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Valorization of Mediterranean Species of Thyme for the Formulation of Bio-Herbicides

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Abstract: This study focused on the evaluation of the phytotoxic activity of four essential oils (EOs) from the Mediterranean species of *Thymus* sp. pl., namely *Thymus algeriensis* Boiss. et Reut., *T. ciliatus* Desf. subspecies *coloratus* (Boiss. et Reut.) Batt., *T. vulgaris* L. ecotype Fasano and *T. vulgaris* cultivar L. Varico 3, to identify new biomolecules with herbicide potential. The chemical characterization of EOs was performed by GC-MS. The evaluation of the phytotoxicity of the EOs was conducted under *in vitro* conditions, and the inhibition of germination and seedling growth of *Lolium perenne* L. and *Amaranthus retroflexus* L. were assessed. Five concentrations (100, 250, 500, 750 and 1000 µL/100 mL) were considered. Phytochemical analysis revealed a great diversity of compounds. *T. algeriensis* and *T. ciliatus* EOs were characterized by the absence of carvacrol and a low content of thymol in *T. ciliatus*. On the contrary, *T. vulgaris* ecotype Fasano and *T. vulgaris* cultivar Varico 3 were characterized by an important content of *p*-cymene, thymol and carvacrol. All the EOs expressed a potent phytotoxic activity against the tested species. The total inhibition of seed germination and seedling growth were recorded for the highest concentrations of all the EOs. *T. vulgaris* ecotype Fasano expressed the most effective activity.

Keywords: thyme; essential oils; GC-MS; phytotoxicity; germination; early growth

Citation: Boukhalfa, R.; Ruta, C.; Messgo-Moumene, S.; Calabrese, G.J.; Argentieri, M.P.; De Mastro, G. Valorization of Mediterranean Species of Thyme for the Formulation of Bio-Herbicides. *Agronomy* **2024**, *14*, 2077. https://doi.org/10.3390/agronomy14092077

Academic Editor: Anestis Karkanis

Received: 23 July 2024
Revised: 4 September 2024
Accepted: 9 September 2024
Published: 11 September 2024

1. Introduction

Today, food security, undermined by a fast-growing human population, is the foremost challenge for governments, policymakers and producers. To increase yields and improve food quality, crop production worldwide is consequently still based on massive chemical inputs. In 2021, 3.53 million tons of pesticides were used, and herbicide consumption reached 1.73 million tons, representing almost half of the total pesticide use [1]. Indeed, weeds are the most damaging category for crop production. They compete for resources such as light, water and nutrients and act as a reservoir of plant pathogens, pests and disease vectors. Therefore, they are considered a major abiotic and biotic cause of yield losses and crop quality depreciation [2–4].

Although several control methods are available, including physical, mechanical and biological approaches, the most common is still the chemical approach, based on the use of herbicides [5]. The overuse of these herbicides has caused negative impacts on human health and the environment [6–8]. Moreover, synthetic chemicals affect both soil organic matter and microbial community composition by decreasing soil bacterial diversity and abundance, especially for rapidly growing species because they can be immobilized in the soil by adsorption or binding to colloids [9,10].

Furthermore, the continuous use of the same molecules, combined with transgenic crops tolerant to herbicides, has contributed to developing increasing herbicidal resistances in many weeds and farmland biodiversity depletion [6,7]. In 2024, 272 herbicide-resistant weed species had been reported in 100 different crops, affecting the efficacy of 168 different herbicides and 21 of the 31 known modes of action (MOAs) [11].

To overcome this issue, several studies have explored new sources of active molecules, which can be used in weed control. These studies have demonstrated that plants, through the release of volatile organic compounds, can alter the physiological processes of neighboring species and affect plant community composition [12]. In this context, plant-based products with herbicidal activity, such as essential oils (EOs), could be an alternative to chemical herbicides due to their allelopathic properties and easy degradation in the environment [8,13,14]. Among bio-herbicides, EOs could be promising candidates due to their high content of monoterpenes, sesquiterpenes and diterpenes, which have important phytotoxic effects, volatility and biodegradability [15].

Within the *Lamiaceae* family, several genera showed interesting potential in terms of molecules with biocidal action. Among these genera, *Thymus* is certainly one of the most important with more than 250 taxa, for which the effectiveness of their EOs and terpenes have been demonstrated to have biological activity, especially antimicrobial properties. Understanding the relationship between the structures of these compounds and their various biological activities is crucial. In an agro-ecological context, it is of great interest to study the potential bioherbicidal activity of certain *Thymus* species to analyze and valorize their biodiversity as an alternative to chemical herbicides with a view to sustainable agriculture. Some thyme species have already been considered for their herbicidal properties in previous research. However, few aspects have been studied, and none of the previous studies have performed large-scale screening [16,17]. Therefore, this genus deserves renewed consideration and further investigations.

Based on the important chemotype variability of *Thymus* species detected in different environments in Algeria and Italy, four species were identified as deserving further attention: two Algerian endemic species, *T. algeriensis* Boiss. et Reut., *T. ciliatus* Desf. subsp. *coloratus* (Boiss. et Reut.) Batt.; an Italian landrace, *T. vulgaris* L. ecotype Fasano; and a Swiss commercial hybrid, *T. vulgaris* L. cultivar Varico 3, cultivated in Italy. In this context, our study aims to perform a preliminary analysis of several thyme species to investigate potential allelopathic activity that could be exploited for sustainable management of weeds. The experiments were conducted under in vitro conditions using two common weed test species.

2. Materials and Methods

2.1. Plant Material and EOs Extraction and Characterization

Aerial part of plant samples of *Thymus* species, namely *T. algeriensis* Boiss. et Reut.; *T. ciliatus* Desf. subsp. *coloratus* (Boiss. et Reut.) Batt.; *T. vulgaris* L. cultivar Varico 3 and *T. vulgaris* L. ecotype Fasano, were collected at the full flowering stage during May and June 2022 (Table 1). *T. algeriensis* Boiss. et Reut. and *T. ciliatus* Desf. subsp. *coloratus* (Boiss. et Reut.) Batt were identified in the Hamma Botanical Garden (Algiers, Algeria) based on the Quezel and Santa (1963) classification. Botanical vouchers were deposited at the herbarium of the garden. *T. vulgaris* L. ecotype Fasano was identified in the Botanical Garden Museum University of Bari Aldo Moro (Italy) and maintained ex situ (field collection) at the experimental farm "Enrico Pantanelli" of the University of Bari A. Moro, located in Policoro (southern Italy, 40°10′20″ N, 16°39′04″). Furthermore, the *T. vulgaris* cultivar Varico 3 is a hybrid that was obtained in 2000 by crossing two accessions from the Agroscope Changins-Wädenswil Research Station (Agroscope ACW—Switzerland) breeding material.

Dried samples were submitted to hydrodistillation for three hours, using a Clevenger-type apparatus, according to the European Pharmacopeia 2022 (https://www.edqm.eu/en/european-pharmacopoeia-ph.-eur.-11th-edition#-, accessed on 24 May 2022). The essential oils (EOs) obtained were yielded and stored at 4 °C until their use.

Table 1. Data on the plant species used in the study.

