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# Evaluation of Allelopathic Activity Interactions of Some Medicinal Plants Using Fractional Inhibitory Concentration and Isobologram

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**Abstract:** Allelopathy is a physiological process with an ecological concept and application. Allelopathy is the result of the production of biologically active molecules by growing plants or their remains, which may have a direct effect on the growth and development of individuals of the same species or other species after changing their shape and entering the environment. As regards, the use of natural compounds in the control of weeds and pests is a priority. In this research, the allelopathic activity of 123 specimens of medicinal and aromatic plants were investigated individually by the dish-pack method using lettuce seeds as a model. Then, the strongest inhibitory ones were selected and their allelopathic interaction effects were investigated for the first time by interacting them together. Two methods were used to evaluate allelopathic interaction effects: calculating Fractional Inhibitory Concentration (FIC) and drawing Isobologram diagrams. Lettuce hypocotyl length, root length, germination percentage, and germination rate were investigated. *Pelargonium graveolens* (leaf) had the greatest inhibitory effect on lettuce radicle growth ($EC_{50}$ = 5.31 mg/well) and *Echinophora platyloba* (stem) had the greatest effect on hypocotyl growth inhibition ($EC_{50}$ = 7.91 mg/well). Also, the lowest lettuce germination percentages were observed in the treatments *Lavandula officinalis* (flower) and *Nepeta binaloudensis* (leaf), respectively (23.61, 22.85%). The highest inhibitory effect by considering lettuce germination rate was detected in *Salvia ceratophylla* (leaf), (12.86 seed/day) and the lowest belonged to *Nepeta binaloudensis* (leaf) and *Lavandula officinalis* (flower), respectively (3.60, 3.32 seed/day). According to FIC calculations and isobolograms, two types of interaction, including synergist (*Nepeta binaloudensis* (leaf) with *Trachyspermum ammi* (fruit) and *Nepeta binaloudensis* (leaf) with *Lavandula officinalis* (flower) and antagonist (*Pelargonium graveolens* (leaf) with *Lavandula officinalis* (flower)), were observed significantly among the plants tested in this research. These interactions can be used to prepare more effective natural herbicides and decrease the use of herbicides.

**Keywords:** allelopathy; lettuce growth; $EC_{50}$; volatile compounds; organic culture; synergist; antagonist; bio-herbicide



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## 1. Introduction

Weeds and crops have growth interactions and cause high expenses for agricultural systems [1]. This played a significant role during the domestication of crops, so weed control measures are required [2]. In most integrated weed management systems, herbicides are widely used [3,4]. Over the last 40 to 50 years, with the commercial production of more than 200 chemical compounds, significant changes in weed control have begun to develop [5].

Medicinal, aromatic, and spice plants and mushrooms produce a wide range of secondary metabolites and the uses of these metabolites as agrochemicals for the control of

pests, diseases, and weeds have been well investigated [6–8]. Secondary metabolites are a plentiful source of the natural compounds that are produced in the special structure in medicinal and aromatic plants and their content and composition are affected by plant species, climate, cultural practices, and harvest time [9–11]. Some of the secondary metabolites have a good potential use in the development of natural herbicides [12]. They are more environmentally friendly than chemical pesticides and are economically viable and can be easily produced in small industries by farmers [13].

The allelopathic compounds are chemicals produced by some plants (especially medicinal and aromatic plants) that can affect the ecosystem in association with other compounds in collaboration with microorganisms [14–16]. In 1999, the International Allelopathy Society offered a precise definition of the allelopathy concept: allelopathy is a science that studies the production of secondary metabolites in plants, algae, bacteria, and fungi and examines their impact on growth in biological and agricultural systems [17].

At the beginning of recognition of plants with allelopathic compounds, due to the lack of rapid methods and knowledge of chemical constituents, it was difficult to describe this important phenomenon [18]. However, in recent years some methods, such as the dish pack method, have be used to detect the allelopathic properties very easily and quickly. In this method, many plant materials can be tested anywhere and in a short period [19].

Although many studies have been conducted on the interaction type (synergistic, antagonistic, and additive potentials) between antimicrobial compounds [20–23], research on the interaction of allelopathic plants or allelochemicals is very rare [6]. The synergistic interaction is a promising combination which could be used to overcome the resistance to herbicides, insecticides, and microbes. It also could decrease the used herbicide volume and increase the herbicidal efficiency and sustainability.

This research aims to evaluate the allelopathic interaction effects of some medicinal plants by the dish pack method by using seed germination and seedling growth of lettuce as a model plant for allelopathic activity.

## 2. Materials and Methods

### *2.1. Plant Materials*

Different parts (leaves, flowers, stems, fruits, roots, flowering branches, and seeds) of 123 specimens belonging to 31 families of volatile and medicinal species were collected from the Research Center for Plant Sciences of Ferdowsi University of Mashhad and Botanical Garden of Mashhad. The plant parts were dried in the oven at 45 °C for 2 days to keep the volatile compounds. After the appropriate drying, the parts were kept in a plastic bag until use.

The effect of the tested plants on radicle and hypocotyl growth of lettuce seeds in comparison with control in different plant families was investigated separately. In each plant family, a comparison was made at two probability levels ($p \leq 0.05$; $p \leq 0.01$).

They were subjected to the analysis of their allelopathic effects using the dish pack method (Figure 1) and the lettuce seeds (Great Lakes 366) were used as the test plant because of their good reliability in germination, sensitivity to inhibitory and stimulatory chemicals, and their convenience of purchase [6,7,19,24].

