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Study of Allelopathic Interaction of Essential Oils from Medicinal and Aromatic Plants on Seed Germination and Seedling Growth of Lettuce

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Received: 25 November 2019; Accepted: 14 January 2020; Published: 23 January 2020

Abstract: Medicinal and aromatic plants have the ability to transmit volatile allelochemicals and affect their surrounding organisms. In this regard, their interaction should also be considered. The inhibitory effects of 112 essential oils on lettuce seed and seedling were investigated by cotton swab method. Germination (G%), Mean germination time (MGT), Lethal of embryo (L%), dormancy (D%), radicle growth (R%), and hypocotyl growth (H%) were measured. Two methods were used for evaluating allelopathic interaction effects: the simplified modified dilution check-board technique (SMCT) and the isobologram. *Thymus daenensis* had the highest inhibitory effect on G% (IC_{50} = 2.9 ppm) and the most lethal effect on the embryo (LC_{50} = 7.2 ppm). *Thymus transcaspicus*, *Dracocephalum moldavica*, *Artemisia sieberi* and *Amomum subulatum* had the greatest effect on MGT. *Ziziphora tenuior*, *Trachyspermum ammi* and *Pelargonium graveolens* had the highest effect on D%. *Origanum vulgare* was the strongest growth inhibitor. The highest synergistic effect on G% was in *A. subulatum* + *Mentha suaveolens*, on H% was related to *Perovskia abrotanoides* + *T. daenensis*, and on R% was observed in *Artemisia vulgaris* + *M. suaveolens*. The results of this study can lead to identification of new phytotoxic compounds in EOs and control weeds more effectively.

Keywords: essential oil; volatile; headspace; phytotoxic; medicinal plant; cotton swab; synergist; antagonist; isobologram; dormancy

1. Introduction

Growth interactions between weeds and crops lead to high costs for agricultural systems [1]. Herbicides have always played a key role in weed management. This event is one of the major causes of environmental damage and public health, leading to environmental pollution, product insecurity and human health hazards [2,3]. Furthermore, every year, a new list of pesticide-resistant weeds is released. According to the 2017 report by the International Herbicide-Resistant Weed site, a list of 36 new cases of weed resistance to herbicides was released [4]. This causes us to always look for new herbicides to control resistant weed populations.

Today, the global effort in modern agriculture is to reduce the use of harmful pesticides by introducing new biological and ecological methods. One of these methods is the use of chemical interactions among plants [5]. Interactions between plants in a common ecosystem are side effects that each plant exerts on its neighboring plants, and these include competition and allelopathy. Competition involves the active absorption of limited resources by one organism, which leads to a decrease in supply and thus to the growth inhibition of other organisms, but when one species stops growing due

to chemicals released from another species, this mechanism is called allelopathy [6,7]. However, some researchers also consider the stimulatory effects of growth in defining allelopathy [8]. Researchers have shown that the use of allelopathic properties of some plant species has been very effective in controlling the growth of other plants, including some weeds and lettuce, which is used as an indicator plant in allelopathic bioassays [9–11]. A large number of allelochemicals from various plant species have been reported, the most important of which are phenolic compounds, benzoxazinoids, sorogoleones, glucosinolates, terpenes, alkaloids, and mamilactones [5]. Since natural herbal compounds, which are mostly safe to environment and human health, cause allelopathy, the use of allelopathy for effective weed control in agricultural systems can play an important role in environmental and community safety.

Aromatic and medicinal plants have a special place among allelopathic plants due to their secondary constituents and their active ingredients. Allelochemicals of higher plants can be released into the environment in various ways, such as volatility (predominant in dry and semi-arid conditions), leaf or stem leaching (through rain, dew, or irrigation), root secretion, and tissue degradation by microorganisms. Allelochemicals then reach the target plant by releasing to the soil, leaching, or diffusion into the air. [12]. The volatile compounds are important secondary metabolites found in medicinal and aromatic plants. Therefore, these plants are capable of transmitting allelochemical constituents in their essential oils (EOs) through diffusion into the air and thus affecting their surrounding organisms. Many studies have shown that EOs play an important role in controlling the growth of microorganisms and can effectively inhibit the growth and germination of spores of bacteria and fungi [13,14]. Studies have also shown that some EOs or their components effectively reduce plant growth [15]. Since EOs and volatile compounds have the potential to be used as fumigant, there will be no residue on the product if used. Therefore, these allelopathic compounds can be very effective in weed control before and after planting crops.

In the evaluation of allelopathic species, their synergistic potential can also be considered. Since plants EOs compounds may have similar or different activity, combining them may cause additive, synergistic, or antagonistic effects. Researchers have shown that there are additive and synergistic interactions between the EOs in a variety of cases, including the effects on the growth of various microorganisms as well as antioxidant effects [16–18]. So far very little research has been done on the interaction of EOs on allelopathic effects on germination and growth of other plants [19]. New research in this area could provide a new context for better understanding of allelopathic effects and achieving natural effective herbicides.

In weed science, most investigations have been done on industrial herbicides [20]. Therefore, further research is needed to identify novel methods. Finding new effective allelopathic species and their inhibitory compounds can be useful in this regard. Further studies to understand the physiological and molecular aspects of allochemicals may reveal the mechanism of allelopathic effects. This paper investigates the inhibitory effects of EOs of some medicinal and aromatic plants of Iran in gaseous phase on seed germination and seedling growth of lettuce. Also in this study, for the first time, bioassay tests were designed and implemented to investigate the allelopathic interaction.

2. Materials and Methods

This study was carried out in three separate experiments, and lettuce was used as test plant because it is a model plant for allelopathic bioassays owing to its short germination period and high sensitivity to phytochemicals.

2.1. Plant Material

Plant samples included 112 specimens of different plant organs including root, rhizome, corm, stem, leaf, flower, fruit, fruit peel, aerial part, or plant exudates such as oleogum belonging to 97 aromatic species from 16 different plant families were collected from different locations of Iran (botanical gardens, plant science research centers and natural habitats) (Table 1). In order to preserve the volatile compounds, the plants were dried according to the type of tissue (in oven at 30–60 °C

for 1 to 3 days). EO extraction was done by hydro distillation method for 4 h using Clevenger-type apparatus at a distillation rate of 3 mL/min according to the European Pharmacopoeia method [21]. The EOs were collected in dark sealed air-tight glass vials, dried with anhydrous sodium sulfate and stored in the refrigerator at 4 °C.

2.2. Evaluation of Allelopathic Effect of EOs on Lettuce Seed and Germination Characteristics

2.2.1. Cotton Swab Method

To evaluate the phytotoxicity of volatile constituents on germination, 112 EOs were evaluated in two different amounts of 1 and 3 µL in vial compared to the control. The experiment was conducted as a factorial experiment in completely randomized design with four replications. Allelopathic effects of volatile compounds were evaluated by cotton swab method [22] (Figure 1). For this purpose, 20 mL glass vials were disinfected in the oven after washing. The 0.75% (w/v) agar solution was prepared using agar powder (Merck Inc., Kenilworth, NJ, USA) and twice distilled water and then sterilized in autoclave. Due to the high sensitivity of agar volume precision in the vial, the vial filling step was carried out after sterilization and when the agar temperature reached about 40 °C. Using a graduated pipette, 10 mL of agar (equal to half the volume of vial) was poured into each vial. After the agar is cooled and solidified, seven lettuce seeds (*Lactuca sativa*, cultivar Great Lakes No. 366) were placed on the agar so that one third of the seed tip was immersed in the agar. Then a double-tipped cotton swab was cut in half and placed in the center of the agar so that its cotton tip was in the middle of the void above the agar surface. The EO was injected into a cotton swab in a specified amount (1 or 3 µL) using a Hamilton capillary syringe. Immediately, the rubber cap and aluminum seal were placed on the vial lid and sealed with Crimper (Figure 1). The vials were placed in a germinator at 21 ± 2 °C under dark conditions. The control vials were filled with agar, seeds were sown in, the swabs were laid, then were sealed and incubated just like other vials. The only difference with the treated vials was that the swabs were not injected with essential oils.

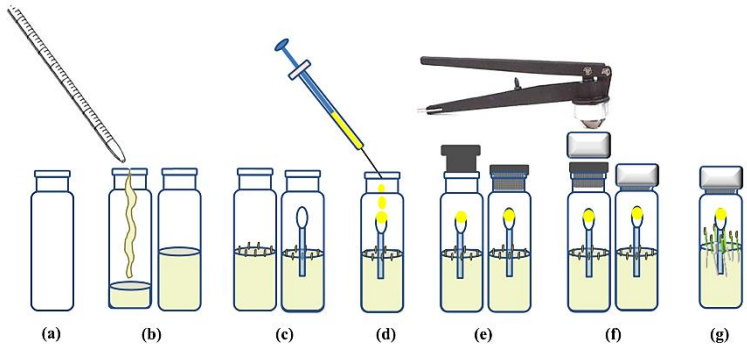

Figure 1. Schematic design of experiment to investigate the allelopathic effect of essential oils (Eos) by cotton swab method: (**a**) 20 mL vial; (**b**) Add 10 mL agar; (**c**) Insert lettuce seeds and cotton swabs after agar cooling and solidification; (**d**) Add EOs to cotton swabs using Hamilton syringe; (**e**) Rubber cap insertion; (**f**) Aluminum sealing and crimping; (**g**) Germination and seed growth after incubation.

Each experiment unit consisted of a vial containing seven seeds in which germination status was monitored daily for five days and germination-related traits were measured.

2.2.2. Germination and Seed Traits

Germination percentage (G%) [23]:

$$G\% = \left(\frac{N}{S}\right) \times 100 \quad (1)$$

G%: germination percentage; N: total germinated seeds by the end of experiment; S: total seeds.

Mean germination time (MGT) [23]:

$$MGT = \frac{\sum T_i N_i}{N} \quad (2)$$

MGT: Mean germination time; N_i: number of germinated seeds on day T_i; T_i: day during germination period; N: Total germinated seeds by end of experiment.

These traits were reported relative to control:

$$V\% = \frac{V_T}{V_C} \times 100 \quad (3)$$

V%: relative value of trait; V_T: average trait of treatment; V_C: average trait of control.

Lethal percentage of seed embryo (L%) and seed dormancy induction (D%); for this purpose, the seeds that did not germinate after five days were cultured in vial without EO for another three days. Seeds that did not germinate after this time were uncoated and immersed in 1% tetrazolium solution (pH = 7) and incubated for 6 h at 30 °C. The endosperm and embryo status were examined using a microscope and the lethality was determined (Formula no. 4). Only seeds that were completely and uniformly colored were considered as live seeds according to the ISTA instructions [24] (Figure 2). With respect to the number of germinated seeds and L%, some seeds were neither germinated nor showed sign of embryo death indicating that they were dormant-induced seeds (Formula no. 5):

$$L\% = \frac{n}{S} \times 100 \quad (4)$$

L%: Lethal percentage; n: non-colored seeds; S: total seeds.

$$D\% = \frac{S - (N + A + L)}{S} \times 100 \quad (5)$$

D%: dormancy percentage; S: total seeds; N: total germinated seeds by the end of experiment; A: germinated seeds after removal of EO treatment; L: Seeds with dead embryos.