Species	Habitat	Code	Collecting Area	Geographical Localization	Altitude
T. algeriensis Boiss. et Reut.	Endemic wild species	T1	Chrea National Park, Blida (Algeria)	N 36°27′08.5″ E 002°54′47.2″	1465 m
T. ciliatus Desf. subsp. *coloratus* (Boiss. et Reut.) Batt.	Endemic wild species	T2	Hammam Melouane-Chrea National Park, Blida (Algeria)	N 36°28′12″ E 003°00′36″	211 m
T. vulgaris L. cultivar Varico 3	Cultivated hybrid	T3	Policoro, Basilicata (Italy)	40°10′28″ N 16°39′26″ E	15 m
T. vulgaris L. ecotype Fasano	Cultivated local species	T4			

Samples of the *Thymus* EOs were analyzed with a trace GC-FID Ultra Thermo Finnigan gas chromatograph equipped with an Agilent DB-5 (J&W Scientific, Milan, Italy) fused silica capillary column (30 m × 0.25 mm; 0.25 µm film thickness). Adopted analytical conditions were as follows: detector temperature was 300 °C; the column temperature was programmed from 60 °C (5 min isothermal) to 280 °C (30 min isothermal) at 4 °C/min. Hydrogen was the carrier gas (35 kP; 2.0 mL/min). Data were processed using a Chrom-Card 32-bit version 2.0 computing software. Analyses were run in the cold on-column mode.

GC-MS analyses were performed with a Hewlett Packard 6890 (MSD)-5973 (GC) GC-MS System interfaced with an HP Chemstation (Agilent, Scientific Instruments, Milan, Italy). The following analytical parameters were used: column oven program of 60 °C (5 min iso-thermal) to 240 °C (15 min isothermal) at 3 °C/min; injector, 280 °C. Helium was the carrier gas (flow rate, 1 mL·min^{-1}). Chromatographic separation was performed with an HP-5 MS capillary column (30 m × 0.25 mm; 0.25 µm film thickness). MS operating conditions were as follows: ion source, 70 eV; ion source temperature, 200 °C; mass spectra acquisition, over 40–800 amu range at 1 scan·s^{-1}. The ion source was operating in electron impact mode. Samples (1 µL) were injected using the splitless sampling technique.

The chemical composition of the analyzed EOs was achieved by a comparison of GC retention times of their constituents with authentic reference compounds in combinations with the Kovats index (KI) and by means of reference mass spectra from standard compounds and/or from library files [18,19].

KI values were calculated using an n-alkane series (C6–C32) under the same GC conditions as that for the samples. The relative amounts of individual components of the oil were expressed as percent peak area relative to the total peak area from the GC-FID analysis of the whole extracts without the use of correction factors. A linear proportion between the areas was used, assuming an equal response factor for all detected compounds.

2.2. Phytotoxicity of the EOs against Target Weeds

Two weed species, *Amaranthus retroflexus* L. and *Lolium perenne* L., were used to test in vitro the phytotoxic effect of the EOs on germination and early growth conditions. Seeds were purchased from Weberseeds Company and stored at 4 °C for use during the experiment.

Seeds were surface-sterilized with sodium hypochlorite (NaClO, 2%—*v*/*v*) for 2 min and rinsed three times with sterile distilled water [20]. The viability of the seeds was assessed by the germination test of 100 seeds in Petri dishes fitted with two layers of Whatman filter paper wetted with distilled water. Petri dishes were sealed immediately with parafilm and placed in a controlled growth chamber (24 ± 1 °C with adjusted light conditions of 16/8 h light/dark cycle).

Subsequently, the phytotoxicity of *Thymus* EOs against the weed test species was estimated in dose–response laboratory bioassays on seed germination and seedling early growth. Suspensions of T1, T2, T3 and T4 were prepared using 2% of Tween® 20 (Sigma-Aldrich, Milan, Italy) as an emulsifying agent, according to Abd-El Gawad et al. [21]. Briefly, five concentrations (100, 250, 500, 750 and 1000 µL/100 mL) of EOs were prepared by diluting the EOs in 2% of Tween 20, and then the volumes were adjusted to 100 mL with sterilized distilled water. A total of 2% of Tween® 20 solution was considered as

negative control, while two pelargonic acid-based bio-herbicides (Finalsan Ultima 18.9% *w*/*w* pelargonic acid and Vithal 51.9% *w*/*w* pelargonic acid) were considered as positive controls. The concentrations of the positive controls were prepared as indicated on the labels of the products (1 L of Finalsan Ultima per 5 L of water and 2.2 L of Vithal per 5 L of water).

2.2.1. Germination Test

Ten seeds of *A. retroflexus* and *L. perenne* were placed in three Petri dishes for each EO and concentration, previously fitted with two layers of Whatman filter paper wetted with 3 mL of each treatment. Petri dishes were immediately sealed with parafilm to prevent loss of moisture and oil volatilization and placed in a controlled growth chamber at 24 ± 1 °C with adjusted light conditions of 16/8 h light/dark cycle and light: LEDs with a broad spectrum between 400 and 700 nm, a white/pink ratio of 2:1 and a light intensity of 50 µm s^{-1} m^{-2}. Trials were conducted in a completely randomized design in triplicate. After 7 days, the number of germinated seeds was counted. Seeds showing radicles of more than 2 mm were considered germinated [22,23]. The results are expressed in a percentage of germinated seeds, determined as follows:

$$GP\ (\%) = (n)/(N) \times 100$$

where GP: Germination percent; n: Number of germinated seeds at final count; N: Total number of seeds [24].

2.2.2. Seedling Early Growth Test

Seeds of both target species were germinated in three Petri dishes (150 mm in diameter) for each EO and concentration. All the Petri dishes were fitted with two layers of filter paper wetted with distilled water and placed in a controlled growth chamber at the seeds' optimal growth conditions for four days. Afterward, ten *A. retroflexus* and *L. perenne* seedlings were placed in 90 mm Petri dishes previously fitted with two layers of Whatman filter paper wetted with 3 mL of EO suspension at different concentrations. The Petri dishes were immediately sealed with parafilm to prevent loss of moisture and oil volatilization and placed in a controlled growth chamber at 24 ± 1 °C with adjusted light conditions of 16/8 h light/dark cycle and light: LEDs with a broad spectrum between 400 and 700 nm, a white/pink ratio of 2:1 and a light intensity of 50 µm s^{-1} m^{-2}. Trials were conducted in a completely randomized design in triplicate. After 7 days, shoot and radical lengths were measured using a ruler and caliper.

2.3. Statistical Analysis

Data obtained were statistically analyzed using Minitab® version 19.2020.1 (Minitab software, State College, PA, USA). Analysis of the variance (one-way ANOVA) of the seed germination percentage and root and shoot growth was performed to assess the phytotoxicity of the treatments. Differences among means was run using Tukey's test. Statistical significance was accepted when the probability of the result, assuming the null hypothesis, (p) was less than 0.05 ($p < 0.05$) [25].

3. Results and Discussion

3.1. Chemical Characterization of the EOs

The hydrodistillation of the aerial part of the samples resulted in different essential oil (EO) yields of the studied *Thymus* species, which ranged from 0.43% to 1.10% (*v*/*w*). The highest yield was observed in *T. ciliatus* subsp. *coloratus* (1.05%) and *T. vulgaris* cv Varico 3 (1.10%), while the lowest ones were found in *T. algeriensis* (0.43%) and *T. vulgaris* ecotype Fasano (0.60%) samples.