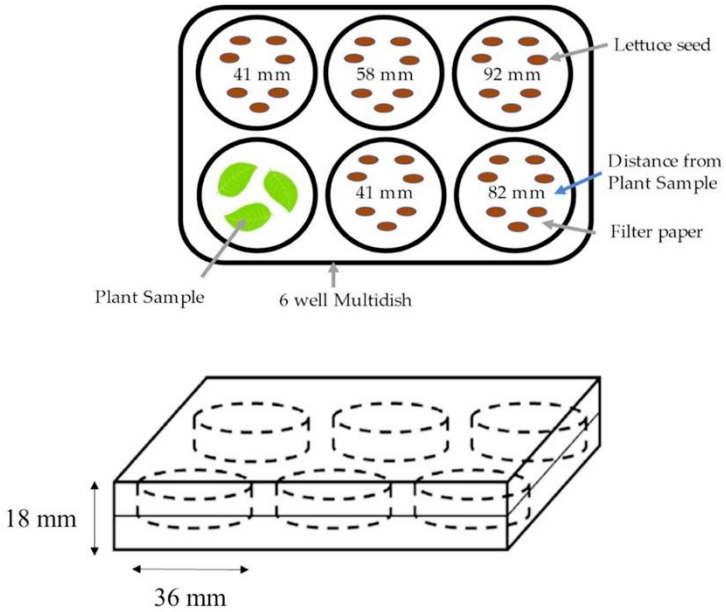



**Figure 1.** The views of multi-well dishes with six wells used in the dish pack method.

### *2.2. Assessment of Allelopathic Effect on Lettuce Seed and Germination Traits*

#### 2.2.1. Dish Pack Method

One of pathways to screen volatile compound secreted from plants is the dish pack method as described by Fujii et al. (2000) [19]. In this method, multi-dishes with six wells (18 mm × 36 mm) (Nunc Company, Tokyo, Japan) were used. The distances from the center of a plant sample well to the center of other wells were 41, 58, 82, and 92 mm, respectively, (Figure 1). Then, 200 mg of dried plant samples were placed in one well (source well), while in the rest of wells no plant sample was added. These wells contained a piece of filter paper that was soaked with 0.7 mL of distilled water. Thereafter, seven lettuce seeds were placed over the filter paper in each well. Five blank dishes were prepared as a control sample according to the above method except that the source well contained no plant. The dishes were sealed with parafilm tape to prevent volatile compound losses and desiccation. Then, they were wrapped in aluminum foil to prevent light penetration and incubated for 3 days at 25 °C [19].

#### 2.2.2. Germination and Seed Traits

The effects of the strongest inhibitory plants in the screening stage on the percentage and rate of germination were investigated. For this reason, the data were recorded every 12 h for a period of 3 days and calculated using the following formulas.

Germination percentage = (Total germinated seeds by the end of experiment/Total seeds) × 100 [25,26].

Germination Rate = (Number of germinated seeds per day/Number of days after planting) × 100 [27].

### *2.3. Assessment of Medicinal Plants' Allelopathic Effects on Lettuce Seedling Growth*

At the end of the incubation period (3 days), to evaluate the growth of hypocotyl and radicle of lettuce seeds, they were placed on checkered paper and photographed and measured by Image J (version 1.331, August 2022) and Excel software (Version 2210) (Figure 2a–c).

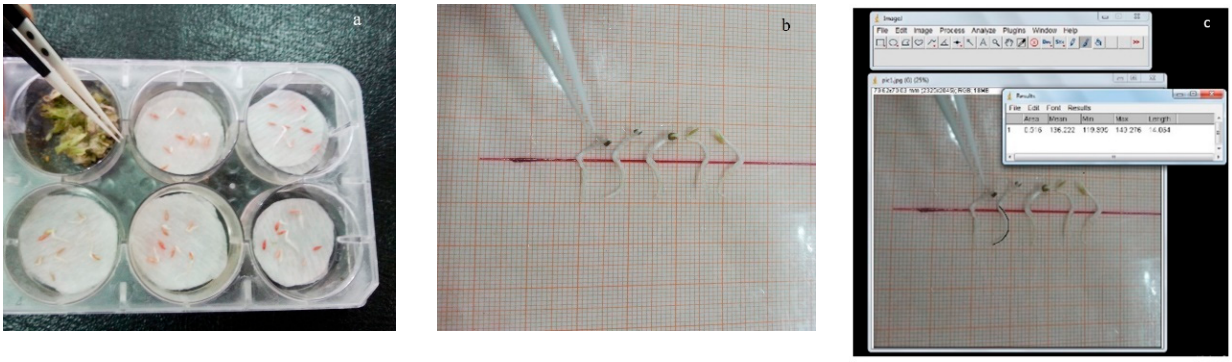


**Figure 2.** (**a**) Examination of germinated lettuce seeds after an incubation period, (**b**) Preparing germinated seeds on checkered paper, and (**c**) Measuring hypocotyl and radicle growth using Image J software.

### *2.4. Statistical Analysis*

The inhibition index of plants on lettuce seed germination factors (Criteria) was defined.