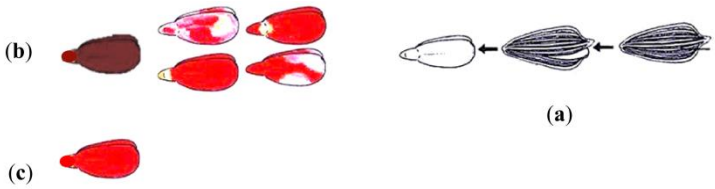

Figure 2. ISTA based embryo death assessment: (**a**) Seed preparation and coat removal method; (**b**) Unacceptable staining types that are considered abnormal seeds; (**c**) Uniform staining of live seed.

2.3. Evaluation of Allelopathic Effect of EOs on Lettuce Seedling Growth

The experiment was conducted as same as the previous one except that in this experiment germinated seeds were used to investigate the effect of EOs on lettuce seedling growth. Lettuce seeds were germinated 24 h prior to the experiment and those with 2 mm radicle were used. Each test unit was a vial containing five germinated seeds that the EO treatment was applied on and sealed. The vials were incubated at 21 °C under dark conditions for three days.

After three days, seedlings were photographed and radicle and hypocotyl length were measured using Image J software using formula no 6 and 7 respectively.

$$R\% = \frac{R_T}{R_C} \times 100 \quad (6)$$

R%: radicle length percentage; R_T: treatment radicle length; R_C: control radicle length.

$$H\% = \frac{H_T}{H_C} \times 100 \quad (7)$$

H%: hypocotyl length percentage; H_T: treatment hypocotyl length; H_C: control hypocotyl length.

Then, according to germination percentage and seedling length, seedling vigor index (VI) was calculated:

$$VI\% = G\% \times S \quad (8)$$

VI: Seedling vigor index; G%: Germination percentage; S: Seedling length (radicle + hypocotyl).

This trait was also reported relative to control.

2.4. Statistical Analysis

The effect of treatments on different traits was compared and classified according to mean and standard deviation (sd) of each trait. The inhibitory effect of treatment on germination and growth increased some traits (MGT%, D%, L%) and decreased some others (G%, H%, R%, VI%). Intensity of inhibitory effect was considered based on the increase and decrease of these traits compare to the mean, respectively. The severity of inhibition was defined at five levels: from the highest to the least effect respectively, level five (*****) Mean ± 2 sd, level four (****) Mean ± 1.5 sd, level three (***) Mean ± 1 sd, Level two (**) Mean ± 0.5 sd, and level one (*) Mean ± 0.25 sd, respectively. It should be noted that MGT was not defined in some test units due to zero germination percent; for this reason, only EOs with at least three replications of germination above zero percent were compared in this trait. SPSS, Graphpad Prism and Excel were used for statistical analysis and graphing.

2.5. Headspace Gas Chromatography-Mass Spectrometry (HS-GC-MS)

Headspace GC-MS was performed to analyze the chemical composition of the strongest samples from the screening stage (experiment 1 and 2). For chemical analysis, 1 microlitre (equivalent to 1 mg) of each EO was added to the tip of a cotton swab, placed in a 20 mL vial then sealed and incubated at 21 °C for 1 h. After 1000 µL of head space from each vial was injected into GC-MS (Shimadzu QP 2010, Tokyo, Japan) using a 5 mL SGE 5MDR-HSV syringe. The injection condition was as follow: equity five capillary columns (30 m × 250 µm × 0.25 µm) and use of helium as carrier gas. GC-MS Operating Conditions: The temperature of the GC cooker was adjusted from 50 to 220 °C with a rise of 3 °C/min, held for 10 min and then increased to 250 °C with a rise of 10 °C/min. The compounds were identified from the mass spectra of NIST/NBS. Mass spectra were recorded at 70 eV with a mass range of 50 to 400 m/z, compared to an internal spectral library (NIST and Wiley). Then they are validated by comparing retention times with authentic standards.

2.6. Allelopathic Interaction Effects of EOs

After screening the EOs the strongest inhibitors on G%, L%, R%, H% and S%, which caused the highest level of inhibition at 1 µL, were identified. In order to investigate the allelopathic interaction of these EOs, effective inhibitory and lethal concentration were first determined.

2.6.1. Determination of IC_{50} and LC_{50}

IC_{50} or inhibitory concentration (the concentration of the EO that results in 50% inhibition) and LC_{50} or effective lethal concentration (the concentration of EO that causes the death of embryo in 50% of the seeds) were determined through applying different amounts of each EO (0.01 µL to 1 µL per vial) so that at least five effective concentrations were in the range of 20% to 80% inhibitory. To increase the accuracy it was necessary to dilute the EOs, which was done with dimethyl sulfoxide (DMSO). The results were presented in ppm and only the void space of each vial was considered to determine the concentration. The results were analyzed using GraphPad Prism 8 software to determine IC_{50} and LC_{50}. In addition, the values of IC_{25} and IC_{90}, and LC_{25} and LC_{90} were calculated with Quick Calcs online software. The most effective EOs were identified after determination of IC_{50} and LC_{50}.

2.6.2. Essential oils Combinations

Some of the most effective EOs were selected and their combination effects were evaluated. The experiment was conducted as factorial in a completely randomized design with four replications. Simplified modified dilution check-board technique (SMCT) in cotton swab method was used and two different EOs (A and B) were evaluated at their IC_{25} concentration. Experiments were performed separately on seed germination, radicle growth, and hypocotyl growth. For germination evaluation the method was similar to the first experiment, and for the radicle and hypocotyl growth it was done like second experiment. The allelopathic interaction effect of the EOs was determined using two methods: determination of the combination effect (CE), and drawing the isobologram curves.

- Combination effect (CE) was calculated using the following equation:

$$CE_{(A,B)} = \frac{I_{(A_{25}+B_{25})}}{\left[I_{(2A_{25})} + I_{(2B_{25})}\right]/2} \quad (9)$$

$CE_{(A, B)}$: combination effect of two EOs (A and B); I: Inhibitory percentage; A_{25} and B_{25}: A and B in their IC_{25} concentration; $2A_{25}$ and $2B_{25}$: A and B in their twice IC_{25} concentration (calculated using Graphpad prism).

In this equation if CE ≥ 1.1 then the interaction was considered as synergistic effect, if 0.9 < CE < 1.1 was then considered as additive effect, and if CE ≤ 0.9 then the interaction was considered as antagonistic effect.

- Isobologram curves

To draw these curves, the inhibitory effect of two EOs (A and B) was considered as the basis, then the concentration of A and B independently leading to similar inhibition was calculated using Quick Calcs software. Thus, there were three concentrations (A alone, B alone, and A or B in combination state) for a fixed amount of inhibition (inhibition caused by the combination of two EOs), using in isobologram curve. This curve can have three different states: (1) no curvature means additive effect, (2) upward curvature means antagonistic effect, and (3) downward curvature means synergistic effect (Figure 3).

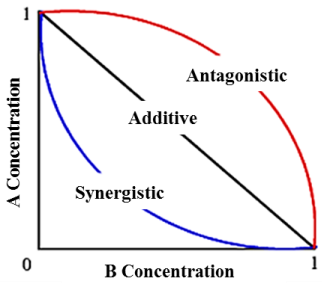

Figure 3. Schematic isobologram curve for determination of allelopathic interactions.

3. Results

3.1. Allelopathic Effects of Essential Oils on Lettuce Seed and Germination Characteristics

All germination traits were significantly affected by plant EO treatment at both amount of 1 and 3 µL (Table 1).

Table 1. The results of evaluation of inhibitory potential of medicinal plants EO on traits related to seed germination and seedling growth of lettuce.