Gas chromatography analysis allowed for the identification of 43 components, representing 91.2–98.9% of the oils, with the remaining components present only in trace amounts (<0.1%). The percentage of the composition and indexes of the components are

listed in Table 2. Based on the dominant compound of each EO, three different types were identified: α-pinene for *T. algeriensis*, linalool for *T. ciliatus* subsp. *coloratus* and *p*-cymene for *T. vulgaris* cv Varico 3 and *T. vulgaris* ecotype Fasano. The chemical analysis of the *T. algeriensis* EO revealed the dominance mainly of α-pinene (19.73%), α-terpenyl acetate (13.21%), borneol (11.31%), camphene (8.64%), isobornyl acetate (5.28%), β-pinene (4.37%), camphor (4.32%) and linalool (3.09%), while the *T. ciliatus* EO was characterized by an important content of linalool (93.06%), β-cedrene (1.98%) and limonene (1.36%). The chemical analysis of *T. algeriensis* (T1) and *T. ciliatus* subsp. *coloratus* (T2) EOs revealed the absence of carvacrol in both samples and a low content of thymol in *T. ciliatus* (0.55%).

Table 2. Chemical composition of the studied *Thymus* EOs.

N°	Compound	KI [1]	KI [2]	T1	T2	T3	T4
01	santolina triene	908	910	0.36	--	--	--
02	trycyclene	926	924	0.47	--	--	--
03	α-thujene	926	924	--	0.20	0.48	0.66
04	α-pinene	936	940	19.73	0.21	1.03	1.15
05	camphene	954	950	8.64	0.29	0.88	0.93
06	verbenene	967	968	0.34	--	0.05	--
07	sabinene	975	975	0.87	0.05	--	--
08	β-pinene	979	980	4.37	0.14	0.21	--
09	myrcene	991	992	2.77	0.36	0.52	1.35
10	α-terpinene	1017	1012	0.25	0.05	0.13	0.24
11	*p*-cymene	1022	1021	0.30	0.13	35.63	23.85
12	limonene	1029	1026	1.95	1.36	0.79	1.33
13	1,8 cineole	1031	1028	2.99	0.16	3.53	3.30
14	β(E)-ocymene	1050	1047	1.17	0.07	--	--
15	γ-terpinene	1059	1058	0.54	0.98	2.35	10.36
16	*cis*-sabinene hydrate	1070	1067	0.45	0.10	--	--
17	terpinolene	1088	1088	0.47	--	0.48	0.17
18	linalool	1096	1103	3.09	93.06	2.57	2.54
19	1-octen-3γlacetate	1110	1116	0.43	--	--	--
20	α-campholenal	1125	1125	0.99	--	--	--
21	camphor	1143	1142	4.32	0.38	1.66	1.20
22	trans-verbenol	1144	1145	2.61	--	--	--
23	pinocarvone	1162	1158	0.56	--	--	--
24	borneol	1165	1166	11.31	--	1.47	1.22
25	ρ-mentha-1,5 dien-8-ol	1170	1174	1.72	--	1.76	1.65
26	terpin-4-ol	1177	1182	0.76	--	--	--
27	α-terpineol	1189	1187	0.96	--	--	--
28	myrtenal	1193	1190	0.90	--	--	--
29	verbenone	1204	1215	0.73	--	--	--
30	isobonyl formate	1233	1243	0.33	--	--	--
31	thymol methyl ester	1235	1234	--	--	1.97	1.48
32	linalool acetate	1257	1262	3.96	--	--	--
33	isobornyl acetate	1285	1289	5.28	--	--	--
34	thymol	1290	1293	--	0.55	20.35	21.77
35	carvacrol	1298	1300	--	--	11.76	18.15
36	trans-carvyl acetate	1328	1337	0.11	--	--	--
37	α-terpenyl acetate	1350	1353	13.21	--	--	--
38	α-copaene	1376	1370	0.49	--	--	--
39	β-bourbonene	1384	1376	0.22	--	--	--
40	β-cedrene	1418	1404	0.51	1.98	7.69	6.56
41	germacrene	1480	1475	1.58	--	--	--
42	Δ-cadinene	1524	1530	0.26	--	--	--
43	caryophyllene oxide	1581	1573	--	--	5.57	2.09
Monoterpene hydrocarbons				42.23	3.77	42.85	40.04
Oxygen-containing monoterpenes				54.71	94.18	43.89	51.31
Sesquiterpene hydrocarbons				3.06	1.98	7.69	6.56
Oxygen-containing sesquiterpenes				-	-	5.57	2.09
Others				-	-	-	-

KI: Kovats index; [1]: the literature; [2]: calculated.

Overall, these results are in agreement with other studies on *Thymus* species. Similar results for *T. algeriensis* were reported by [26–28]. They pointed out that the phytochemical profiles of *T. algeriensis* harvested from Chrea National Park at the same altitude and Medea (north-central Algeria) were characterized by the predominance of terpinyl acetate (18.0%), nerolidol (12.6%), geranyl acetate (16.4%), α-pinene (11.1–27.1%), borneol (9.0%), bornyl acetate (7.7%) and in some samples, the absence of thymol. In contrast, [26] observed that a sample collected in Chrea National Park at 800 m altitude was characterized by thymol as the predominant component. Great variability was also found in *T. algeriensis* populations from the central-northern, central-southern and eastern regions of the country [29–32]. These results confirm the important role that environmental conditions play in the synthesis of different essential oil compounds.

Concerning *T. ciliatus* subsp. *coloratus*, our results indicated that the chemical profile of the EOs of the species growing in Hammam Melouane differed significantly from what has been reported in the literature. Indeed, our findings identified linalool as the major compound (93.06%) for this species for the first time. Generally, the North Algerian *T. ciliatus* EO is characterized by the predominance of thymol, carvacrol, *p*-cymene and γ-terpinene [33–35].

T. vulgaris cv Varico 3 and *T. vulgaris* ecotype Fasano (T3 and T4) have similar chemical compositions, characterized by *p*-cymene (35.63% and 23.85%, respectively), thymol (20.35% and 21.77%, respectively), carvacrol (11.76% and 18.15%, respectively), α-cedrene (7.69% and 6.56%, respectively), caryophyllene oxide (5.57 and 2.09%, respectively), γ-terpinene (2.65% and 10.36%, respectively) and 1,8 cineol (2.35% and 3.30%, respectively) as major components.