According to the results obtained from the average inhibition activity, the mean and standard deviation were calculated, and based on these, the basis of different groupings for the inhibition percentage of plants were defined in 4 levels: (****) Mean + 3 SD, (***) Mean + 2 SD, (**) Mean + 1 SD, (*) Mean < 1 SD. The highest inhibition percentage is related to the group (****), which indicates 3 times the difference with the standard deviation, (***) which indicates 2 times the difference with the standard deviation, (**) which indicates one times the difference with the standard deviation, and (*) which indicates less than 1 times the difference with the standard deviation.

The inhibition index of the samples was calculated and the means of three replications were analyzed statistically on the basis of RCD in each family. The means comparison was performed using Duncan's multiple-range test at 0.05 level of probability. Minitab (version 21.01.0), Graphpad Prism (Version 9.4.1), and Excel (Version 2210) were used for the statistical analysis and graphing. In addition, the variance analysis was performed separately for plants of each family.

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

Headspace GC-MS was performed to investigate the chemical composition of the samples' volatiles with the most allelopathic effects. For this purpose, 200 mg of solid plant samples were incubated in 20 mL glass vials and stored at 20 °C for one hour. Then, 1000 µL of air over each sample was removed by a 5 mL SGE 5MDR-HSV syringe and injected into the GC-MS (Shimadzu QP 2010, Tokyo, Japan). GC analysis was run on a (30 m × 250 µm × 0.25 µm) column with helium gas as the carrier gas. The temperature of the injection was as follows: the oven temperature was 50–150 °C, with an increase rate of 3 °C/min, kept in this mode for 10 min, then, reached a temperature of 200 °C at 10 °C/min. The components of volatile compounds were determined by the device library (NIST/NBS). Mass spectra were registered at 70 eV with a mass range of 50 to 400 $m/z$, in comparison to an internal spectral library (NIST and Wiley). They were then validated by comparing the retention times with the valid standards.

### *2.6. Allelopathic Interaction Effects*

After investigating the allelopathic effects of plants, the strongest inhibitory plants were identified. In order to investigate the allelopathic interaction of these plants, the effective concentration was determined first.

#### 2.6.1. Determination of $EC_{50}$ and $EC_{25}$

$EC_{50}$ is the concentration of the substance that causes 50% of the effect in a process. To evaluate $EC_{50}$, different concentrations (10, 50, 70, 100, 120, 150, and 200 mg) of each

allelopathic plant were examined using the dish pack method. The determination of these values for radical and hypocotyl resistance was performed separately. The results were analyzed by GraphPad Prism 8 software and finally $EC_{50}$ value was calculated. In addition, the values of $EC_{25}$ were calculated with Quick Calcs online software.

#### 2.6.2. Plants Combinations

The combined effects of these plants were performed based on the combination of $EC_{25}$ concentrations. The experiments were conducted to investigate the radicle and hypocotyl growth. Based on the FIC formula and isobologram curves, the mutual behaviors of two plants were investigated.

- Fractional inhibitory concentration (FIC) was calculated using the following equation:

FIC = obtained inhibitory effect in combining two plants expected inhibitory effect in combining two plants

The obtained inhibitory effect of combining two plants indicating the inhibition percentage that was obtained as a result of the combination two plants at a concentration of $EC_{25}$ in the test.

The expected inhibitory effect of combining two plants indicating the inhibition value of 25% in the concentration of $EC_{25}$ for each plant, which is naturally expected to be 50% in combination [28].

- Isobologram curves

To draw the isobologram curves, the inhibitory effect of two plants was considered as base, then the concentrations of each plant A and B that lead to similar inhibition were calculated separately using Quick Calcs software. Therefore, three concentrations (A alone, B alone, and A + B in combination) were used to draw graphs showing the same inhibition percentage in all three modes. This curve can be describe in three different situations: (1) without a curve indicating an additive effect; (2) with an upward curve, meaning an antagonistic effect; and (3) with a downward curve, meaning a synergistic effect (Figure 3) [6].

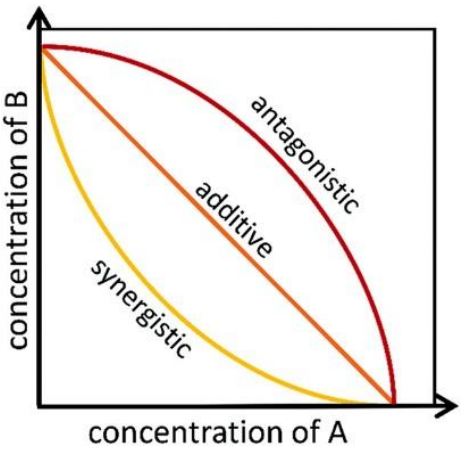



**Figure 3.** Schematic diagram of the isobologram curve to determine allelopathic interactions.

## 3. Results

### *3.1. Allelopathic Effects of Medicinal Plants on Lettuce Seed Growth and Germination Specifications*

The results of allelopathic effects of 123 samples selected from 31 plant families are shown in Table 1. In each of the samples, the part with evidence of the presence of the most volatile compounds was selected and analyzed at close distances (41 mm) and the total average (whole wells).

