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Phytotoxic Effects of Three *Origanum* Species Extracts and Essential Oil on Seed Germinations and Seedling Growths of Four Weed Species

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Abstract: The use of chemical pesticides to protect agricultural products is a global concern because of their adverse effects on the environment and public health. To avoid the dangers of synthetic herbicides, research has turned to natural alternatives. This study was conducted to evaluate the allelopathic effect of essential oil (EO) extracted from *Origanum syriacum*, *Origanum onites*, and *Origanum majorana*. In addition, the chemical composition of the essential oil was elucidated by gas chromatography and mass spectrometry (GC–MS) analysis. A total of 11 different components of *O. syriacum* were identified, and the main components were carvacrol (88.49), *p*-Cymene (5.71), γ-Terpinene (1.63), β-Caryoplhyllene (1.48), and Terpinen-4-ol (0.65), respectively. For *O. onites*, 10 different compounds were identified, and the main components were carvacrol (58.65), Thymol (30.97), Linalool (4.17), *p*-Cymene (1.94), and β-Caryoplhyllene (0.98), respectively. Finally, for *O. majorana*, 14 different compounds were identified, and the main components were carvacrol (40.57), α-Terpineol (29.28), *p*-Cymene (9.02), γ-Terpinene (5.80), and carvacrol methyl ether (3.46). Finally, 14 compounds from the *Origanum majorana* species were identified, with carvacrol (40.57), -Terpineol (29.28), *p*-Cymene (9.02), and -Terpinene (5.80) as the parent compound (3.46). Oxygenated monoterpenes were the highest in all species' EO content. EOs and plant extracts were tested at 5, 10, and 20 L/Petri concentrations against seed germination and seedling growth in four weed species (*Thlaspi arvense*, *Amaranthus retroflexus*, *Rumex cripus*, and *Lactuca serriola*). The concentrations of essential oil were set as 5, 10, and 20 µL/Petri dishes for seed germination. In the greenhouse experiment, the final concentration of solutions was set as 20 µL and the solutions were directly sprayed on the surface of the weeds, and the mortality rates were noted after 24 and 48 h of application. It was observed that increasing the application decreased seed germination. The phytotoxic effects on the seedling germination in the greenhouse were observed, resulting in 48.76–94% mortality rates. Consequently, the essential oil from *Origanum* species could be considered as an alternative bio-herbicide to tested weeds.

Keywords: oregano; herbicidal effect; essential oil; extract

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Citation: Kordali, S.; Kabaagac, G.; Sen, İ.; Yilmaz, F.; Najda, A. Phytotoxic Effects of Three *Origanum* Species Extracts and Essential Oil on Seed Germinations and Seedling Growths of Four Weed Species. *Agronomy* **2022**, *12*, 2581. https://doi.org/10.3390/agronomy12102581

Academic Editor: Antonios Chrysargyris

Received: 24 August 2022
Accepted: 12 October 2022
Published: 20 October 2022

1. Introduction

Crop production has been increasing yearly to supply the food demand, which is a consequence of the fast expansion of the world population [1,2]. Unfortunately, weeds in the modern agricultural systems are one of the major problems worldwide because of yield and crop loss [3]. Globally, around 1800 weed species cause a 31.5% reduction in crop production [4]. Therefore, farmers have tended to use more herbicides to improve yields. Many studies have shown that less than 10% of conventional pesticides target the plant [5] and only 0.1% of these remain long enough to reach the plant, while the rest are dispersed directly into the environment [6,7]. However, intensive usage of synthetic herbicides can contaminate soil and groundwater [8–11], and weeds could also gain resistance against these synthetic herbicides [12,13]. Therefore, the awareness of chemical-free weed control methods has gained importance to keep society healthy. As a result, research on bio-herbicides obtained from aromatic plants and their selective herbicidal mechanism has been conducted in recent years [14–20].

Essential oils (EOs) by nature (as plant secondary metabolites) represent a safer alternative in many applications such as food preservation, biomedicine, cosmetics or agriculture [20,21]. Because of their allelopathic effects, they are often used for biopesticide production [22–27].

Because of their inherent allelopathic effects, essential oils (EOs) are often used for the production of biopesticides [20,21,23–27]. At the same time, because of their specific composition, they are rapidly degraded in the soil and are considered environmentally safe [25–27].

The genus *Origanum* (oregano), which belongs to the *Lamiaceae* family, is widespread worldwide and comprises about 900 species. One of the most common genera of the Lamiaceae family, *Origanum* L. has 21 species (24 taxa) and 13 hybrids in Turkey (5–7). There are about 20 species of the *Origanum* genus in Turkish flora [27–31]. The *Origanum* species has traditionally been used as a spicy additive for food instead of thyme in Turkey. This genus is rich in essential oils and bitter substances [32]. The *Origanum* species has several medicinal properties such as a sedative, diuretics, sweaters, antiseptics, and additionally in the treatment of gastrointestinal diseases and constipation, and it is used traditionally in Turkey [32]. The *Origanum* species is commonly known as "Oregano", and Turkey is the world's largest supplier. Approximately 15,000 tons of *Origanum* species were harvested and exported as raw material and essential oil in 2019 [33,34].

The *Origanum* species has been shown to have anti-diabetic, anti-obesity, anti-hyperlipidemic, hepatoprotective, anti-urolytic, anti-microbial, antioxidant, anti-proliferative, anti-nociceptive, anti-platelet, anti-melanogenic, anxiolytic, anti-inflammatory, memory-enhancing, and cytotoxic properties [35,36]. The chemical properties of the Origanum species were found to be rich in phenolic acids, flavonoids, sesquiterpenes, monocyclic monoterpenes, bicyclic monoterpenes, diterpenoids, and triterpenoids [35]. There also are numerous reports on the chemical composition and the various biological activities of the *Origanum* species [37–42].