T. vulgaris is the most studied *Thymus* species in terms of phytochemistry. Since 2000, more than one hundred articles have been published, but as far as we know and according to the literature, few studies have focused on the *T. vulgaris* cultivar Varico 3 and none on *T. vulgaris* ecotype Fasano. Regarding the *T. vulgaris* cultivar Varico 3, [36] obtained different results than ours. In their study on the chemical composition of the volatile oil and genetic fingerprint of ten *T. vulgaris* clones, they found that *T. vulgaris* Varico 3, cultivated in Switzerland, was rich in thymol (75.44%), with *p*-cymene as the second most abundant compound (8.14%). Previously, [37] revealed that thymol is the main compound (60.2%), followed by *p*-cymene (19.9%) and carvacrol (10.3%) for this species cultivated in the Czech Republic. Generally, the EOs of *T. vulgaris* can have different chemotypes depending on the main compounds. Our results showed that the main compounds of both EOs are *p*-cymene, thymol and carvacrol, suggesting that both belong to the *p*-cymene chemotype. These results are in accordance with those found by [38] in their cluster analysis on the compositions of 85 *T. vulgaris* EOs. In addition to the already-established seven chemotypes (thymol, carvacrol, linalool, geraniol, thujanol-4, terpineol and 1,8-cineole) [39], they recorded *p*-cymene/thymol as the second most common chemotype represented by 18 samples, even though the most dominant chemotype reported by the literature remains the "thymol chemotype".

It is important to note that, apart from the main component that defines the chemotype of the species, some compounds are predictable, as they are metabolically related. For thymol and carvacrol chemotypes, *p*-cymene and γ-terpinene are expected to be present in considerable amounts, as they are their precursors [40]. Indeed, the metabolic pathway for the formation of thymol and carvacrol starts with the autoxidation of γ-terpinene to *p*-cymene, followed by hydroxylation to thymol [41].

The great variability and diversity of compounds observed in the chemical composition of *Thymus* species EOs could be attributed to several factors, such as response mechanisms to abiotic variations. In essence, environmental conditions can affect biosynthetic pathways [29,35,42,43].

3.2. Phytotoxicity of the EOs against Target Weeds

3.2.1. Phytotoxic Effect on Seed Germination

The phytotoxic effect of the different EOs extracted from aerial parts of thyme species on seed germination of *L. perenne* and *A. retroflexus* is summarized in Figure 1. All the treatments prevented seed germination of both species where their influence varied significantly depending on the EO and concentration. Seven days after treatment, T1 and T4 completely inhibited seed germination at 500, 750 and 1000 µL in a similar way to the positive controls. T1 and T4 also reduced seed germination at 100 µL; however, this reduction was not significant compared to the negative control. T3 completely suppressed seed germination at 750 and 1000 µL and reduced it considerably at 500 µL (83% less than negative control). In contrast, at 100 and 250 µL, the seed germination percentage was close to that of the negative control. T2 showed the least significant effect, suppressing seed germination only at 1000 µL. Germination percentages were almost similar to the negative control at 100, 250, and 500 µL. T4 exerted the most potent activity against the treated species (Figure 1a).

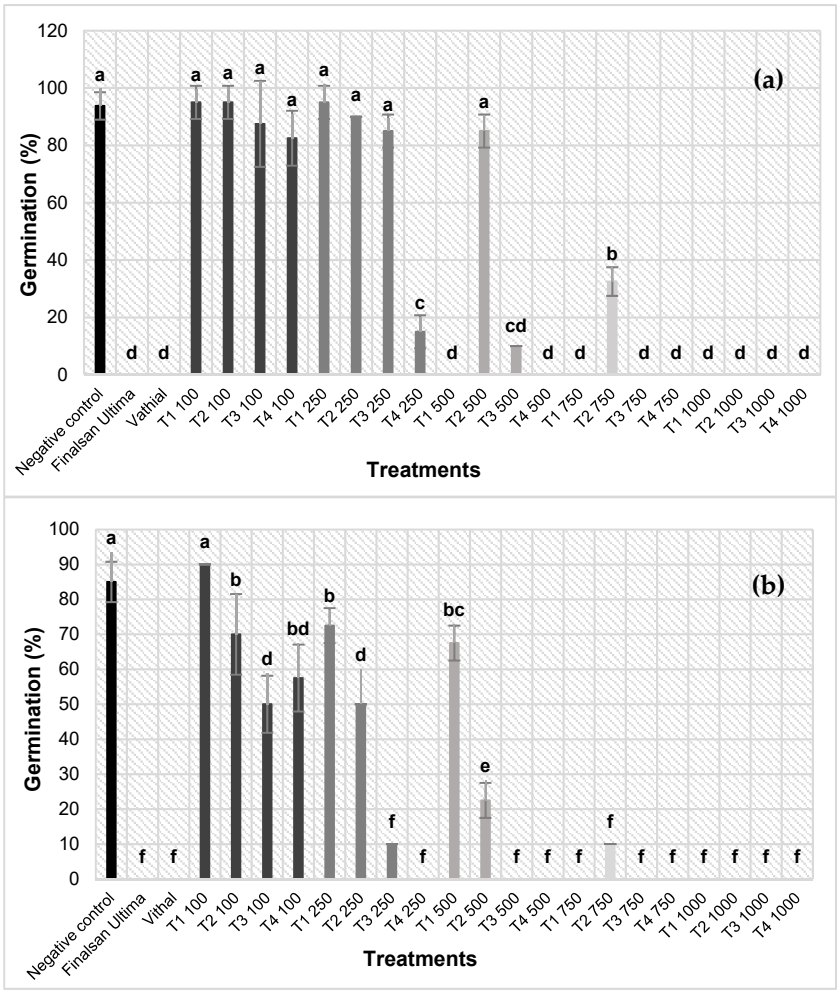

Figure 1. Effect of the different treatments and doses on seed germination of *L. perenne* (**a**) and *A. retrofelxus* (**b**) after 7 days of exposure to a 100–1000 µL/100 mL range of concentrations of the four tested EOs. T1: *T. algeriensis*, T2: *T. ciliatus*, T3: *T. vulgaris* cultivar Varico 3, T4: *T. vulgaris* ecotype Fasano. Data are means of three replicates. At each dose, data followed by different letters are significantly different ($p \leq 0.05$, Tukey's test).

A similar pattern was observed for *A. retroflexus*. T4 completely inhibited seed germination at 250, 500, 750 and 1000 µL and reduced it by almost half at 100 µL. T3 showed strong effectiveness at 500, 750 and 1000 µL, extremely reduced seed germination at 250 µL

and reduced it by half at 100 µL. T1 completely inhibited seed germination at 750 and 1000 µL and reduced it by almost 20% and 13% at 250 and 500 µL, respectively. T2 was the least effective treatment, inhibiting seed germination only at the highest concentration (1000 µL) and slowing it down at the other concentrations. As with *L. perenne*, it was noticeable that T4 exerted the strongest phytotoxic effect against *A. retroflexus* by totally inhibiting seed germination at four concentrations (Figure 1b).

Overall, all the EOs exhibited significant phytotoxic activity against the tested weed species. T4 was the most phytotoxic treatment, while T2 was the least effective.

3.2.2. Phytotoxic Effect on Seedlings' Early Growth

The allelopathic effect of the EOs extracted from aerial parts of thyme species on *L. perenne* L. and *A. retroflexus* L. root and shoot growth is summarized in Figure 2. The results demonstrated a strong inhibitory effect of the EOs on the growth of the target plants. Root and shoot elongation was strongly inhibited in a dose-dependent manner. For *L. perenne*, T1, T2, T3 and T4 showed potent toxicity on root growth at all four concentrations (250, 500, 750 and 1000 µL), with T4 having a particularly strong effect, reducing root elongation by about 30 mm and 45 mm compared to the mean root elongation of the other treatments and the negative control, respectively, even at 100 µL (Figure 2a).