**Table 1.** The results of investigating the inhibitory power of medicinal plants on the growth of lettuce seeds.

| No | Family | Plant Scientific Name | Part Used | Inhibition Activity (%) | | | | Criteria |
|---|---|---|---|---|---|---|---|---|
| | | | | Average (41 mm) | | Average (Whole Wells) | | |
| | | | | Radicle | Hypocotyl | Radicle | Hypocotyl | |
| 1 | Amaranthaceae | *Amaranthus blitoides* | Flower | 30.12 | 36.08 | 30.70 | 40.00 | ** |
| 2 | | *Amaranthus hypochondriacus* | Leaf | 79.03 | 85.62 | 62.52 | 63.52 | *** |
| 3 | | *Atriplex halimus* | Leaf | 36.98 | 16.34 | 29.50 | 20.42 | ** |
| 4 | | *Kochia prostrata* | Leaf | 24.60 | 15.02 | 28.27 | 15.92 | * |
| 5 | Amaryllidaceae | *Allium sativum* | Leaf | 30.03 | 18.04 | 25.27 | 18.25 | * |
| 6 | | *Ungernia trisphaera* | Leaf | 9.72 | −6.32 | −21.64 | −39.28 | + |
| 7 | Apiaceae | *Conium maculatum* | Leaf | 82.57 | 85.93 | 66.70 | 73.57 | *** |
| 8 | | *Echinophora platyloba* | Stem | 100.00 | 100.00 | 84.62 | 82.85 | **** |
| 9 | | *Ferula szowitsiana* | Leaf | 26.94 | 29.22 | 5.57 | −6.90 | * |
| 10 | | *Ferula xylorhachis* | Leaf | 11.85 | 17.78 | −2.08 | 0.77 | + |
| 11 | | *Foeniculum vulgare* | Fruit | 87.03 | 85.23 | 79.91 | 76.73 | *** |
| 12 | | *Seseli transcaucasicum* | Flower | 12.47 | 35.98 | −8.70 | −6.82 | + |
| 13 | | *Seseli transcaucasicum* | Leaf | 37.94 | 38.03 | 1.61 | −22.83 | * |
| 14 | | *Seseli transcaucasicum* | Stem | 33.63 | 29.21 | −8.06 | −55.43 | + |
| 15 | | *Seseli transcaucasicum* | Root | 28.04 | 31.69 | 13.91 | 1.52 | * |
| 16 | | *Trachyspermum ammi* | Fruit | 99.41 | 100.00 | 80.20 | 82.73 | **** |
| 17 | Apocynaceae | *Vinca minor* | Leaf | 77.62 | 80.55 | 52.23 | 51.49 | ** |
| 18 | Asparagaceae | *Asparagus officinalis* | Leaf | 78.25 | 86.54 | 66.35 | 72.66 | *** |
| 19 | Asteraceae | *Achillea nobilis* | Flower | 94.56 | 97.10 | 82.39 | 79.58 | *** |
| 20 | | *Achillea filipendula* | Leaf | 55.23 | 66.35 | 50.82 | 60.69 | ** |
| 21 | | *Achillea biebersteinii* | Leaf | 90.05 | 88.37 | 85.72 | 83.40 | *** |
| 22 | | *Achillea wilhelmsii* | Flower | 87.98 | 91.68 | 62.13 | 62.93 | *** |
| 23 | | *Achillea millefolium* | Flower | 27.90 | 18.02 | 25.82 | 16.74 | * |
| 24 | | *Achillea pachycephala* | Leaf | 70.17 | 65.36 | 60.49 | 53.56 | *** |
| 25 | | *Artemisia absinthium* | Leaf | 39.75 | 37.50 | 40.47 | 50.54 | ** |
| 26 | | *Artemisia scoparia* | Leaf | 40.81 | 35.56 | 35.16 | 27.38 | ** |
| 27 | | *Artemisia tournefortiana* | Leaf | 11.36 | 16.12 | −3.64 | −13.16 | + |
| 28 | | *Calandula officinalis* | Leaf | 50.54 | 46.87 | 58.12 | 59.35 | ** |
| 29 | | *Centaurea behen* | Leaf | 82.11 | 83.86 | 71.31 | 70.17 | *** |
| 30 | | *Cousinia raddeana* | Flower | 26.61 | 27.43 | 34.69 | 37.11 | ** |
| 31 | | *Codonocephalum peacockianum* | Leaf | 36.40 | 20.91 | 30.27 | 30.48 | ** |
| 32 | | *Grindelia robusta* | Leaf | 31.90 | 31.46 | 48.49 | 44.94 | ** |
| 33 | | *Helichrysum italicum* | Leaf | 51.91 | 59.15 | 44.62 | 45.42 | ** |
| 34 | | *Lactuca persica* | Leaf | 20.45 | 03.04 | −12.73 | −9.21 | + |
| 35 | | *Matricaria chamomilla* | Flower | 64.70 | 68.19 | 46.51 | 52.23 | ** |
| 36 | | *Pseudohandelia umbellifera* | Leaf | 55.14 | 59.79 | 44.50 | 40.47 | ** |
| 37 | | *Pulicaria gnaphalodes* | Leaf | 28.55 | 30.69 | 16.11 | 13.50 | * |
| 38 | | *Pulicaria gnaphalodes* | Seed | 91.07 | 89.72 | 84.68 | 86.79 | *** |
| 39 | | *Santolina chamaecyparissus* | Leaf | 85.02 | 92.05 | 66.25 | 69.57 | *** |
| 40 | | *Tanacetum balsamita* | Leaf | 45.24 | 43.94 | 23.17 | 28.87 | * |
| 41 | Berberidaceae | *Berberis integerrima* | Root | 59.00 | 33.91 | 46.26 | 34.12 | ** |
| 42 | | *Berberis vulgaris* | Leaf | 59.07 | 48.68 | 40.30 | 31.62 | ** |
| 43 | Boraginaceae | *Trichodesma incanum* | Leaf | 58.96 | 59.97 | 57.33 | 61.59 | ** |
| 44 | Cannabaceae | *Cannabis sativa* | Leaf | 32.14 | −4.59 | −18.57 | −33.67 | + |
| 45 | Capparaceae | *Capparis spinosa* | Leaf | 87.86 | 92.02 | 57.69 | 68.82 | ** |
| 46 | Cleomaceae | *Cleome chorassanica* | Leaf | 8.63 | 27.69 | 4.59 | 18.94 | * |
| 47 | Ephedraceae | *Ephedra major* | Leaf | −0.86 | −29.55 | −14.81 | −54.96 | + |
| 48 | Euphorbiaceae | *Chrozophora tinctoria* | Leaf | 60.62 | 61.59 | 49.55 | 55.67 | ** |
| 49 | | *Ricinus communis* | Fruit | 56.67 | 49.65 | 20.26 | 27.47 | * |
| 50 | | *Euphorbia petiolata* | Leaf | 7.95 | 4.61 | −10.30 | −18.42 | + |
| 51 | | *Euphorbia serpens* | Leaf | 82.74 | 88.23 | 75.23 | 75.29 | *** |
| 52 | | *Euphorbia aellenii* | Leaf | 26.96 | 10.15 | 20.26 | 12.25 | * |