The several biological activities of *Origanum* species such as antioxidant, antimicrobial, antifungal, phytotoxic, and insecticidal effects were described previously [43–51]. However, it could be considered that the essential oils and extracts obtained from several *Origanum* species could be a useful synthetic herbicide alternative in modern agriculture.

The purpose of this study was to determine the herbicidal properties of essential oils and extracts isolated from *Origanum syriacum* L., *Origanum onites* L., and *Origanum majorana* L. on weed species that cause significant crop losses in agricultural production.

2. Materials and Methods

2.1. Plant Materials and Isolation of Essential Oils

Fresh plant materials of *Origanum syriacum, Origanum onites*, and *Origanum majorana* were collected at the flowering stage from the production areas located in the Kahramanmaraş region of Turkey. The plant materials were identified morphologically and voucher specimens were deposited in the herbarium of Atatürk University, Erzurum. The plant materials were dried in shadow at room temperature and ground to 0.1–0.4 mm by using a grinder.

To obtain the essential oils from the selected plants, the dried plant samples (100 g for each cycle, n = 5) were subjected to hydro-distillation using a Clevenger-type apparatus (DURAN®, Mainz, Germany) for 4 h. The obtained essential oils were extracted into chloroform and the water was removed by using dry sodium sulfate. Then chloroform was removed by using a rotary evaporator (DURAN®, Mainz, Germany) under low temperature and pressure conditions.

In addition to the essential oils, the n-hexane and acetone extracts were obtained. To obtain the extracts from grounded plants, 100 g of dried plant materials were placed in a volumetric flask and 500 mL of n-hexane and acetone were added for each extraction process. The extractions process kept going for 48 h and the processes were repeated 4 times [52–57]. The supernatants were united and the organic solvent was evaporated under low temperature and pressure conditions by using a rotary evaporator.

The essential oils and the extracts were stored at 4 °C until further experiments. The yields of the essential oils and extracts (% referred to dry plant materials) are given in Table 1.

Table 1. The yields of essential oils and extracts (g/100 dried plant materials).

Species	Essential Oils	Extracts	
		Acetone	*n*-Hexane
Origanum syriacum	4.0	13.8	14.3
Origanum onites	4.5	14.2	14.7
Origanum majorana	5.0	14.3	14.8

2.2. GC–MS Analysis

The essential oils were analyzed using a Thermofinnigan Trace GC/A1300 (E.I.) equipped with SGE/BPX5 MS capillary column (30 m × 0.25 mm i.d., 0.25 µm). Diluted samples (1/100, *v*/*v*, in methylene chloride) of 1.0 µL were injected in the splitless mode. Helium was used as the carrier gas, at a flow rate of 1 mL/min. The injector temperature was set at 220 °C. The program used was 50–150 °C at a rate of 3 °C/min, held isothermal for 10 min and finally raised to 250 °C at 10 °C/min [58,59].

2.3. Seed Germination and Seedling Growth Experiments In Vivo and In Vitro Conditions

The bio-herbicidal effects of the essential oil and extracts obtained from the *Origanum* specimen were tested against *Amaranthus retroflexus*, *Rumex crispus*, *Lactuca serriola*, and *Thlaspi arvense.* The seeds of weeds were collected in the Erzurum region (Turkey). Empty and undeveloped seeds were discarded by floating in tap water and the healthy seeds were selected to use in the experiments. To avoid possible inhibition caused by toxins from fungi or bacteria, the seeds were surface-sterilized with 15% sodium hypochlorite for 20 min [60] and then rinsed with abundant distilled water.

To determine herbicidal effects, the essential oils and extracts were dissolved in a 10% dimethyl sulfoxide (DMSO) (Sigma-Aldrich®, Darmstadt, Germany)–water solution, and the final concentrations of stock solutions were set as 5, 10, and 20 µL/Petri dishes. The emulsions were transferred to a Petri dish (9 cm diameter) and placed on the bottom two layers of filter paper (10 mL/Petri dishes). Afterward, 50 disinfected seeds were placed on the filter paper [14,54]. Petri dishes were covered with adhesive tape to prevent volatile compounds from escaping. The Petri dishes were incubated at 23 ± 2 °C and 80% humidity for 12 h consecutive dark and light periods in a growth chamber [61–63]. After 10 days, the number of germinated seeds was determined and the length of the seedling (root and shoot of seedlings) was measured by using a caliper. The germination rates were calculated as a percentage. Additionally, trifluralin (Maga-Tref 48 EC) (5, 10, and 20 µL/Petri) was used as the positive control. Petri dishes containing 10 mL dimethyl sulfoxide-water solution without the essential oils and extracts solutions were used as the negative control. A seed was considered as germinated when the emerging radicle elongated to 2 mm. Germination percentages were recorded every 24 h for 7 days. Rate of germination inhibition was calculated by using following formula:

$$GI = [GC - TG/GC] \times 100$$

where GI is rate of germination inhibition (%); GC is germination rate of control treatment; TG is germination rate in respective essential oil treatment of wheat genotypes or weed species.

The experimental design was a completely randomized design and all experiments were conducted thrice, including controls.

2.4. In Greenhouse Conditions

To test the herbicidal effects in in vitro conditions, twenty µL/pot dosage of essential oil and each extract obtained from *O. syriacum*, *O. onites*, and *O. majorana* were tested against the weeds that had 3–4 leaf stage and were growing in the greenhouse. The pots (10 × 10 cm) were filled with 550 g of sterile soil (organic material ratio: 2.02%; cation change capacity: 43.34 me/100 g; pH = 7.5). Then, 50 seeds of the weeds were sown into the pots and kept under photoperiod conditions (23 ± 2 °C, 12 h consecutive light and dark period) and relative humidity (80% ± 5) in a growth chamber to allow germination and growth of the plant samples [16,64,65]. The pots were irrigated with tap water when necessary. The number of germinated seeds of the respective weed samples in each pot was counted. Afterward, the oil and extracts were emulsified in 10 mL of dimethyl sulfoxide-water solution (10% *v*/*v*). The final concentration of the treatments was 20 µL/Pot. These emulsions were sprayed uniformly with a glass atomizer on the surface of whole plants in each pot in the stage of 2–4 real leaves. The plants in each pot sprayed uniformly with 10 mL of dimethyl sulfoxide-water solution (1%) were used as negative control groups. The plants sprayed with trifluralin (20 µL for each pot) were used as the positive control. Dead plants were counted and recorded at the 24th and 48th hour after sample applications. The treatments were arranged in a completely randomized design with three replications, including controls. The phytotoxicity of the treatments was expressed as the percent mean of dead plants [43].