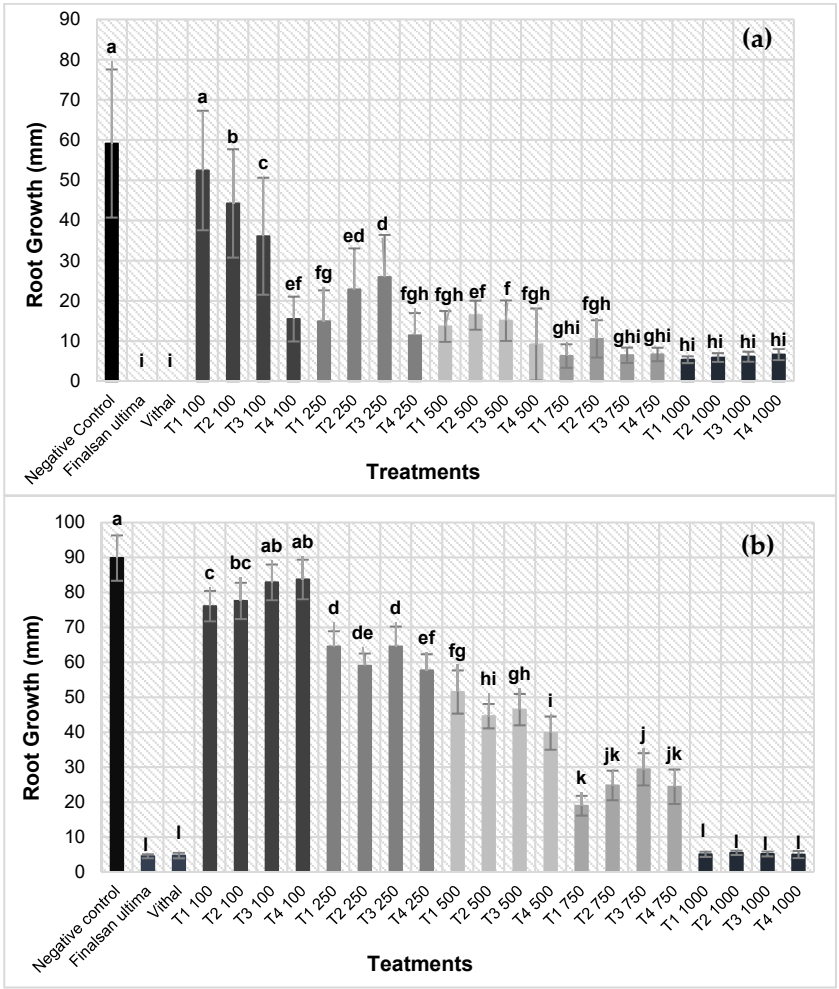

Figure 2. Effect of the different treatments and doses on root elongation of *L. perenne* (**a**) and *A. retrofelxus* (**b**) seedlings after 7 days of exposure to a 100–1000 µL/100 mL range of concentrations of the four tested EOs and active compounds. T1: *T. algeriensis*, T2: *T. ciliatus*, T3: *T. vulgaris* cultivar Varico 3, T4: *T. vulgaris* ecotype Fasano. Data are means of three replicates. At each dose, data followed by different letters are significantly different ($p \leq 0.05$, Tukey's test).

The same effect was recorded for *A. retroflexus*. All the EOs negatively affected root growth. They significantly reduced it at 750 and 1000 µL and decreased it by half at 250 and 500 µL. At 100 µL, no major effect was recorded. At the lower concentration, the effect was almost the same as that of the negative controls (Figure 2b).

For *L. perenne*, shoot growth responded to the application of EOs in a quite similar manner to root growth. T4 completely inhibited shoot growth at 750 and 1000 µL comparable to the positive controls and greatly reduced it at 500, 250 and 100 µL. In particular, the reduction in shoot growth was 25 mm less than the average of the other treatments at 250 µL and 30 mm for 100 µL. T1 also inhibited shoot elongation at 750 and 1000 µL and considerably reduced it at 500 and 250 µL, while at 100 µL, the effect was almost similar to that of the negative control. T3 prevented shoot growth only at 1000 µL but significantly slowed it down at 250, 500 and 750 µL. T2 was the least effective treatment compared to the other three. It did not prevent shoot growth; however, it significantly decreased it at 500, 750 and 1000 µL in comparison to the negative control with decreases of about 36, 39 and 47 mm, respectively (Figure 3).

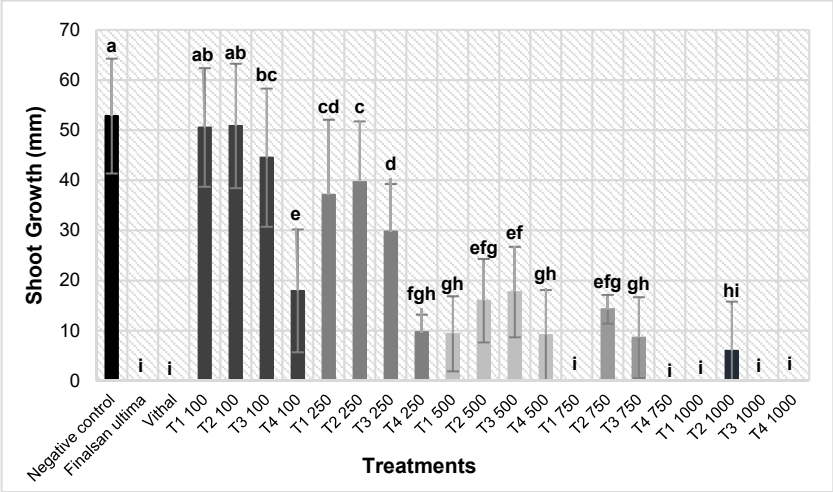

Figure 3. Effect of the different treatments and doses on shoot elongation of *L. perenne* seedlings after 7 days of exposure to a 100–1000 µL/100 mL range of concentrations of the four tested EOs and active compounds. T1: *T. algeriensis*, T2: *T. ciliatus*, T3: *T. vulgaris* cultivar Varico 3, T4: *T. vulgaris* ecotype Fasano. Data are means of three replicates. At each dose, data followed by different letters are significantly different ($p \leq 0.05$, Tukey's test).

It is noteworthy that all the EO-based treatments completely prevented the shoot growth of *A. retroflexus*, and T4 was the most effective in inhibiting or slowing down root and shoot growth of both target species in a dose-dependent manner.

The results obtained from the in vitro trials confirmed the phytotoxic effect of the studied *Thymus* EOs. All treatments inhibited seed germination and seedlings' growth of the target weed species at higher concentrations, with T4 being effective even at lower concentrations. These results are highly consistent with those reported in the literature. Ref. [44] established that the *T. vulgaris* EO possessed potent activity against seven tested plants, negatively influencing their root growth and completely inhibiting their seed germination. Ref. [45] also revealed that the EO of *T. algeriensis* inhibited both shoot and root growth of *Medicago sativa* L. and of *Triticum aestivum* L. seedlings. To the best of our knowledge, no studies have been reported on the phytotoxic effect of *T. vulgaris* cultivar Varico 3, *T. vulgaris* ecotype Fasano and *T. ciliatus* subsp. *coloratus*. Nevertheless, the allelopathic activity of various *Thymus* sp. pl. EOs and their constituents has been reported to affect seed germination and plant growth of several weed species, thereby influencing plant community composition and dynamics [46]. Studies on EOs of *T. pulegioides* L., *T. proximus* Serg., *T. vulgaris*, *T. kotschyanus* Boiss. & Hohen., *T. decussatus* Benth., *T. fontanesii* Boiss. et

Reut. *T. eigii* and *T. daenensis* Celak. revealed significant and strong phytotoxic potential against a wide range of weed species, such as *Amaranthus retroflexus* L., *Poa anuua* L., *Sinapis arvensis* L. and *Avena fatua* L. [16,17,43,47–50].