**Table 1.** *Cont.*

| No | Family | Plant Scientific Name | Part Used | Inhibition Activity (%) | | | | Criteria |
|---|---|---|---|---|---|---|---|---|
| | | | | Average (41 mm) | | Average (Whole Wells) | | |
| | | | | Radicle | Hypocotyl | Radicle | Hypocotyl | |
| 53 | | *Euphorbia granulata* | Leaf | 82.47 | 88.39 | 60.35 | 63.68 | *** |
| 54 | Fabaceae | *Genista tinctoria* | Leaf | 41.69 | 37.77 | 34.12 | 22.73 | ** |
| 55 | Frankeniaceae | *Frankenia* spp | Leaf | 33.52 | 4.76 | 27.63 | 31.92 | * |
| 56 | Geraniaceae | *Pelargonium graveolens* | Leaf | 100.00 | 100.00 | 83.35 | 88.23 | **** |
| 57 | | *Hypericum helianthemoides* | Root | 51.68 | 59.62 | 37.52 | 48.88 | ** |
| 58 | Hypericaceae | *Hypericum perforatum* | Leaf | 71.81 | 63.71 | 49.21 | 52.58 | ** |
| 59 | | *Hypericum scabrum* | Leaf | 34.91 | 35.45 | −11.50 | −36.67 | + |
| 60 | | *Acinos graveolens* | Leaf | 63.97 | 79.21 | 37.26 | 52.02 | ** |
| 61 | | *Ballota nigra* | Leaf | 44.93 | 61.84 | 26.13 | 25.00 | * |
| 62 | | *Hyssopus angustifolius* | Leaf | 18.48 | 3.63 | 9.82 | −7.34 | * |
| 63 | | *Hyssopus angustifolius* | Flower | 39.83 | 28.13 | 30.44 | 28.13 | ** |
| 64 | | *Lavandula officinalis* | Flower | 100.00 | 100.00 | 80.29 | 82.49 | **** |
| 65 | | *Lavandula officinalis* | Leaf | 84.56 | 92.14 | 82.24 | 88.39 | *** |
| 66 | | *Melissa officinalis* | Leaf | −12.36 | −1.22 | 23.64 | 22.37 | * |
| 67 | | *Melissa officinalis* | Root | −12.37 | −1.23 | −13.39 | −1.50 | + |
| 68 | | *Mentha piperita* | Leaf | −10.03 | 10.44 | 0.62 | 18.36 | * |
| 69 | | *Mentha spicata* | Leaf | 18.06 | 29.40 | 9.12 | 7.31 | * |
| 70 | | *Mentha longifolia* | Leaf | 90.03 | 88.55 | 67.20 | 77.57 | *** |
| 71 | | *Nepeta binaloudensis* | Leaf | 100.00 | 100.00 | 96.37 | 98.28 | **** |
| 72 | | *Nepeta sintenisii* | Leaf | 29.52 | 18.82 | 14.06 | 16.37 | * |
| 73 | | *Origanum major* | Leaf | 57.07 | 75.54 | 16.16 | 17.39 | * |
| 74 | | *Origanum vulgare* | Leaf | 64.12 | 92.96 | −26.70 | −26.53 | + |
| 75 | | *Perovskia abrotanoides* | Leaf | 95.32 | 89.65 | 81.81 | 77.13 | *** |
| 76 | Lamiaceae | *Perovskia abrotanoides* | Seed | 31.21 | 20.94 | 20.81 | 16.78 | * |
| 77 | | *Rosmarinus officinalis* | Leaf | 90.67 | 91.63 | 80.86 | 85.41 | *** |
| 78 | | *Salvia aethiopis* | Leaf | 94.62 | 92.56 | 55.38 | 36.90 | ** |
| 79 | | *Salvia tebesana* | Leaf | 12.50 | −22.64 | −1.00 | −31.13 | + |
| 80 | | *Salvia nemorosa* | Leaf | 71.68 | 71.45 | 63.16 | 65.95 | *** |
| 81 | | *Salvia leriifolia* | Root | 86.21 | 78.93 | 55.88 | 54.49 | ** |
| 82 | | *Salvia leriifolia* | Leaf | 67.54 | 65.89 | 85.06 | 83.94 | *** |
| 83 | | *Salvia chloroleuca* | Leaf | 67.16 | 59.85 | 29.36 | 26.37 | ** |
| 84 | | *Salvia ceratophylla* | Leaf | 100.00 | 100.00 | 83.87 | 81.54 | **** |
| 85 | | *Salvia macrosiphon* | Leaf | 71.56 | 70.51 | 57.79 | 51.84 | ** |
| 86 | | *Salvia officinalis* | Leaf | 17.86 | 6.49 | 11.43 | −8.16 | * |
| 87 | | *Salvia virgata* | Leaf | 32.99 | 35.00 | 40.17 | 41.90 | ** |
| 88 | | *Salvia sahendica* | Leaf | 17.85 | 26.07 | 24.52 | 18.59 | * |
| 89 | | *Salvia sclarea* | Leaf | 62.24 | 73.54 | 60.19 | 68.31 | *** |
| 90 | | *Stachys byzantina* | Leaf | 43.43 | 47.05 | 40.05 | 45.20 | ** |
| 91 | | *Stachys lavandulifolia* | Flowering branch | 100.00 | 100.00 | 89.52 | 88.65 | **** |
| 92 | | *Thuspeinanta brahuica* | Leaf | 18.18 | −0.32 | −10.61 | −12.72 | + |
| 93 | | *Teucrium chamaedrys* | Leaf | 21.36 | 7.84 | 17.57 | 13.74 | * |
| 94 | | *Althaea officinalis* | Leaf | 21.94 | 51.12 | 21.61 | 40.32 | * |
| 95 | Malvaceae | *Malva sylvestris* | Flower | 76.13 | 82.20 | 76.75 | 78.11 | *** |
| 96 | | *Malva sylvestris* | Leaf | 49.41 | 54.58 | 16.28 | 10.13 | * |
| 97 | Myrtaceae | *Peganum harmala* | Leaf | 46.20 | 49.02 | 36.22 | 44.65 | ** |
| 98 | | *Eucalyptus globulus* | Leaf | 100.00 | 100.00 | 80.29 | 82.49 | *** |
| 99 | Nitrariaceae | *Eucalyptus globulus* | Seed | −12.99 | −59.00 | 2.01 | −14.22 | * |
| 100 | Onagraceae | *Epilobium hirsutum* | Leaf | 30.02 | 38.02 | 27.22 | 32.99 | * |
| 101 | | *Oenothera biennis* | Leaf | 63.00 | 61.01 | 47.50 | 52.41 | ** |
| 102 | Papaveraceae | *Corydalis aitchisonii* | Leaf | 34.43 | 34.83 | 19.27 | 18.55 | * |
| 103 | | *Glaucium flavum* | Leaf | 14.69 | 10.18 | 14.85 | −8.59 | * |
| 104 | Plantaginaceae | *Plantago major* | Leaf | 29.96 | 28.87 | 14.94 | 17.75 | * |