No.	Plant Scientific Name	Plant Family	Part of Use [a]	G% [b]		MGT (Day) [b]		D% [b]		L% [b]		H% [b]		R% [b]		VI% [b]	
				1 µL	3 µL	1 µL	3 µL	1 µL	3 µL	1 µL	3 µL	1 µL	3 µL	1 µL	3 µL	1 µL	3 µL
1	*Abies alba*	Pinaceae	L	100	100	3.3	2.5	0	0	0	0	27.8	43.2	42.7	63.9	36.9	56
2	*Achillea filipendulina*	Asteraceae	Ap	60.7	96.5	4.3 ***	2.5	0	0	3.6	0	10.8 **	25.8 **	5.7 **	16.6 ***	4.7 **	19.4 **
3	*Achillea wilhelmsii*	Asteraceae	Ap	60.7	93	4.8 ****	3.7 **	0	0	3.6	0	12.1 **	21.2 **	4.8 **	18.2 **	4.6 **	18.0 **
4	*Allium hirtifolium*	Liliaceae	C	0.0 ****	3.5 *****		5	0	0	100 *****	96.4 *****	6.9 **	10.4 ***	1.2 **	5.2 ***	0.0 **	0.3 ***
5	*Amomum subulatum*	Zingiberaceae	Fr	0.0 ****	28.5 ****		4.8 *****	3.6	0	96.4 *****	0	0.8 ***	12.2 ***	1.0 **	10.8 ***	0.0 **	3.2 ***
6	*Anethum graveolens*	Apiaceae	Fr	21.4 ***	92.8	4.7 ****	3.8 **	57.1 *****	0	0	0	4.4 ***	14.6 ***	0.5 **	18.0 **	0.4 **	15.5 **
7	*Apium graveolens*	Apiaceae	Fr	100	89.3	2.2	2.3	0	0	0	3.6	22.2	29.2 *	59.9	61.2	45.4	43.7
8	*Artemisia absinthium*	Asteraceae	L	96.4	100	3.2	2.5	0	0	0	0	28.7	42.2	44.9	49.9	37.3	46.9
9	*Artemisia aucheri*	Asteraceae	Ap	82.1	100	4.7 ****	3.8 **	0	0	0	0	6.9 **	23.0 **	0.0 ***	13.8 ***	2.2 **	17.3 **
10	*Artemisia deserti*	Asteraceae	Ap	89.3	100	3.9 **	3.3 *	0	0	0	0	7.1 **	21.0 **	0.0 ***	16.7 **	2.4 **	18.4 **
11	*Artemisia dracunculus*	Asteraceae	L	100	100	2.5	2.1	0	0	0	0	20.8	39.9	17.5 *	43.6	18.8	42.2
12	*Artemisia ludoviciana*	Asteraceae	L	10.7 ***	96.5	4.8	3.9 ***	7.2	0	7.2	0	4.7 ***	14.1 ***	1.3 **	3.7 ***	0.3 **	7.4 ***
13	*Artemisia scoparia*	Asteraceae	L	25.0 **	82.3	4.8 ****	4.5 ****	3.6	3.6	0	7.2	10.8	19.9 **	2.3 **	16.9 **	1.4 **	14.9 **
14	*Artemisia turanies*	Asteraceae	Ap	82.1	100	4.3 ***	3.6 **	0	0	3.6	0	19.1 *	35.8	20.2	48.6	16.2	43.7
15	*Artemisia vulgaris*	Asteraceae	L	85.7	100	3.4	2.6	0	0	3.6	0	24.7	45.1	14.8 *	33.8 *	15.9	38.1
16	*Artemisia vulgaris*	Asteraceae	F	89.5	100	4.5 ***	3.9 ***	0	0	7.2	0	12.2 **	18.4 **	5.4 **	7.3 ***	7.2 **	11.6 ***
17	*Artemisia sieberi*	Asteraceae	L,S	0.0 ****	50.0 ***		4.7 ****	7.2	0	17.9	7.2	11.3 **	20.7 **	0.6 **	7.6 ***	0.0 **	6.3 ***
18	*Ballota nigra*	Lamiaceae	L,F	100	92.8	2.8	3	0	0	0	0	83.2	93.5	78.9	90.1	80.6	84.9
19	*Bunium persicum*	Apiaceae	Fr	0.0 ****	25.3 ****		4.5 ****	0	0	89.3 *****	10.7	11.2 **	30.6 *	4.3 **	33.1 *	0.0 **	8.0 ***
20	*Cedrus atlantica*	Pinaceae	L	96.4	96.5	3.1	2.7	3.6	0	0	0	40.8	44.3	63.9	67.6	53.1	56.5
21	*Chamaecyparis lawsoniana*	Cupressaceae	L	57.1	93	4.8 ****	3.7 **	0	0	3.6	0	20.9	42.8	27.4	37.5	14.2*	36.7
22	*Chamaecyparis lawsoniana*	Cupressaceae	L	96.4	100	2	2	0	0	0	0	23.5	33.8	30.7	51.2	27	44.5
23	*Chamaecyparis sp.*	Cupressaceae	L	96.4	96.5	3	2.8	0	0	0	0	18.6 *	34	9.3 **	24.3 **	12.4 *	27.0 *
24	*Chenopodium botrys*	Chenopodiaceae	L	100	93	3.1	3.1	0	0	0	3.6	46	50	66.1	66.7	58.4	56
25	*Chrysanthemum morifolium*	Asteraceae	L	92.9	96.5	3.8 **	3.3 *	3.6	0	0	0	18.4 *	35.3	4.2 **	33.3 *	9.0 **	32.8
26	*Citrus × limon*	Rutaceae	Fp	92.9	96.5	3.7 *	2.4	0	3.6	0	0	40.2	67.6	59.7	90.5	48.4	78.8
27	*Citrus × paradisi*	Rutaceae	Fp	92.9	100	2.4	2.3	3.6	0	0	0	26.7	46.1	24.5	57.8	23.5	53.3
28	*Citrus × sinensis*	Rutaceae	Fp	100	96.5	2.7	2.3	0	0	0	0	29.4	71.1	37.4	85.9	34.3	77.3
29	*Citrus aurantifolia*	Rutaceae	Fp	100	100	2.5	2.2	0	0	0	0	28.8	64.3	15.6 *	53.4	20.7	57.6
30	*Citrus aurantifolia*	Rutaceae	L	0.0 ****	29.0 ****		4.5 ***	25.0 ****	3.6	39.3 **	3.6	23.1	30.6 *	13.3 *	45.2	0.0 **	11.3 ***
31	*Citrus aurantium*	Rutaceae	Fp	100	100	2.2	2	0	0	0	0	54.6	70.9	72.6	74.3	65.7	73
32	*Citrus japonica*	Rutaceae	Fp	92.9	96.5	2.2	2.3	3.6	3.6	0	0	32.8	56.2	54.1	87	42.6	72.5
33	*Citrus medica*	Rutaceae	Fp	92.9	96.5	3	2.6	7.2	3.6	0	0	45.2	58.5	77.5	76.8	60.4	67.2
34	*Citrus reticulata*	Rutaceae	Fp	100	93	2.4	2.3	0	7.2 *	0	0	35.3	60.6	35.5	61.2	35.4	56.6
35	*Citrus × tangelo*	Rutaceae	Fp	100	100	2.1	2.1	0	0	0	0	48.6	71.4	57.5	85	54.1	79.7
36	*Citrus × latifolia*	Rutaceae	Fp	89.3	100	3.3	2.8	0	0	0	0	15.6 *	28.2 *	14.3 *	34.1 *	13.2 *	31.8
37	*Coffea arabica*	Rubiaceae	Fr	89.3	100	2.5	2.1	0	0	10.7	0	85.4	94.9	94.2	106.9	81.1	102.3
38	*Coriandrum sativum*	Apiaceae	Fr	50.0 *	93	4.2 **	3.1	0	0	0	0	6.0 **	18.8 **	1.9 **	24.9 **	1.7 **	20.9 **
39	*Cuminum cyminum*	Apiaceae	Fr	3.6 ****	86	5	3.8 **	3.6	0	14.3	3.6	11.6 **	23.2 **	6.0 **	21.2 **	0.3 **	18.9 **
40	*Cupressus arizonica*	Cupressaceae	L	89.3	96.5	4.3 ***	3.7 **	0	3.6	3.6	0	21.8	39.6	19.7 *	47.5	18.3	42.8
41	*Cupressus arizonica*	Cupressaceae	Fr	67.9	100	3.4	3.1	0	0	0	0	37.5	47	74.7	86.1	41	71.1
42	*Cupressus sempervirens*	Cupressaceae	L	100	89.3	3.6 *	2.7	0	0	0	7.2	38.2	51.1	73.2	91.8	59.7	68

Table 1. *Cont.*

No.	Plant Scientific Name	Plant Family	Part of Use [a]	G% [b]		MGT (Day) [b]		D% [b]		L% [b]		H% [b]		R% [b]		VI% [b]	
				1 µL	3 µL	1 µL	3 µL	1 µL	3 µL	1 µL	3 µL	1 µL	3 µL	1 µL	3 µL	1 µL	3 µL
43	*Curcuma longa*	Zingiberaceae	Rh	60.7	86	3.8 **	3.8 **	3.6	0	0	0	47.1	49.2	91.8	99.5	45.3	68.7
44	*Datura stramonium*	Solanaceae	L	100	96.5	3	3.3 *	0	0	0	0	73.1	72.7	65.4	76.6	68.3	72.4
45	*Daucus carota*	Apiaceae	Fr	92.9	96.5	2.9	2.6	0	0	3.6	0	19.6	27.1 *	12.8 **	28.7 **	14.3 *	27.1 *
46	*Dracocephalum moldavica*	Lamiaceae	Ap	0.0 ****	39.5 ***		4.7 *****	0	0	92.9 ****	7.2	4.8 ***	24.2 **	0.7 **	23.8 **	0.0 **	9.4 ***
47	*Durema ammonicum*	Apiaceae	O	92.9	100	2.4	2.4	0	0	0	0	37	49.6	41.5	71.8	36.9	63.3
48	*Elettaria cardamomum*	Zingiberaceae	Fr	100	96.5	3.3	2.4	0	3.6	0	0	12.9 **	27.1 *	11.0 **	38.2	11.8 *	32.7
49	*Eucalptus globulus*	Myrtaceae	L3	100	89.3	3.4	2.6	0	0	0	7.2	20.6	32.2	24.2	46.1	22.8	36.4
50	*Ferula alliacea*	Apiaceae	Ap	96.4	100	3.1	2.6	0	0	3.6	0	24	43	23.6	51.9	22.9	48.5
51	*Ferula foetida*	Apiaceae	O	0.0 ****	93		3.2	0	3.6	100 *****	0	9.3 **	38.5	4.7 **	48.1	0.0 **	41.3
52	*Ferula gumosa*	Apiaceae	O	100	100	2.6	2.6	0	0	0	0	42.2	76	54.8	88.3	50	83.6
53	*Ferula lutensis*	Apiaceae	L	89.3	100	2.9	2.3	0	0	3.6	0	17.8 *	32	10.9 **	30.7 *	12.1 *	31.2
54	*Foeniculum vulgare*	Apiaceae	Fr	100	100	3.2	2.5	0	0	0	0	9.0 **	29.8 *	1.8 **	32.2 *	4.6 **	31.3
55	*Foeniculum vulgare*	Apiaceae	L	78.6	100	3.9 **	3.1	0	0	3.6	0	25.6	42.2	29.1	48.4	21.8	46.1
56	*Grindelia robusta*	Asteraceae	L,F	92.9	93	2.8	2.4	3.6	0	0	0	26.6	51.7	31	72.4	27.2	59.8
57	*Helianthus annuus*	Asteraceae	L	100	100	2.8	2.4	0	0	0	0	26.9	47.5	17.4 *	63.1	21.1	57.1
58	*Helichrysum italicum*	Asteraceae	Ap	53.6 *	96.5	4.1 **	3.6 **	0	0	3.6	0	19.4	31.1 *	27.4	42.4	13.0 *	36.7
59	*Heracleum persicum*	Apiaceae	Fr	25.0 **	100	3.3	2.4	14.3 **	0	3.6	0	7.5 **	29.6 *	3.5	30.1 *	1.3 **	29.9 *
60	*Inula paecockianum*	Asteraceae	R	100	93	2	1.7	0	0	0	0	71.4	70.7	75.1	74	73.7	67.5
61	*Juniperus chinensis*	Cupressaceae	L	92.9	96.5	3.3	2.9	0	3.6	0	0	19.4	27.7 *	23.4	39.2	20.3	33.5
62	*Juniperus excelsa*	Cupressaceae	L	92.9	100	2	2.2	7.2	0	0	0	42.9	61.5	73.8	91.9	57.5	80.2
63	*Juniperus horizontalis*	Cupressaceae	Fr	96.5	100	2.4	2.6	0	0	0	0	45.9	56.5	59.7	69.5	52.5	64.5
64	*Juniperus horizontalis*	Cupressaceae	L	96.4	92.8	2.1	2	3.6	3.6	0	0	15.6 *	31.2 *	22.7	53.3	19.2	41.6
65	*Juniperus sp.*	Cupressaceae	L,F	100	96.5	2.8	2.4	0	3.6	0	0	33.3	38.3	37	36.2 *	35.6	35.7
66	*Lantana camara*	Verbenaceae	L,F	92.9	93	2.9	2.4	3.6	0	0	0	32.4	39.1	53.7	47.5	42.2	41.1
67	*Lavandula angustifolia*	Lamiaceae	L	71.4	93	3.9 **	3.5 **	0	0	3.6	3.6	15.4 *	27.5 *	9.3 **	45.6	8.3 **	35.9
68	*Lavandula angustifolia*	Lamiaceae	F	21.4 ***	96.5	4.1 **	3.8 **	0	0	3.6	0.0	2.0 ***	13.1 ***	0.2 ***	4.1 ***	0.2 **	7.3 ***
69	*Lippia citriodora*	Verbenaceae	L	60.7	96.5	4.4 ***	3.3 *	0	0	10.7	0	7.3 **	33	5.8 **	24.3 **	3.9 **	26.6 *
70	*Magnolia virginiana*	Magnoliaceae	L	96.4	85.8	2.7	3.1	3.6	0	0	10.7	63.6	62.1	70.2	70.7	65.2	57.8
71	*Melissa officinalis*	Lamiaceae	L	0.0 ****	3.5 *****		4	0	0	100 *****	21.5 **	15.0 *	21.1 **	6.3 **	18.8 **	0.0 **	0.7 ***
72	*Mentha longifolia*	Lamiaceae	L	53.6 *	96.5	4.3 ***	3.6 **	0	0	0	0	11.8 **	19.0 **	5.2 **	14.3 ***	4.1 **	15.5 **
73	*Mentha piperita*	Lamiaceae	L,F	32.2 **	60.5 **	4.4 ***	4.2 ***	17.9 ***	3.6	0	3.6	9.4 **	26.8 **	4.7 **	35.6 *	2.1 **	19.6 **
74	*Mentha pulegium*	Lamiaceae	L,F	53.6 *	96.5	4.4 ***	3.7	7.2	0	0	0	7.3 **	21.3 **	3.3 **	20.2 **	2.6 **	19.9 **
75	*Mentha suaveolens*	Lamiaceae	L	0.0 ****	10.8 *****		4.5	0	14.3 ***	92.9 *****	3.6	12.2 **	15.8 ***	6.5 **	7.6 ***	0.0 **	1.2 ***
76	*Microcephala lamellata*	Asteraceae	L,F	89.3	100	2.7	2.3	0	0	3.6	0	39.5	46.4	43.5	75.4	37.5	64.2
77	*Myristica fragrans*	Myrtaceae	Fr	96.4	100	3.1	2.5	0	0	0	0	25.3	41.8	38.2	54.5	32	49.6
78	*Nepeta binaludensis*	Lamiaceae	Ap	10.7 ***	78.5	5	4.6 ****	3.6	3.6	3.6	0	26.9	57.4	38.2	66.6	3.6 **	49.5
79	*Nepeta cataria*	Lamiaceae	L,F	14.3 ***	68.0 *	4.3	4.4 ****	42.9 *****	3.6	14.3	0	24.8	55.4	10.9 **	54.7	2.3 **	37.3
80	*Origanum vulgare subsp. viridi*	Lamiaceae	F	46.4 *	89.5	4.5 ***	3.3 *	0	0	7.2	3.6	5.6 ***	5.0 ****	0.1 ***	2.3 ****	1.0 **	3.0 ***
81	*Origanum vulgare subsp. vulgare*	Lamiaceae	L	0.0 ****	7.0 *****		5	0	0	100 *****	85.7 *****	4.6 ***	10.5 ***	0.2 ***	8.4 ***	0.0 **	0.7 ***