To explain this biological activity, research confirmed that this potential is due to the presence of terpenic compounds, most of which are volatile [51,52]. In fact, [21] established a structure–activity relationship between phytotoxicity and EO chemical composition based on chemometric analysis. Data analysis revealed that mono- and sesquiterpenes play major roles in the phytotoxicity of EOs, concluding that α- and β-pinene, 1,8 cineole, linalool and carvacrol are the most effective monoterpenes exhibiting an allelopathic effect on many plants. Additionally, caryophyllene, a sesquiterpene compound, and its metabolites, such as germacrene, spathulenol and hexahydrofarnesyl acetone, play an important role in the phytotoxic potential of EOs. This was also reported by [21], where monoterpenes like linalool and 1,8 cineole delayed weed germination time and reduced their development. This is in accordance with our results, where *T. algeriensis, T. vulgaris* cultivar Varico 3 and *T. vulgaris* ecotype Fasano, which are rich in α-pinene, *p*-cymene, thymol and carvacrol, respectively, exhibited the most significant activity.

In addition, it was assumed that the biological properties of EOs are generally induced mainly by their major components. However, recent research has demonstrated that compounds present in minor and trace amounts can also influence plant defense strategies and could act synergistically to enhance the biological potential of EOs [53]. *T. ciliatus* was the least effective treatment, even though it contained more than 90% of linalool. Furthermore, our EOs were generally rich in certain compounds, such as α-pinene, borneol, linalool, thymol, carvacrol and caryophyllene, but their chemical compositions were particularly different. *T. algeriensis* and *T. ciliatus* did not contain thymol and carvacrol, two compounds commonly well known for their strong allelopathic activity, or contained these only in trace amounts. This supports the view that there was a synergy between the major and minor compounds of the examined EOs that affected the germination and early growth of *L. perenne* and *A. retroflexus*.

4. Conclusions

The object of this study was to exploit plant sources to obtain active ingredients for use in crop protection, particularly for weed control. With this aim, a preliminary assessment of the phytotoxic potential of some Mediterranean *Thymus* sp. pl. EOs was performed, and the presence of bioactive compounds, potentially useful for weed management, was evaluated. The chemical analysis demonstrated the high variability of secondary metabolites in *Thymus* sp. pl essential oils (EOs). Forty-three chemical compounds were identified. The Algerian species were characterized by the absence of carvacrol and traces of thymol in *T. ciliatus* subsp. *coloratus* and the predominance of linalool, α and β-pinene, α-terpenyl acetate, borneol and camphene, while *p*-cymene, thymol, carvacrol and γ-terpinene were found as the main constituents of *T. vulgaris* species. Biologically, all the EOs demonstrated a significant phytotoxic effect on *L. perenne* and *A. retroflexus*. Thyme EOs strongly inhibited the seed germination and seedling growth of both weed species. *T. algeriensis* Boiss. et Reut., *T. vulgaris* cultivar Varico 3 and *T. vulgaris* ecotype Fasano completely inhibited the seed germination of both test species at 500, 750 and 1000 µL/100 mL, while *T. ciliatus* subsp. *coloratus* considerably reduced it at 500 and 750 µL/100 mL and completely suppressed it at 1000 µL/100 mL. The seedling growth of *L. perenne* and *A. retroflexus* was drastically slowed down under the effect of thyme EOs in a dose-dependent manner; at 1000 µL/100 mL, all the EOs almost inhibited the seedling growth. Overall, *T. vulgaris* ecotype Fasano exhibited the most potent phytotoxic activity, while *T. ciliatus* subsp. *coloratus* was the least effective.

This initial screening of species of genus *Thymus* endowed with biologically active compounds represents the first stage in a process of introducing into cultivation certain species that are currently under-utilized but have the potential to become industrial crops. The high cost of the natural product could thus be reduced in favor of a more efficient and less costly method of obtaining the raw material. All of this is perfectly in line with

sustainability goals (Agenda 2030), including the replacement of synthetic compounds with plant-based compounds in agriculture, as in other contexts.

Further studies and applicative investigations on the effectiveness, selectivity, modes of action and application of thyme EOs under in vivo conditions, as well as their effects on crops and soil, are necessary to develop ongoing, tailored strategies for more sustainable weed control.

Author Contributions: Conceptualization, G.D.M., C.R. and S.M.-M.; data curation, R.B., S.M.-M., M.P.A., C.R. and G.D.M.; investigation, R.B., S.M.-M., M.P.A., C.R. and G.D.M.; project administration, G.D.M. and G.J.C.; supervision, R.B., S.M.-M., G.J.C., M.P.A., C.R. and G.D.M.; validation R.B., S.M.-M., M.P.A., C.R. and G.D.M.; writing—original draft preparation, R.B. and S.M.-M.; writing—review and editing, R.B., S.M.-M., G.J.C., M.P.A., C.R. and G.D.M. All authors have read and agreed to the published version of the manuscript.

Funding: This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)—MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4—D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