The results are reported as the lethality percentage (LP) using the following formula:

$$LP = [N - n/N] \times 100$$

where N is number of healthy individuals before treatment; n is number of alive individuals after treatment.

2.5. Statistical Analysis

The SPSS 10.0 software package was used to carry out the statistical test. The statistically significant differences among the herbicidal activity assays were analyzed in the Analysis of Variances (ANOVAs) test. When the statistical significance was observed, the Duncan test was used as a posthoc test.

3. Results

3.1. Chemical Composition of the Essential Oils

The essential oil components of *Origanum syriacum*, *O. onites* and *O. majorana* are given in Table 2. The major components of the essential oils were found as carvacrol representing 88.49% in *O. syriacum*, 58.65% in *O. onites*, 40.57% in *O. majorana*. *Origanum majorana* had a higher level of α-Terpineol with 29.28% while *O. onites* included thymol with 30.97% (Table 2). Additionally, each essential oil contained relatively high amounts of oxygenated monoterpenes.

Table 2. Chemical compositions of essential oils.

Compound	Essential Oil Compounds (%)		
	O. syriacum	*O. onites*	*O. majorana*
α-Pinene	0.30	-	0.40
Myrcene	0.21	-	0.57
3-Octanol	0.28	-	0.42
3-Octanone	-	-	0.17
α-Terpinene	0.37	0.19	0.85
p-Cymene	**5.71**	**1.94**	**9.02**
1,8-Cineole	-	-	2.20
γ-Terpinene	**1.63**	0.95	**5.80**
Terpinen-4-ol	**0.65**	0.58	2.15
α-Terpineol	0.27	0.21	**29.28**
Thymol	0.35	**30.97**	-
Carvacrol	**88.49**	**58.65**	**40.57**
Carvacrol methyl ether	-	-	**3.46**
β-Caryoplhyllene	**1.48**	**0.98**	1.76
Linalool	-	**4.17**	0.85
Borneol	-	0.64	-
Class composition (%)			
Monoterpene hydrocarbons	8.22	3.08	16.64
Oxygenated monoterpenes	**89.76**	**95.22**	**78.51**
Sesquiterpene hydrocarbons	1.48	0.98	1.76
Oxygenated hydrocarbons	-	-	-
Aliphatic compounds	0.28	-	0.59
Total	99.74	99.28	97.50

The essential oil rate of *Origanum syriacum* was found to be 99.74%. Eleven compounds from the essential oil of *O. syriacum* were identified, and these compounds are: 88.49% carvacrol; 5.71% *p*-Cymene; 1.63% γ-Terpinene; 1.48% β-Caryoplhyllene; 0.65% terpinen-4-ol; 0.37% α-Terpinene; 0.35% thymol; 0.30% α-Pinene; 0.28% 3-octanol; 0.27% α-Terpineol; 0.21% Myrcene agents. The class composition of the essential oil was observed as monoterpene hydrocarbons 8.22%, oxygenated monoterpenes, 89.76%, sesquiterpene hydrocarbons 1.48%, aliphatic compound 0.28% (Table 2).

The essential oil contents of *Origanum onites* were found as 99.28%. The main compounds were 58.65% carvacrol; 30.97% thymol; 4.17% linalool; 1.94% *p*-Cymene; 0.98% β-Caryoplhyllene; 0.95% γ-Terpinene; borneol 0.64%; 0.58% terpinen-4-ol; 0.21% α-Terpineol; 0.19% α-Terpinene agents. The class compositions were monoterpene hydrocarbons 3.08%, oxygenated monoterpenes 95.22%, and sesquiterpenes hydrocarbons 0.98% (Table 2).

The essential oil contents of *Origanum majorana* were found as 97.50%. Fourteen of the main compounds were identified, and these compounds were carvacrol 40.57%, α-Terpineol 29.28%, *p*-Cymene 9.02%, γ-Terpinene 5.8%, carvacrol methyl ether 3.46%, 1,8-Cineole 2.20%, terpinen-4-ol 2.15%, β-Caryoplhyllene 1.76%, α -Terpinene 0.85%, linalool 0.85%, Myrcene 0.57%, 3-octanol 0.42%, α-Pinene 0.40%, and 3-octanol 0.17%. The compound class was monoterpene hydrocarbons 16.64%, oxygenated monoterpenes 78.51%, and sesquiterpene hydrocarbons 1.76% (Table 2).

3.2. Herbicidal Effects of the Oil and Extracts

The essential oils, *n*-hexane, and acetone of the extracts isolated from *O. syriacum*, *O. onites* and *O. majorana* were tested on seed germinations and seedling growths of *A. retroflexus*, *L. serriola*, *R. crispus*, and *T. arvense*, important weeds in cultivated areas in agriculture. Different degrees of the inhibition of germinations and seedling growths of the weeds were observed when compared with control groups.

The results showed that, in particular, the oils have a potent inhibitory effect on the seed germinations and seedling growths of all weeds tested. The current results also showed that the *n*-hexane and acetone extracts have a low herbicidal effect against the weeds tested as compared with those of the essential oils.