**Table 1.** *Cont.*

| No | Family | Plant Scientific Name | Part Used | Inhibition Activity (%) | | | | Criteria |
|---|---|---|---|---|---|---|---|---|
| | | | | Average (41 mm) | | Average (Whole Wells) | | |
| | | | | Radicle | Hypocotyl | Radicle | Hypocotyl | |
| 105 | Polygonaceae | *Polygonum aviculare* | Leaf | 68.71 | 59.09 | 45.12 | 47.84 | ** |
| 106 | | *Polygonum patulum* | Leaf | 53.91 | 60.01 | 48.00 | 54.02 | ** |
| 107 | Rosaceae | *Filipendula ulmaria* | Leaf | 16.07 | 2.08 | 18.18 | 20.58 | * |
| 108 | | *Rosa foetida* | Leaf | 58.44 | 61.15 | 60.81 | 57.81 | *** |
| 109 | Rutaceae | *Haplophyllum furfuraceum* | Leaf | 76.91 | 76.12 | 37.13 | 27.99 | ** |
| 110 | | *Ruta graveolens* | Leaf | 33.52 | 41.44 | 24.40 | 30.36 | * |
| 111 | Solanaceae | *Datura innoxia* | Leaf | 37.72 | 40.01 | 39.16 | 33.78 | ** |
| 112 | | *Datura stramonium* | Leaf | 12.33 | 12.88 | −3.40 | 2.00 | + |
| 113 | | *Lycium depressum* | Leaf | 52.60 | 47.90 | 45.99 | 48.99 | ** |
| 114 | | *Lycium ruthenicum* | Leaf | 55.37 | 55.35 | 59.24 | 61.67 | *** |
| 115 | | *Solanum nigrum* | Leaf | 38.83 | 36.60 | 32.42 | 28.80 | ** |
| 116 | Urticaceae | *Urtica dioica* | Leaf | 77.65 | 70.97 | 68.39 | 71.68 | *** |
| 117 | | *Urtica dioica* | Root | −45.30 | 21.10 | −30.00 | 20.01 | + |
| 118 | Verbenaceae | *Lippia citriodora* | Leaf | 91.35 | 87.32 | 33.02 | 22.83 | ** |
| 119 | | *Vitex pseudo-negundo* | Leaf | 53.90 | 45.75 | 23.99 | 20.33 | * |
| 120 | | *Vitex pseudo-negundo* | Seed | 20.22 | 38.54 | 15.22 | 12.02 | * |
| 121 | | *Lantana montevidensis* | Leaf | 19.67 | 15.33 | 29.50 | 36.49 | ** |
| 122 | Zygophyllaceae | *Tribulus terrestris* | Leaf | 56.88 | 67.94 | 46.14 | 41.68 | ** |
| 123 | | *Zygophyllum fabago* | Leaf | 12.94 | 11.27 | 20.22 | 16.21 | * |

1—The intensity of the inhibitory effect on lettuce seed germination was defined by the standard deviation value in four levels: Criteria (****) Mean + 3 SD; (***) Mean + 2 SD; (**) Mean + 1 SD; (*) Mean < 1 SD. 2—Negative numbers indicate stimulating effects on lettuce seed germination.