Data Availability Statement: The original contributions presented in this study are included in the article, and further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. FAO. Pesticides Use. Available online: https://www.fao.org/faostat/en/#data/RP (accessed on 10 May 2024).
2. Fried, G.; Chauvel, B.; Reynaud, P.; Sache, I. Decreases in crop production by non-native weeds, pests and pathogens. In *Impact of Biological Invasions on Ecosystem Services*; Vilà, M., Hulme, P.E., Eds.; Springer: Cham, Switzerland, 2017; pp. 83–101.
3. Stewart, C.N., Jr. Becoming weeds. *Nat. Genet.* **2017**, *49*, 654–655. [CrossRef] [PubMed]
4. Byron, M.; Treadwell, D.; Dittmar, P. Weeds as reservoirs of plant pathogens affecting economically important crops. *EDIS* **2019**, *5*, 7. [CrossRef]
5. Müller-Schärer, H.; Collins, A.R. Integrated weed management. In *Managing Soils and Terrestrial Systems*; CRC Press: Boca Raton, FL, USA, 2020; pp. 439–447.
6. Liu, J.; Zhou, J.H.; Guo, Q.N.; Ma, L.Y.; Yang, H. Physiochemical assessment of environmental behaviors of herbicide atrazine in soils associated with its degradation and bioavailability to weeds. *Chemosphere* **2021**, *262*, 127830. [CrossRef] [PubMed]
7. Qu, R.Y.; He, B.; Yang, J.F.; Lin, H.L.; Yang, W.C.; Wu, Q.Y.; Li, Q.X.; Yang, G.F. Where are the new herbicides? *Pest Manag. Sci.* **2021**, *77*, 2620–2625. [CrossRef] [PubMed]
8. Kong, Q.; Zhou, L.; Wang, X.; Luo, S.; Li, J.; Xiao, H.; Zhang, X.; Xiang, T.; Feng, S.; Chen, T.; et al. Chemical composition and allelopathic effect of essential oil of *Litsea pungens*. *Agronomy* **2021**, *11*, 1115. [CrossRef]
9. Ntalli, N.; Koliopoulos, G.; Giatropoulos, A.; Menkissoglu-Spiroudi, U. Plant secondary metabolites against arthropods of medical importance. *Phytochem. Rev.* **2019**, *18*, 1255–1275. [CrossRef]
10. Kanissery, R.; Fenn, R.; Gairhe, B.; Kadyampakeni, D. Understanding the fate and Persistence of herbicides in soils. *Citrus Ind News*, 3 August 2020.
11. Heap. The International Survey of Herbicide Resistant Weeds. Available online: https://www.weedscience.org/Home.aspx (accessed on 7 May 2024).
12. Araniti, F.; Miras-Moreno, B.; Lucini, L.; Landi, M.; Abenavoli, M.R. Metabolomic, proteomic and physiological insights into the potential mode of action of thymol, a phytotoxic natural monoterpenoid phenol. *Plant Physiol. Biochem.* **2020**, *153*, 141–153. [CrossRef]
13. Assaeed, A.; Elshamy, A.; El Gendy, A.E.N.; Dar, B.; Al-Rowaily, S.; Abd-ElGawad, A. Sesquiterpenes-rich essential oil from above ground parts of *Pulicaria somalensis* exhibited antioxidant activity and allelopathic effect on weeds. *Agronomy* **2020**, *10*, 399. [CrossRef]
14. Casella, F.; Vurro, M.; Valerio, F.; Perrino, E.V.; Mezzapesa, G.N.; Boari, A. Phytotoxic effects of essential oils from six *Lamiaceae* species. *Agronomy* **2023**, *13*, 257. [CrossRef]
15. Fotsing, Y.S.F.; Kezetas, B. Terpenoids as important bioactive constituents of essential oils. In *Essential Oils-Bioactive Compounds, New Perspectives and Applications*; De Oliveira, M.S., Da Costa, W.A., Eds.; IntechOpen: London, UK, 2020; pp. 1–15.
16. Zeynep, U.; Menderes, C.; Huseyin, I.; Murat, Y. Antimicrobial and herbicidal activities of the essential oil from the Mediterranean *Thymus eigii*. *J. Essent. Oil Bear. Plants* **2018**, *21*, 214–222. [CrossRef]
17. Zhou, S.; Han, C.; Zhang, C.; Kuchkarova, N.; Wei, C.; Zhang, C.; Shao, H. Allelopathic, phytotoxic, and insecticidal effects of *Thymus proximus* Serg. essential oil and its major constituents. *Front. Plant Sci.* **2021**, *12*, 689875. [CrossRef] [PubMed]

18. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th ed.; Allured Publishing Corporation: Carol Stream, IL, USA, 2007; 469p.
19. Avato, P.; Laquale, S.; Argentieri, M.P.; Lamiri, A.; Radicci, V.; D'Addabbo, T. Nematicidal activity of essential oils from aromatic plants of Morocco. *J. Pest Sci.* **2017**, *90*, 711–722. [CrossRef]
20. Liu, M.; Childs, M.; Loos, M.; Taylor, A.; Smart, L.B.; Abbaspourrad, A. The effects of germination on the composition and functional properties of hemp seed protein isolate. *Food Hydrocoll.* **2023**, *134*, 108085. [CrossRef]
21. Abd-El Gawad, A.M.; El Gendy, A.E.-N.G.; Assaeed, A.M.; Al-Rowaily, S.L.; Alharth, A.S.; Mohamed, T.A.; Nassar, M.I.; Dewir, Y.H.; Elshamy, A.I. Phytotoxic effects of plant essential oils: A systematic review and structure-activity relationship based on chemometric analyses. *Plants* **2020**, *10*, 36. [CrossRef]
22. Franco, C.D.J.P.; Ferreira, O.O.; Cruz, J.N.; Varela, E.L.P.; de Moraes, Â.A.B.; Nascimento, L.D.D.; Cascaes, M.M.; Souza Filho, A.P.D.S.; Lima, R.R.; Percário, S.; et al. Phytochemical profile and herbicidal (phytotoxic), antioxidants potential of essential oils from *Calycolpus goetheanus* (*Myrtaceae*) specimens, and in silico study. *Molecules* **2022**, *27*, 4678. [CrossRef]
23. Li, J.; Chen, L.; Chen, Q.; Miao, Y.; Peng, Z.; Huang, B.; Guo, L.; Liu, D.; Du, H. Allelopathic effect of *Artemisia argyi* on the germination and growth of various weeds. *Sci. Rep.* **2021**, *11*, 4303. [CrossRef]
24. Islam, A.K.M.M.; Kato-Noguchi, H. Phytotoxic activity of *Ocimum tenuiflorum* extracts on germination and seedling growth of different plant species. *Sci. World J.* **2014**, *1*, 676242. [CrossRef]
25. Shedden, K. *Generalized Linear Models*; Creative Commons Attribution Share Alike 3.0 License; Department of Statistics, University of Michigan: Ann Arbor, MI, USA, 2015; 35p.
26. Hazzit, M.; Baaliouamer, A. Essential oil composition of *Thymus algeriensis* Boiss. et Reut. and *Thymus numidicus* Poiret from Algeria. *Mozzo Riv. Ital. EPPOS* **2007**, *43*, 11–18.
27. Giordani, R.; Hadef, Y.; Kaloustian, J. Compositions and antifungal activities of essential oils of some Algerian aromatic plants. *Fitoterapia* **2008**, *79*, 199–203. [CrossRef]
28. Hazzit, M.; Baaliouamer, A.; Veríssimo, A.R.; Faleiro, M.L.; Miguel, M.G. Chemical composition and biological activities of Algerian *Thymus* oils. *Food Chem.* **2009**, *116*, 714–721. [CrossRef]
29. Kouache, B.; Brada, M.; Saadi, A.; Fauconnier, M.L.; Lognay, G.; Heuskin, S. Chemical composition and acaricidal activity of *Thymus algeriensis* essential oil against *Varroa destructor*. *Nat. Prod. Commun.* **2017**, *12*, 1934578X1701200138. [CrossRef]
30. Zouaoui, N.; Chenchouni, H.; Bouguerra, A.; Massouras, T.; Barkat, M. Characterization of volatile organic compounds from six aromatic and medicinal plant species growing wild in North African drylands. *NFS J.* **2020**, *18*, 19–28. [CrossRef]
31. Ouakouak, H.; Benarfa, A.; Messaoudi, M.; Begaa, S.; Sawicka, B.; Benchikha, N.; Simal-Gandara, J. Biological properties of essential oils from *Thymus algeriensis* Boiss. *Plants* **2021**, *10*, 786. [CrossRef] [PubMed]
32. Adouane, S.; Mehaoua, M.S.; Bouatrous, Y.; Tudela, J.; Flamini, G.; Mechaala, S. Natural insecticides from native plants of the Mediterranean basin and their activity for the control of the date moth *Ectomyelois ceratoniae* (Zeller) (*Lepidoptera: Pyralidae*). *J. Plant Dis. Prot.* **2022**, *129*, 775–782. [CrossRef]
33. Souadia, A. Chemical composition and antioxidant activity of *Thymus ciliatus* (Desf.) Benth. essential oils of Algeria. *Nat. Prod. Commun.* **2022**, *17*, 1934578X221080337. [CrossRef]
34. Zatout, A.; Djibaoui, R.; Flamini, G.; Ascrizzi, R.; Benbrahim, C.; Mazari, H.E.; Benkredda, F.; Mechaala, S.; Kassah-Laouar, A. Chemical composition analysis of essential oils of four plants from Aurès region of Algeria and their antibacterial and antibiofilm activities against coagulase-negative staphylococci. *Afr. J. Clin. Exp. Microbiol.* **2022**, *23*, 278–289. [CrossRef]
35. Benomari, F.Z.; Sarazin, M.; Chaib, D.; Pichette, A.; Boumghar, H.; Boumghar, Y.; Djabou, N. Chemical variability and chemotype concept of essential oils from Algerian wild plants. *Molecules* **2023**, *28*, 4439. [CrossRef]
36. György, Z.; Incze, N.; Pluhár, Z. Differentiating *Thymus vulgaris* chemotypes with ISSR molecular markers. *Biochem. Syst. Ecol.* **2020**, *92*, 104118. [CrossRef]
37. Pavela, R.; Sedlák, P. Post-application temperature as a factor influencing the insecticidal activity of essential oil from *Thymus vulgaris*. *Ind. Crop. Prod.* **2018**, *113*, 46–49. [CrossRef]
38. Satyal, P.; Murray, B.L.; McFeeters, R.L.; Setzer, W.N. Essential oil characterization of *Thymus vulgaris* from various geographical locations. *Foods* **2016**, *5*, 70. [CrossRef]
39. Torras, J.; Grau, M.D.; López, J.F.; de las Heras, F.X.C. Analysis of essential oils from chemotypes of *Thymus vulgaris* in Catalonia. *J. Sci. Food Agric.* **2007**, *87*, 2327–2333. [CrossRef]
40. Krause, S.T.; Liao, P.; Crocoll, C.; Boachon, B.; Förster, C.; Leidecker, F.; Wiese, N.; Zhao, D.; Wood, J.C.; Buell, C.R.; et al. The biosynthesis of thymol, carvacrol, and thymohydroquinone in *Lamiaceae* proceeds via cytochrome P450s and a short-chain dehydrogenase. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2110092118. [CrossRef]
41. Alizadeh, A.; Alizadeh, O.; Amari, G.; Zare, M. Essential oil composition, total phenolic content, antioxidant activity and antifungal properties of Iranian *Thymus daenensis* subsp. *daenensis* Celak. as in influenced by ontogenetical variation. *J. Essent. Oil Bear. Plants* **2013**, *16*, 59–70. [CrossRef]
42. Figueiredo, A.C.; Barroso, J.G.; Pedro, L.G.; Salgueiro, L.; Miguel, M.G.; Faleiro, M.L. Portuguese *Thymbra* and *Thymus* species volatiles: Chemical composition and biological activities. *Curr. Pharm. Des.* **2008**, *14*, 3120–3140. [CrossRef] [PubMed]
43. Vaiciulyte, V.; Loziene, K.; Svediene, J.; Raudoniene, V.; Paskevicius, A. α-Terpinyl acetate: Occurrence in essential oils bearing *Thymus pulegioides*, phytotoxicity, and antimicrobial effects. *Molecules* **2021**, *26*, 1065. [CrossRef] [PubMed]