In general, the toxic effects of the extracts isolated from the aerial parts on the germinations and seedling growths of the weeds increased with an increase in the application concentrations of the extracts.

The essential oils and extracts obtained from the *Origanum* species and the application dosages have statistically significant effects on the seed germination rate of all tested weeds ($p < 0.01$). The seeds of *A. retroflexus*, *Lactuca serriola*, and *Rumex crispus* could not germinate with the 20 µL of essential oils of each tested *Origanum* species, whereas the seeds of *Thlaspi arvense* were not germinated with 10 µL essential oil application (Figure 1). The n-hexane and acetone extraction of all *Origanum* species were not effective as the tested essential oils (Figure 1), and none of them totally inhibited the seed germination of weeds.

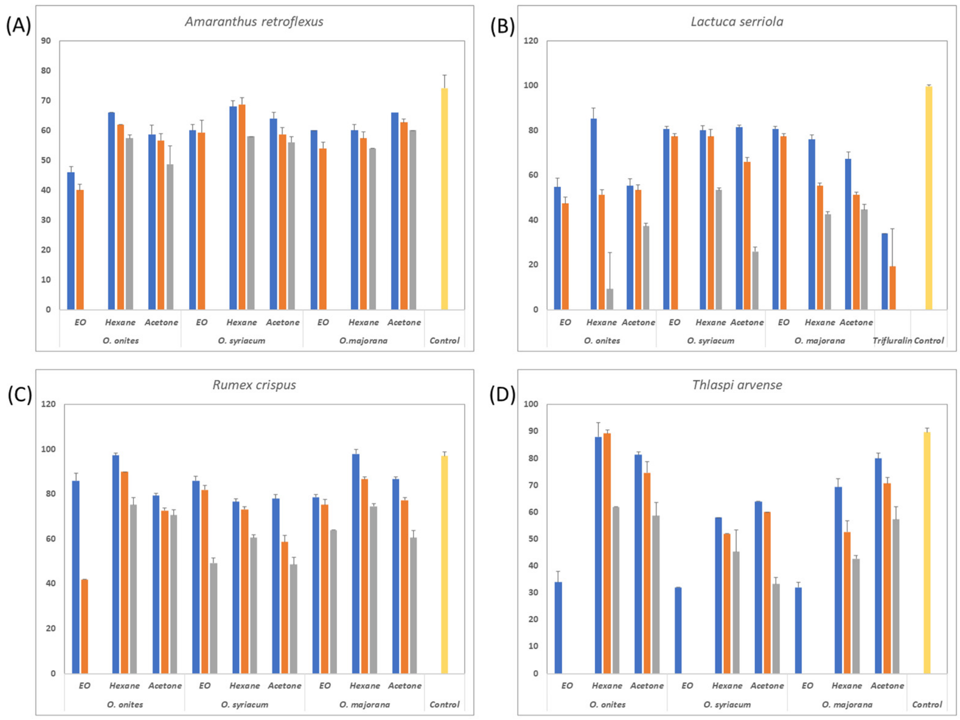

Figure 1. The seed germination rates (%) of (**A**) *Amaranthus retroflexus*, (**B**) *Lactuca serriola*, (**C**) *Rumex crispus*, (**D**) *Thlaspi arvense*. Blue bars indicate 5 µL dose application while red bars 10 µL, grey bars 20 µL. The yellow bars indicate control application.

The essential oils obtained from all *Origanum* species inhibited root and shoot development ($p < 0.01$). In particular, the 20 µL dose of essential oils was effective for root and shoot development control. In addition, 10 µL of essential oils showed the best results for *Thlapsi arvense* root and shoot control (Figures 2 and 3). All extracts did not inhibit the root and shoot development.

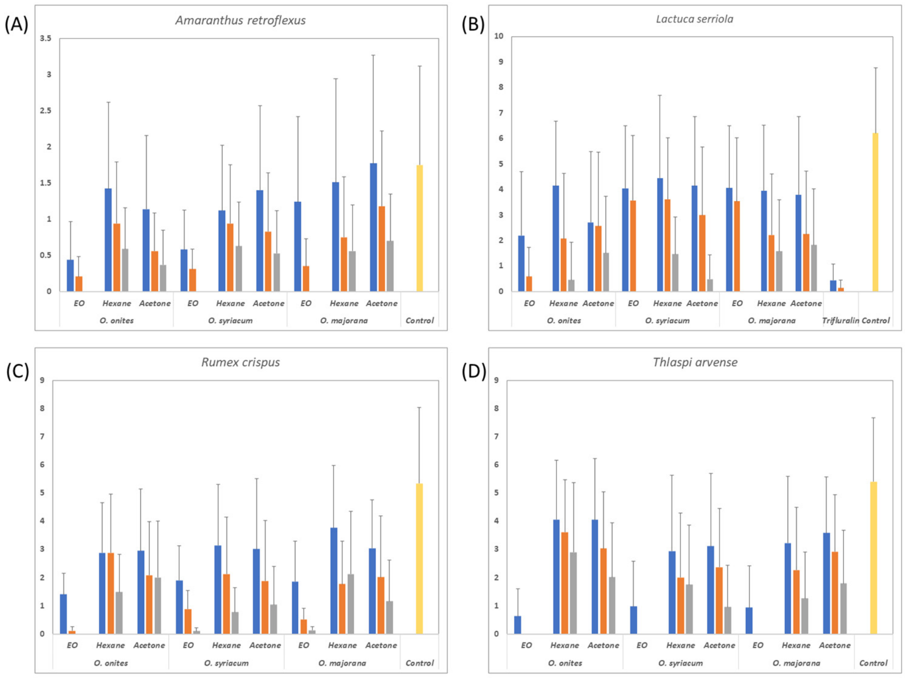

Figure 2. The root development of the weeds (cm). (**A**) *Amaranthus retroflexus*, (**B**) *Lactuca serriola*, (**C**) *Rumex crispus*, (**D**) *Thlaspi arvense*. Blue bars indicate 5 µL dose application while red bars 10 µL, grey bars 20 µL. The yellow bars indicate control application.