Table 1. *Cont.*

No.	Plant Scientific Name	Plant Family	Part of Use [a]	G% [b]		MGT (Day) [b]		D% [b]		L% [b]		H% [b]		R% [b]		VI% [b]	
				1 µL	3 µL	1 µL	3 µL	1 µL	3 µL	1 µL	3 µL	1 µL	3 µL	1 µL	3 µL	1 µL	3 µL
82	*Origanum vulgare subsp. vulgare*	Lamiaceae	F	100	100	2.2	2.1	0	0	0	0	44.1	45.3	61	62.1	54.5	55.7
83	*Pelargonium graveolnes*	Geraniaceae	L	3.6 ****	7.0 *****	5	3.5	25.0 ****	57.1 *****	71.4 ****	0	14.3 **	19.0 **	26.6	27.8 **	0.8 **	1.7 ***
84	*Pelargonium graveolnes*	Geraniaceae	L,F	0.0 ****	0.0 *****			14.3 **	32.2 *****	82.1 *****	32.2 ***	16.4 *	13.7 ***	18.4 *	25.6 **	0.0 **	0.0 ***
85	*Perovskia abrotanoides*	Lamiaceae	F	53.6 *	89.3	4.8 ****	3.8 **	3.6	0	0	0	7.8 **	6.7 ****	1.0 **	4.3 ***	1.9 **	4.7 ***
86	*Perovskia abrotanoides*	Lamiaceae	L	89.3	100	4.3 ***	3.3	0	0	0	0	31.8	35.9	24.9	43.4	24.6	40.5
87	*Petroselinum sativum*	Apiaceae	Fr	96.4	96.5	2.5	2.4	0	0	3.6	3.6	19.4	31.2	36.5	85	28.8	62
88	*Pimpinella anisum*	Apiaceae	Fr	0.0 ****	0.0 *****			0	0	10 0 *****	10 0 *****	1.9 ***	5.8 ****	0.5 **	4.2 ***	0.0 **	0.0 ***
89	*Pinus eldarica*	Pinaceae	O	100	100	2	2.3	0	0	0	0	23.1	58.7	13.6 *	65.7	17.2	63
90	*Pinus eldarica*	Pinaceae	L	100	89.3	3	3	0	7.2 *	0	0	66.7	71.8	81.4	79.7	75.8	68.5
91	*Pinus eldarica*	Pinaceae	L	96.4	96.5	3.4	3	0	0	0	0	44.6	32.2	53.6	25.6 **	48.4	27.1 *
92	*Pistacia vera*	Anacardiaceae	Fp	100	100	3	2.4	0	0	0	0	47.3	51	60.2	69.2	55.2	62.2
93	*Rosmarinus officinalis*	Lamiaceae	L	35.7 **	89.3	5.0 ****	4.5 ****	10.7 *	0	0	0	8.8 **	28.1 *	0.8 **	12.8 ***	1.4 **	16.7 **
94	*Rosmarinus officinalis*	Lamiaceae	F	17.9 ***	89.5	5.0 ****	4.1 ***	10.7 *	0	0	0	19.4	39.2	11.2 **	45.6	2.6 **	38.5
95	*Ruta graveolens*	Rutaceae	L	3.6 ****	14.5 *****	4	2.5	42.9 *****	10.7 **	39.3 **	10.7	14.0 **	18.7 **	15.2 *	23.0 **	0.5 **	3.1 ***
96	*Salvia nemorosa*	Lamiaceae	Ap	67.9	100	4.1 **	3.2	0	0	7.2	0	11.7 **	18.7 **	0.3 ***	11.7 ***	3.2 **	14.4 **
97	*Salvia officinalis*	Lamiaceae	Ap	96.4	100	4.2 **	3.4 *	0	0	0	0	5.6 ***	14.3 ***	0.5 **	6.7 ***	2.4 **	9.6 ***
98	*Salvia syriaca*	Lamiaceae	Ap	67.9	78.5	4.5 ***	3	3.6	0	3.6	3.6	26	40.2	44.6	65.9	25.4	44
99	*Santolina chamaecyparissus*	Asteraceae	L,F	92.9	100	3	2.4	0	0	3.6	0	24.8	31.2 *	27	38.8	24.3	35.8
100	*Syzygium aromaticum*	Myrtaceae	F	46.4 *	93	2.1	2.4	0	3.6	21.5	0	10.6 **	20.9 **	16.0 *	29.5 **	6.5 **	24.3 *
101	*Tanacetum balsamita*	Asteraceae	L,F	3.6 ****	100	5	3.3	0	0	25.0 *	0	3.7 ***	11.0 ***	0.0 ***	10.2 ***	0.1 **	10.5 ***
102	*Thuja occidentalis*	Cupressaceae	L	71.4	92.8	4.7 ****	4.0 ***	0	0	3.6	3.6	14.3 **	24.7 **	3.1 **	26.2 **	5.3 **	23.8 **
103	*Thuja orientalis*	Cupressaceae	L	96.4	89.3	3.1	3.2	3.6	0	0	3.6	21.1	32.3	16.4 *	30.5 *	17.6	27.9 *
104	*Thymus daenensis*	Lamiaceae	L	0.0 ****	0.0 *****			0	0	100 *****	100 *****	0.8 ***	5.8 ****	0.0 ***	0.5 ****	0.0 **	0.0 ***
105	*Thymus transcaspicus*	Lamiaceae	L	0.0 ****	10.5 *****		5.0 *****	0	3.6	96.4 *****	57.1 *****	13.5 **	22.5 **	15.4 *	26.7 **	0.0 **	2.7 ***
106	*Trachyspermum ammi*	Apiaceae	Fr	3.6 ****	10.5 *****	4	3.7 **	67.8 *****	39.3 *****	14.3	28.6 ***	13.5 **	35.8	13.6 *	49.6	0.5 **	4.7 ***
107	*Vitex agnus castus*	Verbenaceae	Fr	96.4	89.5	2.6	2.4	0	0	3.6	0	29.7	45.3	50.5	49.9	40.9	42.9
108	*Xanthium strumarium*	Asteraceae	L	64.3	96.5	4.0 **	3.3 *	0	0	0	0	24.1	44.4	13.3 **	43.5	11.2 *	42.3
109	*Zataria multiflora*	Lamiaceae	L,F	0.0 ****	39.5 ***		4.0 ***	0	0	100 *****	53.6 *****	6.7 **	11.1 ***	3.9 **	8.2 ***	0.0 **	3.7 ***
110	*Zingiber officinale*	Zingiberaceae	Rh	64.3	100	3.5	2.8	7.2	0	3.6	0	19.4	25.7 **	17.2 *	30.6 *	11.6 *	28.7 *
111	*Ziziphora clinopodioides*	Lamiaceae	L	7.2 ***	93	4.5	3.8 **	28.6 ****	3.6	10.7	0	11.0 **	28.7 *	8.2 **	40.5	0.7 **	33.4
112	*Ziziphora tenuior*	Lamiaceae	L	0.0 ****	10.8 *****		3.5	71.4 *****	67. 8 *****	28. 6 *	7.2	9.8 **	19.8 **	14.5 *	21.9 **	0.0 **	2.3 ***

[a]: Part of use for EO extraction: Ap (Aerial part), C (Corm), F (Flower), Fr (Fruit), Fp (Fruit peel), L (Leaf), S (Stem), O (Oleogum), Rh (Rhizome), R (Root); [b]: The abbreviations of variables: G% (Germination percentage), MGT (Mean germination time), D% (Seed Dormancy Induction) L% (Lethal percentage of seed embryo), H% (Hypocotyl length compare to control), R% (Radicle length compare to control), VI (Vigor Index); *: The severity of the inhibitory effect on traits was defined by deviation value at five levels: (*****) Mean ± 2sd, (****) Mean ± 1.5 sd, (***) Mean ± 1 sd, (**) Mean ± 0.5sd, and (*) Mean ± 0.25 sd.