44. Synowiec, A.; Kalemba, D.; Drozdek, E.; Bocianowski, J. Phytotoxic potential of essential oils from temperate climate plants against the germination of selected weeds and crops. *J. Pest Sci.* **2017**, *90*, 407–419. [CrossRef]
45. Ali, I.B.; Chaouachi, M.; Bahri, R.; Chaieb, I.; Boussaid, M.; Harzallah-Skhiri, F. Chemical composition and antioxidant, antibacterial, allelopathic and insecticidal activities of essential oil of *Thymus algeriensis* Boiss. et Reut. *Ind. Crop. Prod.* **2015**, *77*, 631–639. [CrossRef]
46. Linhart, Y.B.; Gauthier, P.; Keefover-Ring, K.; Thompson, J.D. Variable phytotoxin effects of *Thymus vulgaris* (*Lamiaceae*) terpenes on associated species. *Int. J. Plant Sci.* **2015**, *176*, 20–30. [CrossRef]
47. Benchaa, S.; Hazzit, M.; Zermane, N.; Abdelkrim, H. Chemical composition and herbicidal activity of essential oils from two Labiatae species from Algeria. *J. Essent. Oil Res.* **2019**, *31*, 335–346. [CrossRef]
48. Ghasemi, G.; Alirezalu, A.; Ghosta, Y.; Jarrahi, A.; Safavi, S.A.; Abbas-Mohammadi, M.; Barba, F.J.; Munekata, P.E.; Domínguez, R.; Lorenzo, J.M. Composition, antifungal, phytotoxic, and insecticidal activities of *Thymus kotschyanus* essential oil. *Molecules* **2020**, *25*, 1152. [CrossRef]
49. Saleh, I.; Abd-ElGawad, A.; El Gendy, A.N.; Aty, A.A.; Mohamed, T.; Kassem, H.; Aldorsi, F.; Elshamy, A.; Hegazy, M.E.F. Phytotoxic and antimicrobial activities of *Teucrium polium* and *Thymus decussatus* essential oils extracted using hydrodistillation and microwave-assisted techniques. *Plants* **2020**, *9*, 716. [CrossRef] [PubMed]
50. Portuguez-García, M.P.; Agüero-Alvarado, R.; González-Lutz, M.I. Herbicidal activity of three natural products on four weed species. *Agron. Mesoam.* **2021**, *32*, 991–999. [CrossRef]
51. Amri, I.; Hamrouni, L.; Hanana, M.; Gargouri, S.; Fezzani, T.; Jamoussi, B. Chemical composition, physico-chemical properties, antifungal and herbicidal activities of *Pinus halepensis* Miller essential oils. *Biol. Agric. Hortic.* **2013**, *29*, 91–106. [CrossRef]
52. Maldaner, J.; Steffen, G.P.K.; Missio, E.L.; Saldanha, C.W.; De Morais, R.M.; Steffen, R.B. Rue and Brazilian peppertree essential oils inhibit the germination and initial development of the invasive plant lovegrass. *Int. J. Environ. Stud.* **2020**, *77*, 255–263. [CrossRef]
53. Araniti, F.; Lupini, A.; Sorgonà, A.; Conforti, F.; Marrelli, M.; Statti, G.A.; Menichini, F.; Abenavoli, M.R. Allelopathic potential of *Artemisia arborescens*: Isolation, identification and quantification of phytotoxic compounds through fractionation-guided bioassays. *Nat. Prod. Res.* **2013**, *27*, 880–888. [CrossRef]