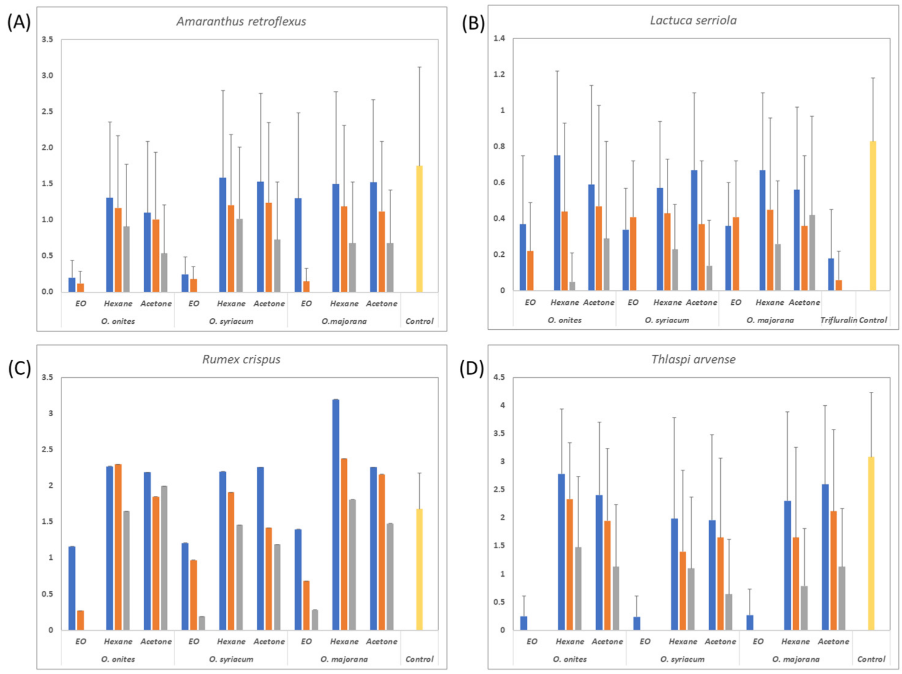

Figure 3. The shoot development of the weeds (cm). (**A**) *Amaranthus retroflexus*, (**B**) *Lactuca serriola*, (**C**) *Rumex crispus*, (**D**) *Thlaspi arvense*. Blue bars indicate 5 µL dose application while red bars 10 µL, grey bars 20 µL. The yellow bars indicate control application.

In addition, the positive control of the experiment, Trifularin, inhibited totally the seed germination, root and shoot development of the weeds except for *Lactuca serriola* (the Trifularin was not shown in the Figure except for *L. serriola*). It was observed that only a 20 µL dose of Trifularin totally inhibited the seed germination, and root and shoot development of *Lactuca serriola*.

In the nursery conditions, the essential oils and the extract can control weed development ($p < 0.01$). The tested essential oils showed better control against *Amaranthus retroflexus* after 24 - and 48 h application (Table 3). After 48 h of Trifluralin application, the mortality rate was observed as 76.67%, while *O. majorana* essential oils killed 79.33% of *A. retroflexus*. The essential oil obtained from *O. syriacum* and *O. onites* killed 73.33% of weeds. The extracts prepared with acetone yielded the best results from *O. onites and O. syriacum* species, while the extracts prepared with n-hexane yielded the best results from *O. majorana*.

Table 3. The mortality rate (%) of *Amaranthus retroflexus* in nursery.

Species	Applications	24 h	48 h
Origanum onites	Essential oil	50.00 ± 4.00 ^{bcd*}	**73.33** ± 1.15 ^{b}
	Acetone	37.33 ± 4.16 ^{ef}	61.33 ± 2.31 ^{d}
	Hexane	36.00 ± 2.00 ^{f}	58.67 ± 2.31 ^{d}
Origanum syriacum	Essential oil	55.33 ± 1.15 ^{bc}	**79.33** ± 1.15 ^{a}
	Acetone	44.00 ± 4.00 ^{de}	68.00 ± 2.00 ^{c}
	Hexane	42.00 ± 4.00 ^{ef}	66.67 ± 1.15 ^{c}
Origanum majorana	Essential oil	56.67 ± 0.58 ^{b}	**79.33** ± 1.15 ^{a}
	Acetone	49.33 ± 1.15 ^{cd}	68.67 ± 1.15 ^{c}
	Hexane	50.00 ± 2.00 ^{bcd}	69.33 ± 1.15 ^{c}
	Control	0.00 ± 0.00 ^{g}	0.00 ± 0.00 ^{e}
	Trifluralin	69.33 ± 3.06 ^{a}	**76.67** ± 3.06 ^{ab}

* The letters indicate the Duncan test results.

The mortality rate of *Lactuca serriola* was observed at 90% 48 h after *O. onites* essential application, while Trifluralin killed 94% of plants (Table 4). The essential oil of *O. onites* showed the best herbicidal effects against *L. serriola*. In addition, the essential oils of *O. syriacum* and *O. majorana* can be useful for controlling *L. serriola* with 87.33% and 84.67%, respectively. The use of extracts from Origanum species in *L. serriola* greenhouse trials was similarly to *A. retroflexus* greenhouse trials.

Table 4. The mortality rate (%) of *Lactuca serriola* in nursery.