3.1.1. Germination Percentage (G%)

Most EOs reduced G% and some had no effect on it. In 1 µL, 36 EOs were ineffective on this trait and 76 EOs decreased the index compared to the control. At this amount, the highest degree of reduction (less than 22% compared to control) was observed in 12 EOs of Lamiaceae (*Melissa officinalis*, *Thymus daenensis*, *Thymus transcaspicus*, *Origanum vulgare* leaf, *Ziziphora tenuior*, *Mentha suaveolens*), Geraniaceae (*Pelargonium graveolens* leaf and flower), Apiaceae (*Pimpinella anisum* and *Trachyspermum ammi*), Liliaceae (*Allium hirtifolium*) and Rutaceae (*Ruta graveolens*). The inhibitory effects were greater in 3 µL, 89 EOs reduced this trait and 23 EOs were ineffective compared to control. At this amount, the highest degree of reduction (less than 5% compared to control) was observed in 21 EOs of Lamiaceae (*Dracocephalum moldavica* and *Zataria multiflora* in addition to six EOs that were effective at 1 µL), Geraniaceae (as same as the EOs that were effective at 1 µL), Apiaceae (*Cuminum cyminum*, *Bunium persicum*, and *Ferula foetida* in addition to two EOs that were effective at 1 µL), Liliaceae (*A. hirtifolium*) and Rutaceae (*R. graveolens* and *Citrus aurantifolia* leaf), Asteraceae (*Artemisia sieberi* and *Tanacetum balsamita*), and Zingiberaceae (*Amomum subulatum*).

3.1.2. Mean Germination Time (MGT)

The EOs also had a significant effect on MGT. The values of this trait were 1.7 to 5 days in 1 µL, 2 to 5 days in 3 µL, and 2.1 days in control. In 1 µL, 95 EOs increased and four EOs decreased this trait compared to control and the rest had no effect. At this amount, the highest increase (more than 224% compared to control) belonged to four EOs of Lamiaceae (*T. transcaspicus* and *D. moldavica*), Asteraceae (*A. sieberi*), and Zingiberaceae (*A. subulatum*). At 3 µL, 70 EOs increased and 40 EOs reduced MGT compared to control. At this amount, the highest degree of increase (more than 204% compared to control) belonged to nine EOs of Lamiaceae (*Rosmarinus officinalis* leaf and flower, and *Perovskia abrotanoides*), Asteraceae (*A. wilhelmsii, Artemisia aucheri*, and *Artemisia scoparia*), Cupressaceae (*Chamaecyparis lawsoniana* and *Thuja occidentalis*), and Apiaceae (*Apium graveolens*).

3.1.3. Lethal Percentage of Seed Embryo (L%)

The lethal percentage of seed embryo (L%) was also affected by EO treatment. At 1 µL, 32 EOs showed lethal effects. The highest lethality (over 45%) belonged to six EOs of Lamiaceae (*O. vulgare* leaf, *T. daenensis*, *Z. multiflora*, and *T. transcaspicus*), Apiaceae (*P. anisum*), and Liliaceae (*A. hirtifolium*). Only *T. daenensis* and *P. anisum* EOs were 100% lethal. At 3 µL, 51 EOs showed lethal effects. The highest lethality (over 78%) belonged to the 13 EOs of the families Lamiaceae (*D. moldavica, M. officinalis*, and *M. suaveolens* in addition to four EOs that were effective at 1 µL), Apiaceae (*C. cyminum*, *F. foetida*, and *P. anisum*), Liliaceae (*A. hirtifolium*), Geraniaceae (*P. graveolens* leaf and flower), and Zingiberaceae (*A. subulatum*). In this amount EOs of *F. foetida*, *P. anisum*, *M. officinalis*, *O. vulgare* leaf, *T. daenensis*, *Z. multiflora,* and *A. hirtifolium* caused 100% lethality.

3.1.4. Seed Dormancy Induction (D%)

Some of the EOs in this experiment induced seed dormancy. The D% in the amount of 1 µL was 0–67.8% and in 3 µL was 0–71.4%. In 1 µL, 25 EOs induced seed dormancy. The highest degree of seed dormancy induction (more than 22%) belonged to four EOs of Lamiaceae (*Z. tenuior*), Apiaceae (*T. ammi*) and Geraniaceae (*P. graveolens* leaf and flower). In 3 µL, 35 EOs showed dormant effects on seed. The highest degree of dormancy induction of seed (over 30%) belonged to five EOs of Lamiaceae (*Z. tenuior* and *Nepeta cataria*), Apiaceae (*T. ammi* and *Anethum graveolens*), and Rutaceae (*R. graveolens*).

3.2. Allelopathic Effects of Essential Oils on Lettuce Seedling Growth

Seedlings growth were also significantly affected by treatments (Table 1).

3.2.1. Hypocotyl Growth (H%)

All EOs decreased hypocotyl length compare to control (H%). Hypocotyl length was 16.6 mm in control, 0.8–15.7 mm in 1 µL, and 0.1–14.1 mm in 3 µL. At 1 µL the highest degree of reduction (less than 8% compared to control) was observed in four EOs of Lamiaceae (*O. vulgare* flower, *P. abrotanoides* flower, and *T. daenensis*) and Apiaceae (*P. anisum*). At 3 µL, the highest degree of reduction (less than 6% compared to control) was observed in 11 EOs from Lamiaceae (*D. moldavica*, *Lavandula angustifolia* flower, *O. vulgare* flower and leaf, *T. daenensis*, and *Salvia officinalis*), Apiaceae (*P. anisum* and *A. graveolens*), Zingiberaceae (*A. subulatum*), and Asteraceae (*T. balsamita* and *A. ludoviciana*).

3.2.2. Radicle Growth (R%)

Radicle length compare to control (R%) was also affected by different amounts of the EOs. The radicle length was 26.5 mm in control, 0.1–28.3 mm in 1 µL, and 0–24.9 mm in 3 µL. The highest radicle inhibitory degree (less than 3.4% compared to control) in 1 µL was observed in two EOs of Lamiaceae (*O. vulgare* flower and *T. daenensis*). The only EO that increased radicle growth at 1 µL was coffee EO, which increased 6.9% this trait compared to control. At 3 µL, the highest radicle growth inhibition (less than 0.5% compared to control) belonged to eight EOs of Lamiaceae (*L. angustifolia* flower, *O. vulgare* flower and leaf, *Salvia nemorosa*, and *T. daenensis*) and Asteraceae (*T. balsamita*, *A. aucheri*, and *A. deserti*). In this amount of EOs *T. balsamita*, *A. aucheri*, and *A. deserti* and *T. daenensis* were the main radicle growth inhibitors of 100%.

3.2.3. Vigor Index (VI)

Vigor index (VI) was also significantly affected by essential oil treatment. The index was 4300 in control, 0–0.4399 in 1 µL, and 0–3487 in 3 µL. At 1 µL the highest degree of reduction (less than 12% compared to control) was observed in 25 EOs of Lamiaceae (*D. moldavica*, *L. angustifolia*, *O. vulgare* flower and leaf, *M. officinalis*, *M. suaveolens*, *P. abrotanoides* flower, *S. officinalis*, *T. transcaspicus*, *T. daenensis*, *Z. multiflora*, and *Z. tenuior*), Asteraceae (*A. ludoviciana, A. vulgaris* flower, *A. sieberi*, and *T. balsamita*), Apiaceae (*B. persicum*, *T. ammi*, and *P. anisum*), Geraniaceae (*P. graveolens* flower and leaf), Liliaceae (*A. hirtifolium*), Zingiberaceae (*A. subulatum*), Rutaceae (*R. graveolens* and *C. aurantifolia* leaf). At 3 µL the highest degree of reduction (less than 10% compared to control) was observed in 50 EOs of Lamiaceae (*Mentha pulegium*, *N. cataria*, *N. binaludensis*, *Mentha longifolia*, *Mentha piperita*, *R. officinalis* flower and leaf, *Ziziphora clinopodioides*, *S. nemorosa* in addition to those were effective in 1 µL), Asteraceae (*Achillea filipendulina*, *Achillea wilhelmsii*, *A. aucheri*, *A. deserti*, *A. scoparia*, *Chrysanthemum morifolium*, in addition to those were effective in 1 µL), Apiaceae (*A. graveolens*, *Coriandrum sativum*, *C. cyminum*, *F. foetida*, *Foeniculum vulgare* fruit, *Heracleum persicum*, in addition to those were effective in 1 µL), Geraniaceae (*P. graveolens* flower and leaf), Liliaceae (*A. hirtifolium*), Zingiberaceae (*A. subulatum*), Rutaceae (*R. graveolens* and *C. aurantifolia* leaf), Cupressaceae (*Thuja occidentalis*), Myrtaceae (*Syzygium aromaticum*), and Verbenaceae (*Lippia citriodora*).

3.3. Headspace Gas Chromatography-Mass Spectrometry (HS-GC-MS)

Headspace analysis was performed for 16 EOs that inhibit seed germination and seedling growth (Table 2). The results showed that alpha and beta pinene and limonene were among the most common constituents in essential oils, which were observed in seven EOs (alpha pinene = 11–47%, beta pinene = 7–24%, limonene = 11–48%). Eucalyptol was the most volatile compound in four EOs (15–39%). Thujone, camphor, camphene, and borneol were the two most volatile constituents of the two EOs (21–23%, 11–33%, 11–23%, and 30–40%, respectively). Other compounds found in headspace analysis were found in only one EO, but some were highly potent, such as carvacrol, which was 91% of the volatile compounds in *T. daenensis* EO.

Table 2. The main components of EOs based on Headspace Gas Chromatography-Mass Spectrometry (HS-GC-MS) analysis.