Among these samples, seven species showed a strong inhibitory effect on the germination of lettuce seeds. Some samples also stimulated the germination of lettuce seeds compared to the control.

#### 3.1.1. Radicle Growth (R %)

The most inhibitory effects on radicle growth were observed in the families Lamiaceae (*N. binaloudensis* leaf); Asteraceae (*A. nobilis* flower, *A. biebersteinii* leaf and *P. gnaphalodes* seed); Apiaceae (*E. platyloba* stem, *C. maculatum* leaf, *F. vulgare* fruit, and *T. ammi* fruit); Euphorbiaceae (*E. serpens* leaf, and *E. granulata* leaf); Solanaceae (*L. ruthenicum* leaf); Amaranthaceae (*A. hypochondriacus* leaf); Malvaceae (*M. sylvestris* leaf) and Hypericaceae (*H. perforatum* leaf). In the family Verbenaceae, it can be concluded that the individual species had an inhibitory effect on radicle growth (although in some cases small), but no significant difference between them was detected.

#### 3.1.2. Hypocotyl Growth (H %)

The most inhibitory effects on hypocotyl growth were observed in the families Lamiaceae (*N. binaloudensis* leaf); Asteraceae (*P. gnaphalodes* seed); Apiaceae (*E. platyloba* stem, *C. maculatum* leaf, *F. vulgare* fruit, and *T. ammi* fruit); Euphorbiaceae (*E. serpens* leaf); Solanaceae (*L. ruthenicum* leaf) and *L. depressum* leaf); Amaranthaceae (*A. hypochondriacus* leaf); Malvaceae (*M. sylvestris* leaf); Verbenaceae (*L. montevidensis* leaf) and Hypericaceae (*H. perforatum* leaf and *H. helianthemoides* root) (Figures S1–S9).

#### 3.1.3. Germination Percentage (G %)

The comparison of the average germination percentage of lettuce seeds in the vicinity of strong inhibitory plants in the period of 3 days of the experiment is shown in Figure 4. In the treatment of *S. ceratophylla* (leaf), most germinated seeds were observed compared to other treatments, about 72.76%. The highest degree of reduction in lettuce seed germination

percentages were observed in the treatment *L. officinalis* (flower) and *N. binaloudensis* (leaf), respectively, (23.61, 22.85%).

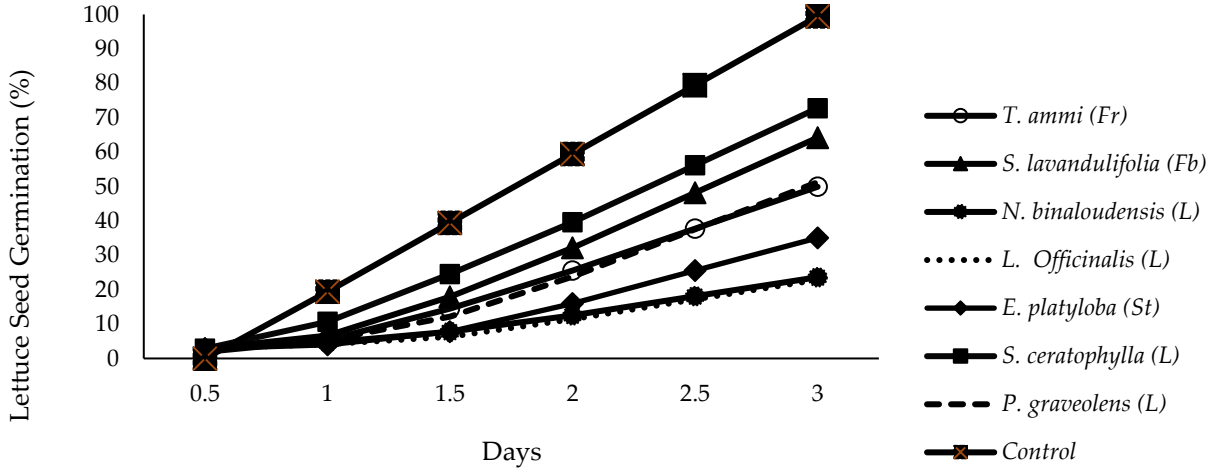



**Figure 4.** Effect of the strongest inhibitory plants on germination percentage of lettuce seeds.

### 3.1.4. Germination Rate

As it can be seen in Figure 5, *S. ceratophylla* (leaf), in comparison to other treatments showed the least effect on reducing the germination rate of lettuce seeds (12.86), while the highest effects belong to *N. binaloudensis* (leaf) and *L. officinalis* (flower), respectively, (3.60 and 3.32). The results showed that there is a significant difference between the plants at the probability level of 5%.