Species	Applications	24 h	48 h
Origanum onites	Essential oil	62.67 ± 1.15 ^{b*}	**90.00** ± 0.00 ^{ab}
	Acetone	52.67 ± 1.15 ^{c}	74.00 ± 2.00 ^{c}
	Hexane	49.33 ± 1.15 ^{cd}	76.00 ± 0.00 ^{c}
Origanum syriacum	Essential oil	63.33 ± 3.06 ^{b}	**87.33** ± 5.03 ^{b}
	Acetone	50.67 ± 1.15 ^{cd}	74.67 ± 1.15 ^{c}
	Hexane	45.33 ± 2.31 ^{d}	70.00 ± 0.00 ^{c}
Origanum majorana	Essential oil	71.33 ± 1.15 ^{a}	**84.67** ± 1.15 ^{b}
	Acetone	49.33 ± 1.15 ^{cd}	70.67 ± 2.31 ^{c}
	Hexane	26.00 ± 5.29 ^{e}	48.67 ± 6.11 ^{d}
	Control	0.00 ± 0.00 ^{f}	0.00 ± 0.00 ^{e}
	Trifluralin	70.67 ± 1.15 ^{a}	**94.00** ± 2.00 ^{a}

* The letters indicate the Duncan test results.

After 48 h of the essential oil and the application of the extract against *Rumex crispus*, *Origanum onites* essential oil showed the best results to control this weed with an 81.33% mortality rate in the greenhouse conditions (Table 5). The mortality rate of *R. crispus* was observed as 71.33% for *O. syriacum* essential oil and 67.33% for *O. majorana* essential oil application. In addition, the n-hexane extraction of *O. majorana* showed better results than the essential oils of it. When the negative control application, Trifluralin, was applied, the mortality rate of *R. crispus* was observed as 91.33%. The extracts obtained with acetone yielded the best results from *O. syriacum* and *O. majorana* species, while the extracts prepared with n-hexane yielded the best results from *O. onites*. Additionally, acetone extraction of all *Origanum* species outperformed n-hexane extraction.

Table 5. The mortality rate (%) of *Rumex crispus* in nursery.

Species	Applications	24 h	48 h
Origanum onites	Essential oil	56.67 ± 0.58 [b*]	**81.33** ± 1.15 [b]
	Acetone	49.33 ± 1.15 [bc]	68.67 ± 1.15 [c]
	Hexane	47.33 ± 1.15 [c]	**68.67** ± 1.15 [c]
Origanum syriacum	Essential oil	55.33 ± 1.15 [bc]	**71.33** ± 1.15 [c]
	Acetone	52.67 ± 4.16 [bc]	70.67 ± 1.15 [c]
	Hexane	46.67 ± 1.15 [c]	66.67 ± 3.06 [c]
Origanum majorana	Essential oil	52.67 ± 6.43 [bc]	**67.33** ± 6.11 [c]
	Acetone	51.67 ± 4.73 [bc]	**69.33** ± 3.06 [c]
	Hexane	50.67 ± 6.11 [bc]	58.67 ± 2.31 [d]
	Control	0.00 ± 0.00 [d]	0.00 ± 0.00 [e]
	Trifluralin	77.33 ± 1.15 [a]	**91.33** ± 1.15 [a]

* The letters indicate the Duncan test results.

Thlaspi arvense was killed by using the essential oils and extracts of *Origanum* species (Table 6). Similarly, the essential oils showed better results by comparing the extracts. After 48 h of the application of Trifluralin, 83.33% of *T. arvense* plants were dead, while the mortality rate of the plants was observed at 80.67% for *O. onites* essential oil, 78% for *O. syriacum* essential oil, and 77.33% for *O. majorana* essential oil. The extracts obtained with acetone yielded the best results from *O. majorana*, while the extracts prepared with n-hexane yielded the best results from *O. syriacum*.

Table 6. The mortality rate (%) of *Thlaspi arvense* in nursery.

Species	Applications	24 h	48 h
Origanum onites	Essential oil	56.67 ± 0.58 [b]	**80.67** ± 1.15 [ab]
	Acetone	46.67 ± 2.31 [ef]	66.67 ± 1.15 [f]
	Hexane	48.67 ± 1.15 [de]	67.33 ± 1.15 [f]
Origanum syriacum	Essential oil	56.67 ± 0.58 [b]	**78.00** ± 2.00 [bc]
	Acetone	44.00 ± 2.00 [f]	68.67 ± 1.15 [ef]
	Hexane	50.67 ± 1.15 [cde]	**71.33** ± 1.15 [de]
Origanum majorana	Essential oil	54.67 ± 1.15 [bc]	**77.33** ± 1.15 [c]
	Acetone	49.33 ± 1.15 [de]	**72.67** ± 2.31 [d]
	Hexane	52.67 ± 1.15 [bcd]	70.67 ± 1.15 [de]
	Control	0.00 ± 0.00 [g]	0.00 ± 0.00 [g]
	Trifluralin	72.00 ± 4.00 [a]	**83.33** ± 1.15 [a]

* The letters indicate the Duncan test results.

4. Discussion

In this study, essential oil and crude extract content analyses from three *Organum* species were performed, and the effects of the obtained essential oils on seed germination and development against weeds of *Thlaspi arvense*, *Amaranthus retroflexus*, *Rumex crispus*, and *Lactuca serriola* were investigated.

The major component obtained from *O. syriacum* was carvacrol (88.49%), followed by other components: *p*-Cymene (5.71%), γ-terpinene (1.63), β-caryophyllene (1.48), Terpinen-4-ol (0.65%) (Table 2). The most abundant carvacrol component was found to be in previous studies: 81.38% [66], 60.80% [67], 22.29% [68], 60.01% [69], 39.87% [42], 74.21–90.22% [70], 82.60% [71], 44.49% [72], 61.18% [73], 35.80% [74].

In our study, the other main components, carvacrol [74,75], *p*-Cymene [66–71,73,75,76], γ-terpinene [42,66–71,73–75], β-caryophyllene [67,72,75], and terpinen-4 ol [66], were in line with previous studies.