Plant Scientific Name	Part of Use [a]	Main Components of EOs Based on HS-GC-MS			
Amomum subulatum	Fr	Dihydrocarveol (32.1%)	β-Pinene (23.1%)	α-Pinene (18.4%)	
Anethum graveolens	Fr	1,2-Diisopropenylcyclobutane (74.7%)			
Artemisia ludoviciana	L	Borneol (44.2%)	Camphor (33.3%)	Eucalyptol (22.4%)	
Artemisia vulgaris	L,F	D-Limonene (33.7%)	Thujone (22.5%)	β-Pinene (14.9%)	
Citrus aurantifolia	L	β-Pinene (20.8%)	Linalyl anthranilate (20.8%)	Linalol (16.2%)	Limonene (11.2%)
Dracocephalum moldavica	Ap	Orthodene (32.6%)	Limonene (20.3%)	α-Pinene (17.4%)	4-Carene (11.3%)
Mentha suaveolens	L	Limonene (47.9%)	α-Pinene (16.7%)	β-Pinene (14.0%)	
Origanum vulgare subsp. vulgare	L	Carvacrol (44.4%)	Thymol (23.0%)	o-Cymene (18.8%)	
Perovskia abrotanoides	F	Borneol (30.1%)	Eucalyptol (21.9%)	Camphor (11.1%)	
Pimpinella anisum	Fr	Trans- anethole (93.3%)			
Ruta graveolens	L	β-Terpinyl acetate (40.8%)	β-Pinene (16.5%)	α-Pinene (10.7%)	
Tanacetum balsamita	L,F	Limonene (40.2%)	Thujone (21.0%)		
Thymus daenensis	L	Carvacrol (90.9%)			
Thymus transcaspicus	L	Camphene (23.0%)	α-Pinene (11.0%)		
Zataria multiflora	L,F	o-Cymene (22.7%)	α-Pinene (22.1%)	Thymol (20.4%)	
Ziziphora tenuior	L	β-Pinene (24.2%)	α-Pinene (21.4%)	Limonene (20.6%)	

[a]: Part of use for EO extraction: Ap (Aerial part), C (Corm), F (Flower), Fr (Fruit), Fp (Fruit peel), L (Leaf), S (Stem), O (Oleogum), Rh (Rhizome), R (Root)

3.4. Determination of Effective Concentration of EOs

Determination of effective concentration of EOs on germination inhibitory (IC_{50}) and Lethality (LC_{50}) was performed in 16 EOs including *P. graveolens* flower and leaf, *A. subulatum*, *C. aurantifolia* leaf, *R. graveolens*, *F. foetida*, *T. ammi*, *P. anisum*, *D. moldavica*, *O. vulgare* leaf, *M. officinalis*, *M. suaveolens*, *T. transcaspicus*, *T. daenensis*, *Z. multiflora*, and *Z. tenuior*. According to the results (Table 3 and Figure 4), *T. daenensis* EO had the lowest IC_{50} (IC_{50} = 2.9 ppm), indicating that this EO has the highest allelopathic potential in this regard. Thereafter, the lowest effective concentrations were related to *P. anisum*, *Z. multiflora*, *O. vulgare* and *Z. tenuior* EOs (IC_{50} = 7.5 ppm, IC_{50} = 7.9 ppm, IC_{50} = 11.4 ppm and IC_{50} = 13.7 ppm, respectively). *T. daenensis* EO was the most lethal EO with the lowest lethal concentration (LC_{50} = 7.2 ppm). Subsequently, the EOs of *O. vulgare*, *Z. multiflora*, *P. anisum*, *T. transcaspicus* had the lowest lethal concentrations (LC_{50} = 16.2 ppm, LC_{50} = 21.6 ppm, LC_{50} = 22.2 ppm, and LC_{50} = 23.3 ppm, respectively).

Determination of effective concentration of EOs on hypocotyl inhibition (HIC), radicle inhibition (RIC), and seedling inhibition (SIC) was performed in 20 EOs including *P. graveolens* flower and leaf, *A. hirtifolium*, *A. subulatum*, *A. graveolens*, *P. anisum*, *A. aucheri*, *A. ludoviciana*, *A. sieberi*, *T. balsamita*, *L. angustifolia* flower, *M. suaveolens*, *O. vulgare* leaf and flower, *P. abrotanoides* flower, *R. officinalis* leaf, *S. nemorosa*, *S. officinalis*, *Z. multiflora*, and *Z. tenuior* was done on radicle (Table 4 and Figure 5). Results showed the strongest EO with the least IC_{50} of hypocotyl, radicle, and seedling growth was *O. vulgare* flower EO (HIC_{50} = 12.9 ppm, RIC_{50} = 9.6 ppm, SIC_{50} = 10.5 ppm). Afterwards, *A. hirtifolium*, *L. angustifolia* flower, and *P. graveolnes* leaf and flower EOs were the most effective on hypocotyl inhibition (HIC_{50} = 18.1 ppm, HIC_{50} = 22.8 ppm, HIC_{50} = 27.8 ppm, and HIC_{50} = 30.1 ppm, respectively); *A. ludoviciana*, *P. abrotanoides* flower, *S. officinalis*, and *P. anisum* had the highest effective on radicle growth (RIC_{50} = 18.3 ppm, RIC_{50} = 19.1 ppm, RIC_{50} = 23.4 ppm, and RIC_{50} = 27.8 ppm, respectively); and *P. abrotanoides* flower, *A. ludoviciana*, *L. angustifolia* flower, and *S. officinalis* EOs

showed the most effect on seedling growth (SIC_{50} = 20.7 ppm, SIC_{50} = 24.9 ppm, SIC_{50} = 28.7 ppm, and SIC_{50} = 29.1 ppm, respectively).

Table 3. The effective concentration of EOs on seed germination inhibition and embryo lethal by cotton swab method.

Plant Scientific Name	Part of Use [a]	Seed Germination Inhibition			Embryo Lethal Effect		
		IC_{25}	IC_{50}	IC_{90}	LC_{25}	LC_{50}	LC_{90}
		ppm					
Amomum subulatum	Fr	33.1	50.0	114	115	137	196
Citrus aurantifolia	L	9.0	20.9	114	23.2	64.9	510
Dracocephalum moldavica	Ap	13.3	21.0	52.3	69.4	126	414
Ferula foetida	O	88.6	115	193.0	119	138	183
Melissa officinalis	L	36.3	48.0	83.8	64.9	102	248
Mentha suaveolens	L	22.3	32.0	66.0	57.6	93.2	244
Origanum vulgare subsp. vulgare	L	8.0	11.4	23.4	12.2	16.2	28.5
Pelargonium graveolnes	L	15.8	26.3	73.1	34.8	64.9	226
Pelargonium graveolnes	L,F	20.8	30.0	63.0	66.7	116	351
Pimpinella anisum	Fr	7.0	7.5	8.5	9.4	22.9	136
Ruta graveolens	L	31.8	45.6	93.7	116	340	2916
Thymus daenensis	L	1.8	2.9	8.0	3.8	7.2	25.3
Thymus transcaspicus	L	12.9	17.3	30.8	18.3	23.1	36.7
Trachyspermum ammi	Fr	18.0	25.0	48.3	52.6	153	1294
Zataria multiflora	L,F	5.1	7.9	18.4	6.9	21.6	213
Ziziphora tenuior	L	8.6	13.7	35.4	30.3	49.2	130

[a]: Part of use for EO extraction: Ap (Aerial part), C (Corm), F (Flower), Fr (Fruit), Fp (Fruit peel), L (Leaf), S (Stem), O (Oleogum), Rh (Rhizome), R (Root)

Table 4. The effective concentration of EOs on radicle, hypocotyl, and seedling inhibition by cotton swab method.

Plant Scientific Name	Part of Use [a]	Hypocotyl Inhibition			Radicle Inhibition			Seedling Inhibition		
		IC_{25}	IC_{50}	IC_{90}	IC_{25}	IC_{50}	IC_{90}	IC_{25}	IC_{50}	IC_{90}
		ppm								
Allium hirtifolium	C	3.4	18.1	512	34.9	62.2	197	18.9	46.2	278
Amomum subulatum	Fr	44.6	69.3	168	50.8	70.2	134	48.8	70.7	148
Anethum graveolens	Fr	20.6	46.7	238	30.4	64.4	289	24.8	56.2	287
Artemisia aucheri	L	39.7	79.7	321	63.8	73.0	95.6	50.8	72.3	147
Artemisia ludoviciana	L	15.4	37.8	227	10.5	18.3	54.8	11.6	24.9	116
Artemisia vulgaris	L, F	15.2	59.1	899	33.5	79.4	448	24.3	71.3	612
Artemisia sieberi	L,S	11.3	38.4	446	10.5	28.4	209	10.8	31.6	270
Lavandula angustifolia	F	9.9	27.8	221	17.6	29.3	80.5	14.5	28.7	113
Mentha suaveolens	L	16.0	31.5	122	19.0	32.8	97.0	17.9	32.2	105
Origanum vulgare subsp. viridi	F	5.4	12.9	73.9	6.2	9.6	23.2	6.0	10.5	31.6
Origanum vulgare subsp. vulgar	L	10.3	36.3	450	18.2	45.0	276	15.2	42.1	322
Pelargonium graveolnes	L,F	7.4	30.1	503	9.8	54.5	1693	8.3	42.2	1093
Perovskia abrotanoides	F	8.1	22.8	182	13.5	19.1	38.4	12.8	20.7	54.8
Pimpinella anisum	Fr	16.6	34.3	147	14.5	27.8	103	18.9	34.1	111
Rosmarinus officinalis	L	49.6	90.4	300	63.1	104	279	57.9	98.7	287
Salvia nemorosa	Ap	33.8	67.9	275	54.6	74.3	138	51.3	74.1	155
Salvia officinalis	Ap	16.1	44.6	342	10.2	23.4	124	11.2	29.1	196
Tanacetum balsamita	L,F	16.7	44.3	313	24.6	56.6	300	20.8	51.3	313
Thymus daenensis	L	15.1	30.7	127	25.1	45.9	153	22.1	40.9	140
Zataria multiflora	L,F	26.0	50.6	193	12.7	33.8	238	17.3	40.5	221

[a]: Part of use for EO extraction: Ap (Aerial part), C (Corm), F (Flower), Fr (Fruit), Fp (Fruit peel), L (Leaf), S (Stem), O (Oleogum), Rh (Rhizome), R (Root).

3.5. Allelopathic Interaction Effect of EOs

Based on the results of screening experiments, the interaction effect on seed germination was evaluated in eight EOs. Also, this effect was studied on root and hypocotyl growth separately in six EOs.

3.5.1. Simplified Modified Dilution Check-Board Technique (SMCT)

Results of SMCT and combination effect (CE) showed different states of the synergistic and antagonistic effect. In the combination of essential oils regarding germination inhibition, most of the combinations resulted in antagonistic interaction (Table 5). The highest antagonistic effect was observed in the combination of *R. graveolens* + *D. moldavica* (CE = 0.56) followed by the *R. graveolens* + *T. transcaspicus,* and *C. aurantifolia* + *T. daenensis* (CE = 0.59 and CE = 0.66, respectively). The synergistic effect was observed only in five combinations, the strongest of which was the combination of *A. subulatum* + *M. suaveolens* (CE = 1.25). There were also different combination effects of essential oil on hypocotyl growth inhibition. Of the 15 compounds, five had additive interaction, 6 had synergistic effects, and 4 had antagonistic effects (Table 5). The most synergistic interaction was related to *P. abrotanoides* + *T. daenensis* (CE = 1.25), and the highest antagonistic effect was observed in *A. subulatum* + *T. balsamita* (CE = 0.72). The effects of essential oil combination on radicle growth inhibition were also different. Most of the combinations showed additive interaction. Among 15 combinations, 4 showed synergistic effect, seven showed additive and 4 other showed antagonistic interaction (Table 5). The highest synergistic effect was observed in *A. vulgaris* + *M. suaveolens* (CE = 1.22), and the strongest antagonistic interaction was in *A. subulatum* + *P. abrotanoides* (CE = 0.68).

