, 0.75, 1.00, and 1.50 $\mu\text{L mL}^{-1}$, with distilled water as the control. Values represent mean seedling length (mm) $\pm$ SE. Seedling length was assessed after 14 days of incubation for *S. oleraceus* and after 10 days for *L. rigidum*. Different letters indicate significant differences among concentrations according to Fisher's LSD test ($p < 0.05$).

Essential oils can serve as effective herbicides when their aromatic profiles contain certain components known for high biological activity. The presence of these compounds depends on various factors, such as genotype and growing environment, as noted by Angelini et al. [21]. In the present study, only *T. vulgaris* completely inhibited germination of all three species. However, this does not imply that the other EOs lack herbicidal potential, as seedling growth is also a crucial factor. Underdeveloped weed seedlings are less likely to compete with the main crop for light, water, and nutrients [49,50]. The inhibition of germination and seedling growth observed here may be related to the multi-target nature of EO phytotoxicity. Essential oils and their terpene constituents have been reported to alter membrane integrity and permeability, promote the accumulation of reactive oxygen species and oxidative damage, and interfere with processes such as respiration, enzyme activity, hormonal regulation, and cell division [46,51–54]. However, the mechanisms underlying the activity of a whole EO are difficult to identify because its constituents may interact synergistically, additively, or antagonistically, and their effects may vary according to EO composition, concentration, target species, and developmental stage. Therefore, although these mechanisms may contribute to the responses observed in this study, they cannot be directly confirmed, as no specific physiological, biochemical, or molecular analyses have been conducted.

Plant-derived products and other bioactive compounds are increasingly investigated as more environmentally friendly alternatives to conventional pesticides [52]. However, their practical use requires adequate efficacy, selectivity, stability, and validation under agronomic conditions. Their effectiveness under realistic agronomic conditions may be limited by high volatility, low persistence, and reduced environmental stability, as noted by Verdeguer et al. [53]. Another crucial step is to assess their effects on non-target plants,

particularly crops, since a promising bioherbicide should control weeds without causing unacceptable damage to cultivated species.

4. Conclusions

Mediterranean MAPs provide a valuable source of EOs with complex and distinctive volatile profiles that are strongly influenced by species identity, environmental conditions, and cultivation area. This chemical complexity is reflected in a wide range of biological properties, including allelopathic and phytotoxic activities, making essential oils from Mediterranean *Lamiaceae* promising candidates for the development of plant-based weed management solutions.

In the present study, EOs extracted from thyme, lavender, and sage inhibited weed germination and early seedling growth under in vitro conditions. Thyme EO exhibited the strongest phytotoxic activity, lavender EO displayed a notable but more species-dependent effect, and sage EO caused a more gradual inhibition in the two species tested. These findings confirm that the effectiveness of EOs depends not only on their chemical composition and concentration but also on the sensitivity of the target weed species. Seedling growth is a particularly informative parameter in phytotoxicity assays, as EO treatments may impair early plant development even when germination is not entirely suppressed. The results also emphasize that the bioherbicidal activity of EOs cannot be generalized only based on the botanical family or the monocotyledonous/dicotyledonous classification of the target weed. Instead, it depends on the interaction between EO chemical composition, applied concentration, weed species sensitivity, and the specific developmental stage considered. This study represents the first evaluation of the bioherbicidal potential of these Mediterranean EOs under controlled in vitro conditions. Further research is required to validate these results in greenhouse and field experiments, where additional factors can be assessed, including crop selectivity, effectiveness under soil conditions, persistence and degradation of active compounds, interactions with soil microbial communities, and possible effects on non-target plants. Concurrently, the economic feasibility of EO-based weed management solutions should be evaluated in relation to extraction costs, biomass availability, and field-scale efficacy. These aspects will be essential to determine whether EOs from Mediterranean MAPs can progress from promising in vitro phytotoxic agents to practical tools within integrated and sustainable weed management systems.

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