The major component of obtained from *Origanum majorana* was carvacrol (40.57%), followed by other components: α-Terpineol (28.29%), *p*-Cymene (9.02%), carvacrol methyl ether (3.46%), y-Terpinene (5.80%), 1,8 Cineole (1.20%) (Table 2.). The most abundant carvacrol component was found to be the first most abundant compound in previous studies: 84.00% [51], 78.27% [77], 75.30% [51], 40.57% [78], 34.14% [79]. In our study, the other main components, α-Terpineol [45,78,80–82], *p*-Cymene [51,80,83–86], α-Terpineol [45,78,80–82], y-Terpinene [46,51,77–81,83–88], and 1,8 Cineole [45,80,83,89,90], were in line with previous studies.

The major component of obtained from *Origanum onites* was carvacrol (58.65%), followed by other components: Thymol (30.97%), Linalool (4.17%), *p*-Cymene (1.94%), γ-terpinene (0.95%) (Table 2). The most abundant carvacrol component was found to be the first most abundant compound in previous studies: 88.71% [47], 83.30% [91], 81.01% [92], 78.40% [50], 72.12% [93], 59.87% [94], 57.63% [49], 57.01% [79] 47.99% [95], 26.91% [96].

In our study, the other main components, Thymol [91,92,94,96,97] *p*-Cymene [47,50,79, 91–97] Linalool [50,79,93,96], and γ-terpinene [47,49,50,79,91,92,94–96], were in line with previous studies.

The reasons for the differences in EO content and rates are geographical, climatic (macroclimatic–microclimatic factors), soil properties, as well as plant collection time [98,99], drying conditions, analysis methods, and geographical or ontogenesis variations [21,100–104].

In this study, the herbicidal effects of the essential oils and the extracts obtained from *Origanum syriacum*, *O. onites,* and *O. majorana* were determined against *Amaranthus retroflexus*, *Lactuca serriola*, *Rumex crispus*, and *Thlaspi arvense,* which cause several detrimental effects in the cropland. All *Origanum* essential oils tested in this study showed herbicidal effects on seed germination, and root and shoot development. In particular, the application of 10 µL for *T. arvense* and 20 µL for other weeds completely inhibited the seed germination, root, and shoot development. Moreover, 5 µL essential oil application can be useful for weed control.

The extracts obtained from *Origanum* species comparatively need a higher application dose for totally herbicidal effects. Even though the tested dose of these extracts was not inhibited totally, it was observed that the higher dose of these extracts can be useful for controlling these weeds. In addition, it was observed that the herbicidal effects of the extraction agent differed depending on the weeds. For instance, the acetone extract of *O. onites* showed the best results for *T. arvense,* while the n-hexane extract of *O. onites* was more suitable for seed germination and shoot development of *R. crispus*.

In the nursery experiment, 20 µL of essential oils and 20 mg of each extract were used because this dose was the best effective application in Petri experiments. It was observed that the essential oils obtained from *Origanum* species could be a good bio-herbicide candidate against the tested weed.

Tanacetum aucheranum and *Tanacetum chiliophyllum* var. chiliophyllum 30 mL/Petri [30], *Zataria multiflora* Boiss. 320–640 mL/L^{-1} [105], *Satureja hortensis* L. essential oil nanoemulsion (NE) 1000 µL/L^{-1} [17] determined that *Amaranthus retroflexus* weed species inhibited seed germination, and root and shoot development by 100%. In another study, (*Ruta graveolens* L.) and Bergamot (*Citrus bergamia* Risso et Poiteau) essential oils were found to be more toxic at doses of *A. retroflexus* L., 20 µL/mL^{-1}, inhibiting seed germination, and root and shoot development 100% [19]. Kordali et al. [43] found that the essential oil obtained from *Origanum acutidens* and carvacrol and thymol compounds in *A. retroflexus* 10 µL/Petri application dose, 9.8 µL/Petri carvacrol and 10 µL/*Petri* thymol petri doses of *Amaranthus retroflexus* completely inhibited seed germination and seedling growth of *Nepeta meyeri* Benth. Kordali et al. [16,52] reported that EO *A. retroflexus* L., which was obtained, completely affected the germination of the *Myrtus communis* seed and the effect was in parallel with the dose amount. It was reported that *Thymus kotschyanus* EO $\geq$ 500 ppm inhibited the germination and subsequent development of *A. retroflexus* seeds and showed an herbicidal effect [106]. An application of 600 and 800 mg/L^{-1} of *Foeniculum vulgare* essential oil showed the highest inhibitory effect against the *A. retroflexus* weed [107]. *Tagetes minuta* essential oil showed the highest inhibition of Amaranthus retrofexus seed germination at 600 μL/L^{-1} concentrations [21]. Yilar et al. [94] reported that *Origanum onites* essential oil completely inhibited seed germination, and root and shoot growth in *Amaranthus retroflexus* L. at a concentration of 15 L/Petri.

Kordali et al. [43] reported that the essential oil obtained from *Origanum acutidens* and carvacrol and thymol compounds in *Rumex crispus* 10 μL/Petri application dose, 9.8 μL/Petri carvacrol and 10 μL/Petri thymol petri doses of *Rumex crispus* completely inhibited seed germination and seedling growth. Doses of 10, 15, 20 g/cm^2 of essential oil obtained from Allium sativum germination and root growth of *Rumex crispus* 100% have inhibited, as have all doses of *Cuminum cyminum* L., *Mentha longifolian* essential oils (10, 15, 20 g/cm^2) 100% [62]. For *Tanacetum aucheranum* and *Tanacetum chiliophyllum* var. chiliophyllum 30 mL/Petri [30], Tursun et al. [108] used essential oils of thyme (*Origanum syriacum* L.) and laurel (*Laurus no bilis* L.) and their main components, carvacrol, 1,8-cineole, and pinene, to investigate the inhibitory effects of essential oils on three separate weeds. The seed germination tests showed that oregano essential oil and carvacrol completely inhibited weed germination at all concentrations ranging from 1 to 5 μL/Petri dish, whereas seed germination of test weeds decreased significantly with increasing concentrations of laurel essential oil and its main components, 1,8-cineol and -pinene ranging from 5 to 20 L/Petri dish. In another study, the most efficient essential oil dosages for inhibiting *Amaranthus palmeri* seed germination were determined to be 2–4 μL/Petri dish. The most successful (100%) application of all essential oils in suppressing seed germination was achieved with an *Origanum syriacum x Origanum onites* hybrid grown at 800 ppm [109].