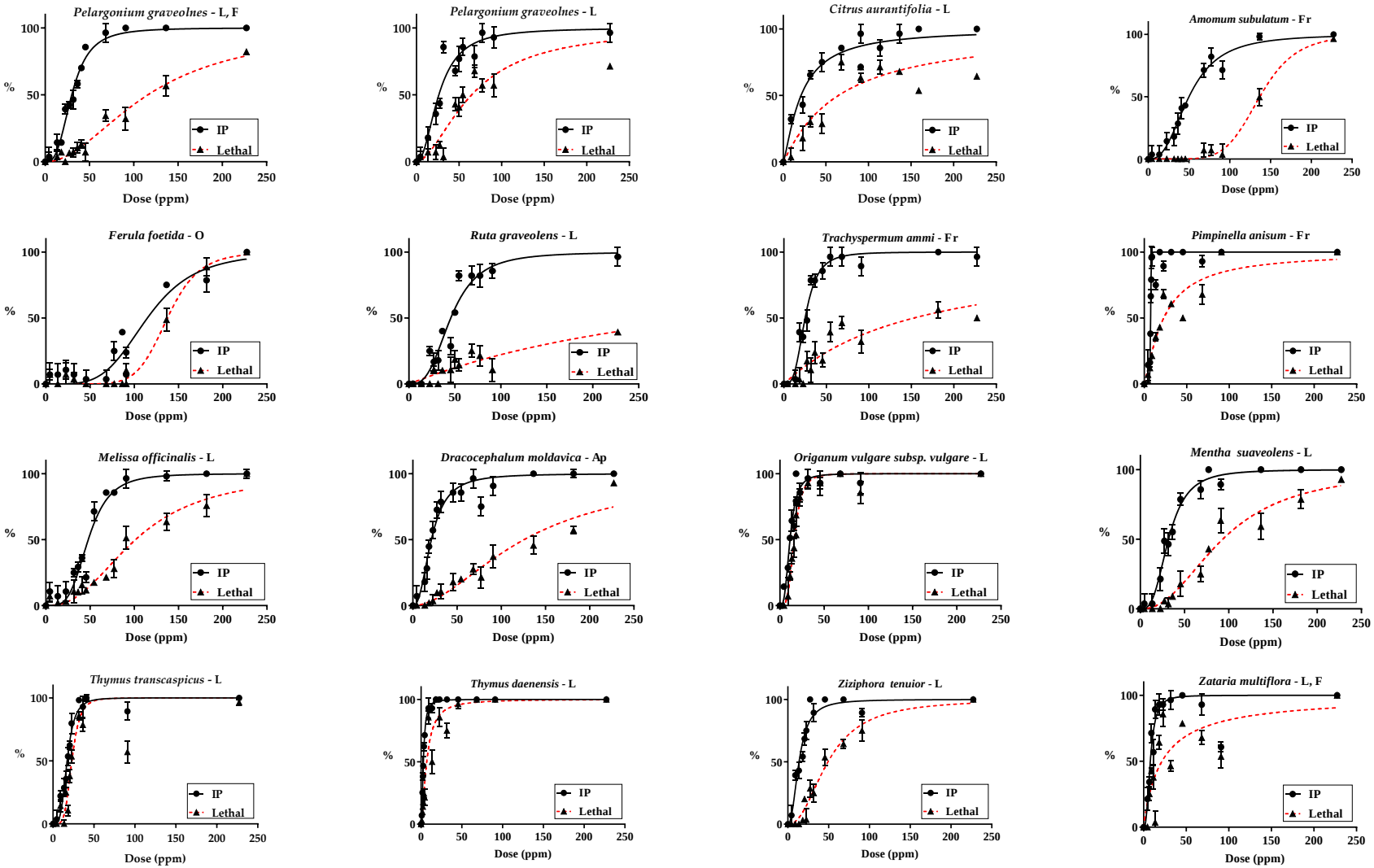

Figure 4. Dose–response diagrams of germination inhibition and embryo lethality of lettuce affected by 16 EOs in cotton swab method. IP: Inhibition Percentage, Lethal: Lethal percentage.

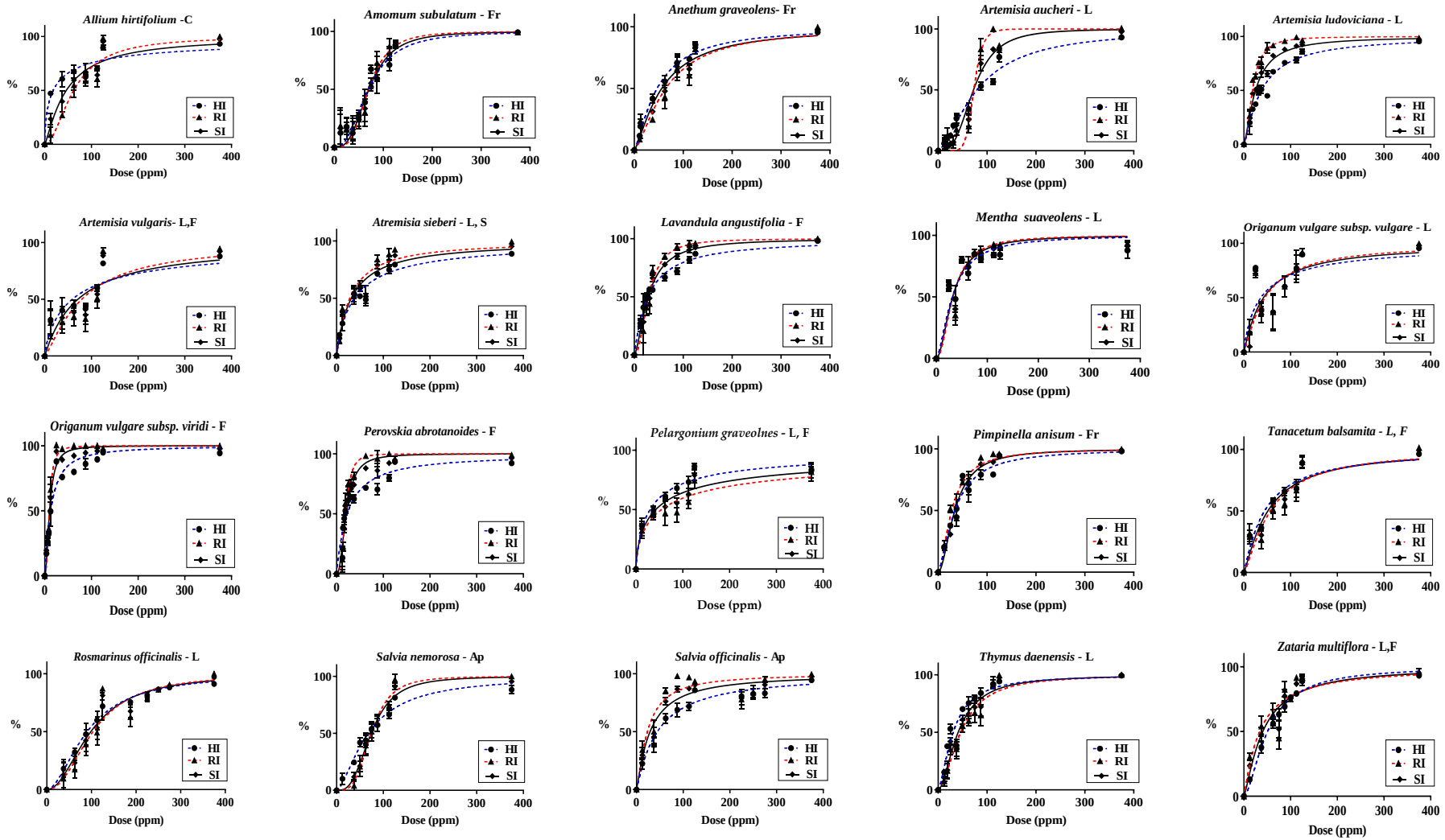

Figure 5. Dose–response diagrams of lettuce seedling growth affected by 20 EOs in cotton swab method. HI: Hypocotyl Inhibition, RI: Radicle Inhibition, SI: Seedling Inhibition.

Table 5. Allelopathic effects of essential oils mixtures on seed germination, hypocotyl and radicle growth of lettuce based on simplified modified dilution check-board technique (SMCT) by cotton swab method.

Germination Interaction	***Amomum subulatum* -(Fr)**	***Citrus aurantifolia* -(L)**	***Ruta graveolens* -(L)**	***Dracocephalum moldavica* -(Ap)**	***Mentha suaveolens* -(L)**	***Thymus daenensis* -(L)**	***Thymus transcaspicus* -(L)**	***Ziziphora tenuior* -(L)**
Amomum subulatum -(Fr) [a]								
Citrus aurantifolia -(L)	1.09							
Ruta graveolens- (L)	0.76 [A]*	0.84 [A]						
Dracocephalum moldavica -(Ap)	0.82 [A]	1.06	0.56 [A]					
Mentha suaveolens -(L)	1.25 [S]	0.85 [A]	1.15 [S]	0.96				
Thymus daenensis -(L)	0.84 [A]	0.66 [A]	0.81 [A]	1.11 [S]	0.86 [A]			
Thymus transcaspicus -(L)	0.82 [A]	0.72 [A]	0.59 [A]	0.84 [A]	1.09	0.97		
Ziziphora tenuior -(L)	0.88 [A]	1.14 [S]	1.02	0.79 [A]	1.19 [S]	0.94	0.80 [A]	

Hypocotyl Growth Interaction	***Amomum subulatum* -(Fr)**	***Anethum graveolens* -(Fr)**	***Artemisia ludoviciana* -(L)**	***Tanacetum balsamita* -(L,F)**	***Perovskia abrotanoides* -(F)**	***Thymus daenensis* -(L)**
Amomum subulatum -(Fr)						
Anethum graveolens -(Fr)	0.89 [A]*					
Artemisia ludoviciana -(L)	0.79 [A]	1.07				
Tanacetum balsamita -(L,F)	0.72 [A]	0.78 [A]	0.94			
Perovskia abrotanoides -(F)	0.96	1.19 [S]	1.31 [S]	1.17 [S]		
Thymus daenensis -(L)	1.00	1.02	1.27 [S]	1.13 [S]	1.25 [S]	

Radicle Growth Interaction	***Amomum subulatum* -(Fr)**	***Artemisia ludoviciana* -(L)**	***Artemisia vulgaris* -(L,F)**	***Mentha suaveolens* -(L)**	***Perovskia abrotanoides* -(F)**	***Thymus daenensis* -(L)**
Amomum subulatum -(Fr)						
Artemisia ludoviciana -(L)	0.84 [A]*					
Artemisia vulgaris -(L,F)	0.94	1.01				
Mentha suaveolens -(L)	0.98	1.17 [S]	1.22 [S]			
Perovskia abrotanoides -(F)	0.68 [A]	1.10	0.80 [A]	0.82 [A]		
Thymus daenensis -(L)	0.97	1.00	1.13 [S]	1.16 [S]	0.95	

[a]: Part of use for EO extraction: Ap (Aerial part), C (Corm), F (Flower), Fr (Fruit), Fp (Fruit peel), L (Leaf), S (Stem), O (Oleogum), Rh (Rhizome), R (Root); *: The letters on the numbers indicate the state of interaction between the essential oils: S: synergistic effect (CE > 1.1), [A]: antagonistic effect (CE < 0.9).

3.5.2. Isobologram Curves

Isobologram curves showed different type of allelopathic interaction of EOs and they appropriately confirm the SMCT result (Figures S1–S3). In the isobologram curves of germination inhibition (Figure S1), eight EOs showed synergistic effects, 16 EOs had antagonistic effects and four others showed additive effects. Among the EOs, *M. suaveolens* had the most synergistic effect with other EOs (four synergistic effects), while *A. subulatum*, *R. graveolens* and *T. transcaspicus* EOs had the most antagonistic effects with the other EOs (five antagonistic effects). In the hypocotyl inhibitory isobologram (Figure S2), four EOs indicating antagonistic status, seven curves indicating synergistic status between EOs, and four other curves indicating additive effects. *A. subulatum* showed the highest number of antagonistic effects with the other EOs (three antagonistic effects), so that black cardamom did not have synergistic effect with any other EOs. *P. abrotanoides* and *T. daenensis* had the highest number of synergistic effects in combination with other EOs (four and three synergistic effects, respectively), so that they showed no antagonistic effect with any other EOs. And finally, in the isobologram curves of radicle growth inhibition (Figure S3) four curves showed antagonistic effects between EOs, five curves indicating synergistic effects and six other curves indicating additive effects. The highest number of antagonistic effects was related to *P. abrotanoides* EO (three antagonistic effects) and *M. suaveolens* EO had the highest number of synergistic effects with other EOs (three synergistic effects). In this regard, black cardamom EO had no synergistic effect, and *T. daenensis* EO showed no antagonistic effect.

4. Discussion

The results of this experiment confirm, in many cases, other researchers' findings on the allelopathic effects of EOs and their compounds on seed germination. Monoterpenes can affect the germination of seeds at very low concentrations [25]. The EOs of *T. daenensis* and *P. anisum* were among the strongest germination inhibitors in this experiment. The potent microbicidal effects of these species, as well as their antimicrobial, inhibitory or stimulatory effect on enzymatic activity, toxic effects of estragol have been reported in previous studies [26–29]. Phytotoxic effects of *T. daenensis* EO have been reported in other allelopathic studies. The germination of some weed species has been showed to be strongly inhibited by this species EO [30], so that complete germination inhibition of *Avena fatua* occurred at 600 µL/L and complete germination inhibition of *Amaranthus retroflexus* and *Datura stramonium* occurred at 800 µL/L. Antibacterial effects on *E. coli* using this EO, have also been reported [28], achieving complete bacterial death within five minutes. These results seem to be caused by thymol, carvacrol, and carvone which are the compounds of *T. daenensis* EO that showed highest inhibitory effect on seed germination [31].

The insecticidal effects of *P. anisum* and *P. graveolens*, especially on larvae killing and oviposition-deterrent, are already known [32,33], as well as their fumigant-pesticide effect [34,35]. In our experiment, these species' EOs showed strong inhibitory and phytotoxic effects. According to Dhima et al. [36], *P. anisum* had significant inhibitory effect on germination and seedling of *Echinochloa crus-galli*, although Azirak and Karaman [31] observed effectiveness against seed germination only at high concentration, and not at low ones. Trans-anethole, the dominant constituent of *P. anisum* EO, is a type of phenylpropanoids that is toxic for some insects [37]. Since this compound was very high in headspace analysis of *P. anisum* EO (90%), it is most likely to be responsible for inhibition of the seed germination. Despite this, in another study, trans-anethole had no significant effect on seed germination inhibition [38]. The differences in the results may be, in part, due to the use of different plant species in the tests: and it should be noted that lettuce seed is more sensitive to allelochemicals. The way EO was applied in each study, fumigation in ours *vs* soaking in the others, might have also explain the differences in the results. Concerning the allelopathic effects of *O. vulgare*, its EO has potent antifungal effects on a wide range of fungi that have been attributed to thymol and carvacrol, among others [39]. These compounds also had germination inhibitory effects on some plant species, whereas p-cymene showed no phytotoxic effect [40]. But Elshafie et al. [41] saw moderate phytotoxic effects of *O. vulgare* EO. According to other studies, *Z. multiflora* significantly reduced germination rate,

seedling length, root and shoot weight [42]. Allelopathic effects of leaves of this species on sandwich method have also been reported [43]. The antifungal effects of *Z. multiflora* EO were also investigated and showed complete inhibition of fungi growth [44]. Allelopathic studies of other researchers have reported phytotoxic effects of *P. abrotanoides* [11,43]. Its flower was one of the most effective inhibitors of lettuce seedling growth in sandwich method [43], and in dish pack method, it had an inhibitory effect of more than 80% on root growth, which, according to the experimental method, is due to its volatile compounds [11]. For other plants, many herbaceous species of *Artemisia* contain active compounds that have allelopathic and antifungal effects, and their allelochemicals are mainly volatile ethers and alkaloids. The allelopathic effect of *A. ludoviciana* showed inhibitory effects on plant growth [45]. The EO of this plant also had antibacterial effects and at a concentration of 10 µg/mL inhibited the growth of some bacteria [46].

Among major volatile compounds resulting from HS-GC-MS, alpha and beta pinene, which were dominant compound in most EOs, are oxygenated bicyclic monoterpenes, which are insoluble in water and highly volatile, and their allelopathic effects on the environment and insect repellent effects have been reported [47,48]. Other compounds, like limonene, an aliphatic and cyclic monoterpene, also present phytotoxic effects [49]. Eucalyptol or 1,8-cineole is an ether cyclic monoterpene that has insecticidal and insect repellent effects [50,51], as well as strong growth inhibitory effects on plants, affecting the activity of the asparagine synthase enzyme, thus being one of the most important allelochemical constituents [52]. The toxicity and lethal effects of eucalyptol on rats have been tested and, although it has a relatively high lethal dose (LD_{50} = 2.48 g/kg bw, rat), its long-term effects on living cells can be extremely damaging and should be carefully considered [53] before using it against weeds. Borneol is an alcoholic monoterpene with reports of its inhibitory effects on *Schizachyrium scoparium* germination (8%) and radicle growth (47%), whereas it did not affect *Leptochloa dubia* germination and seedling growth [54]. Except for *O. vulgare* and *P. abrotanoides*, which inhibited the growth of both radicle and hypocotyl, the other EOs did not have the same inhibitory effects on these two seedling parts. *Allium hirtifolium*, *L. angustifolia* and *P. graveolens* had the greatest effect on decreasing hypocotyl growth, while *A. ludoviciana*, *S. officinalis*, and *P. anisum* were more effective in inhibiting radicle growth. This difference is probably due to the different role of the EOs in disrupting hormonal balance, and the effect of their compounds on growth regulators biosynthetic pathways.

Another notable point in this experiment was the different effect of the EO of different parts of a plant. In 11 plants, the EOs were extracted from two different parts of them, which in some cases had different effects. About flowers and leaves EO, in *P. abrotanoides*, *A. vulgaris,* and *L. angustifolia*, the allopathic effect of flower EO was greater than that of leaf, in *O. vulgare* the effect of the leaf EO was more than the flower, and there was no difference in geranium and rosemary in this regard. On the effect of leaf and fruit EO on germination, in *C. aurantifolia*, *F. vulgare* and *Cupressus arizonica* the inhibitory effect of leaf oil was greater than that of fruit, and in *Juniperus horizontalis* there was no difference. Regarding their differences in inhibition of seedling growth, in *Juniperus horizontalis* and *C. arizonica* the inhibitory effect of leaf EO was greater than fruit, in *F. vulgare* the fruit EO effect was more than leaf and in *C. aurantifolia* there was no difference. Regarding the different effects of *Pinus eldarica* leaf EO with its oleogum EO, the former had more inhibitory effects on germination than the later.

In this study, synergistic, additive and even antagonistic effects between plant EOs were observed. Synergistic effects between the EOs of some species of Apiaceae family such as Coriander and Cumin have been reported for antibacterial and antioxidant activity [16]. In another study, antifungal synergistic effects were observed between the EO of marjoram and thyme, peppermint and tea tree, and thyme and cinnamon [55]. Antimicrobial synergistic effects between EO components such as eugenol and linalool, eugenol and menthol, and carvacrol and thymol have also been reported [18].

5. Conclusions

This study represents new results in the allelopathy database. The results showed significant inhibitory effects of essential oils even at 1 µL concentration on the studied traits in gaseous phase. *P. graveolens*, *P. anisum* and *T. daenensis* EOs had the greatest inhibitory effect on germination; *A. subulatum*, *A. sieberi*, *D. moldavica* and *T. transcaspicus* EOs had the greatest effect on germination delay; *P. anisum* and *T. daenensis* EOs had the greatest effect on seed embryo lethality. *P. anisum*, *O. vulgare*, *P. abrotanoides* and *T. daenensis* EOs caused the greatest inhibition of seedling growth. In addition, the results of allelopathic interaction of the EOs showed that there were different antagonistic, synergistic, and additive interaction in their combination. *M. suaveolens* in inhibition of germination and radicle growth, and *P. abrotanoides* in inhibition of hypocotyl growth, showed the highest synergistic ability in combination with other EOs. *A. subulatum*, *R. graveolens*, and *T. transcaspicus* EOs had the highest antagonistic ability in combination with others. The interaction effects between the EOs can be of great interest in plant-based herbicides production. Such effects have a particular role in determining the appropriate dose so that synergistic effects can more effectively prevent germination and weed growth and reduce herbicide consumption.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/2/163/s1, Figure S1: Isobologram curves of allelopathic interaction effects of EOs on lettuce seed germination inhibition by cotton swab method, Figure S2: Isobologram curves of allelopathic interaction effects of EOs on lettuce hypocotyl growth inhibition by cotton swab method, Figure S3: Isobologram curves of allelopathic interaction effects of EOs on lettuce radicle growth inhibition by cotton swab method, Table S1: Analysis of variance of variables studied in the experiment to investigate the effects of 112 essential oils concentrations on seed germination and seedling growth of lettuce.

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

Funding: This research received funding from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japan. This work was also partly supported by JST CREST Grant Number JPMJCR17O2 and JSPS KAKENHI Grant Number 26304024. "This work was also partly supported by Ferdowsi University of Mashhad under grant number: 38489."

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

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