According to Said et al. [110], the component of *Thymus capitatus* essential oil has an allelopathic effect on *Lactuca sativa* L. seed germination. *Eucalyptus globulus* essential oil has the potential to be examined as a biological pesticide with phytotoxic effects against *Lactuca sativa* weed [111]. Ruiz-Vásquez et al. [112] reported that EOs from *Peruvian casapiense*, *P. reticulatum*, *P. sancti-felicis*, and *P. mituense*, effectively inhibited the root growth of Lactuca sativa. The essential oil obtained from *P. soledadense* caused a decrease in root growth of *Lactuca sativa*. These results show strong selective herbicidal potential of the EOs tested against monocotyledonous plants. Azizan et al. [113] reported that applying *Wedelia trilobata* essential oil to *Lactuca sativa* L. caused changes in fatty acid compositions and could lead to root growth inhibition, as well as providing information on *L. sativa* metabolic responses and the potential mechanisms of action of *W. trilobata* EO as bioherbicides. In another study, *Origanum vulgare* subsp hirtum Letswaart (Greek thyme) essential oil evaluated the seed germination inhibition of *Lolium perenne* L. and *Trifolium pratense* L. weeds in the form of aqueous solutions at concentrations ranging from 0.5 to 3.0 μL/mL, and a 1.5 μL/mL dose application provided complete inhibition. They also evaluated thyme oil in terms of its inhibitory activity on seed germination under field conditions. It was observed that the weight of the plants decreased by 77% as a result of the observations made 1 month after the essential oil was applied as an aqueous solution at 3, 5 and 10 μL/mL concentrations on the super absorbent Teravet, mixed with the seeds of the target plants and planted in the field. They concluded that teravet (super-absorbent) application of essential oil is a good way of herbicidal effect in field conditions [114].

It is known that the essential oils and the extracts have bio-herbicidal effects on the weeds. In particular, the other *Origanum* species have a bio-herbicidal effect. For instance, Kordali et al. [43] tested thymol and carvacrol obtained from *Origanum acutidens* against *Amaranthus retfoflexus,* and these components of the essential oil inhibited the seed germination and seedling development. In the present study, we observed a similar effect. Even though thymol and carvacrol contents were observed from *Origanum syriacum* and *O. onites* (Table 2), thymol could not be isolated from *O. majorana* while for carvacrol contents it was 40.57%. However, the essential oils and extracts of *O. majorana* have bio herbicidal effects. Therefore, the other components of the essential oils obtained from *O. majorana* could inhibit the seed and seedling development.

The use of EOs in weed control is based on the fact that they contain allelochemical compounds, primarily terpenoids, that can inhibit weed species germination and growth [115,116]. Terpenoids, at least those that can disrupt mitosis, are a type of mitotic disrupting bioherbicide [117]. Monoterpenes were found in high concentrations in all of the EOs tested in our study.

Essential oils have phytotoxic potential in the form of hydrocarbons, alcohols, aldehydes, ketones, ethers, esters, peroxides, and phenols. This is attributed to terpenoids (primarily mono and 14 sesquiterpenes) as the main constituents [26–104,115–118].

Therefore, even though EO monoterpenes are known to be phytotoxic and to cause membrane integrity, cell division, and elongation, we observed non-incidental damage and necrosis in shoot and root tissues [119–121].

During using allelochemicals, the most studied response variables are weed seed shoot and root length [122]. The shoot and root lengths in the control treatment are generally longer than those in the treated seeds.

The results of the allelopathic activities of EOs and their effects on shoot and root lengths varied. In addition, when compared to the control treatment, the germinated seeds did not develop normally. Our findings confirmed that sprout and root length can vary depending on the type of EOs, concentrations tested, environmental conditions, and weed species [118–123]. Although essential oils appear to perform well in controlled laboratory conditions, their practical use for weed control in the field is limited due to their low water solubility and high volatility [118,124].

To overcome these drawbacks and improve essential oil performance, researchers recommend using essential oils as solid emulsions to prevent the loss of biological properties of essential oil components [124].

5. Conclusions

In recent years, essential oils have been considered a chemical alternative herbicide [62,117,120–122]. However, the herbicidal efficiency of essential oils and extracts is changing, depending on application dose and weeds. The main reasons for these differences in essential oil herbicide efficacy may be component differences and target weed species, but also application dose. Therefore, the dose and formulations should be designed to directly use these essential oils and extracts in agricultural fields [115]. However, in order to get the best results from field applications, more comprehensive research on the ways in which essential oils can be applied to the target plants is necessary. The limitation of our study is that we did not test individual or combinations of essential oil components to determine the exact source of the herbicide effect. We believe that if different essential oil blends or direct single composition applications can be made in future studies, weed control will become more effective, reducing product loss from weeds in agriculture. However, the herbicidal potential of the *Origanum* species was observed.

Author Contributions: Conceptualization, S.K. and A.N.; methodology, S.K., G.K. and F.Y.; software, G.K.; validation, F.Y.; formal analysis, S.K., G.K., F.Y., İ.S. and A.N.; resources, İ.S.; data curation, S.K.; writing—original draft preparation, G.K., F.Y. and İ.S.; writing—review and editing, S.K. and A.N.; visualization, G.K., F.Y. and İ.S.; supervision, S.K. and A.N.; project administration, S.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data sharing is not applicable to this article.

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

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