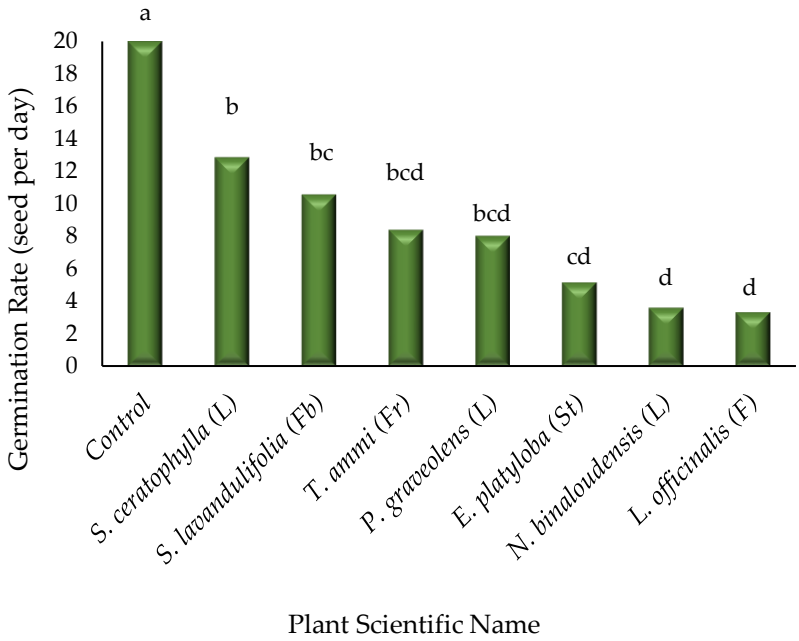



**Figure 5.** Effect of the most inhibitory plants on the germination rate of lettuce seeds. Columns followed by the same letter (a–d) are not significantly different ($p \leq 0.05$) by Duncan's multiple range tests.

### *3.2. Headspace Gas Chromatography-Mass Spectrometry (HS-GC-MS)*

In order to identify the compounds causing allelopathy in the most inhibitory plants (seven specimens), the headspace analysis was performed. The results showed that some phenolic compounds, such as Thymol, Carvacrol, P-Cymene and 1,8-Cineole, were the most common important components in these plants (Table 2).

**Table 2.** The main components of the most inhibitory plants based on Headspace Gas Chromatography-Mass Spectrometry (HS-GC-MS) analysis.

| Sample | Part of Use | Main Identified Components | RT | %Area |
|---|---|---|---|---|
| *Stachys lavandulifolia* | Flowering branch | Thymol | 20.13 | 7.96 |
| *Salvia ceratophylla* | Leaf | Carvacrol | 17.9 | 1.42 |
| | | trans-Caryophyllene | 18.14 | 0.7 |
| | | Thymol acetate | 20.13 | 11.82 |
| | | Chavicol | 25.13 | 0.98 |
| *Echinophora platyloba* | Stem | Thymol acetate | 20.11 | 5.31 |
| *Trachyspermum ammi* | Fruit | P-Cymene | 8.82 | 0.82 |
| | | β-Pinene | 10.10 | 0.69 |
| | | Borneol | 14.46 | 0.53 |
| | | Carvacrol Methyl Ether | 17.9 | 0.77 |
| | | α-Ionone | 18.12 | 0.35 |
| | | Thymol | 20.12 | 40.02 |
| | | Carvacrol | 20.48 | 0.92 |
| | | trans-Caryophyllene | 25.13 | 0.62 |
| | | Propene | 33.98 | 0.56 |
| | | α-terpinolene | 35.29 | 1.18 |
| | | 4-Cumylphenol | 35.49 | 1.02 |
| | | s-Indacene | 37.42 | 0.95 |
| *Lavandula officinalis* | Flower | 1,8-Cineole | 9.1 | 14.3 |
| | | Camphor | 13.55 | 2.46 |
| | | 1-Borneol | 14.47 | 1.04 |
| | | Carvacrol Methyl Ether | 17.9 | 0.42 |
| | | Thymol | 20.11 | 35.71 |
| | | Linalool | 20.49 | 0.71 |
| | | Chromolaenin | 35.28 | 1.3 |
| | | α-Amorphene | 35.49 | 1.12 |
| | | Bornyl acetate | 37.4 | 0.7 |
| *Nepeta binaloudensis* | Leaf | p-Cymene | 8.84 | 0.57 |
| | | 1,8-Cineole | 9.11 | 8.47 |
| | | α-Pinene | 10.15 | 0.34 |
| | | Carvacrol Methyl Ether | 17.92 | 0.21 |
| | | Verbenone | 19.2 | 0.14 |
| | | Thymol | 20.11 | 28.44 |
| | | α-iso-methyl ionone | 20.5 | 0.71 |
| | | 4-Cumylphenol | 35.27 | 0.86 |
| | | β-Caryophyllene | 35.49 | 0.92 |
| | | α-Terpinene | 37.4 | 0.79 |

**Table 2.** *Cont.*

| Sample | Part of Use | Main Identified Components | RT | %Area |
|---|---|---|---|---|
| *Pelargonium graveolens* | Leaf | β-pinene | 7.17 | 1.22 |
| | | 1,8-Cineole | 9.14 | 1.13 |
| | | α-Pinene | 10.13 | 0.7 |
| | | Thymol | 20.12 | 13.93 |
| | | Carvacrol | 20.49 | 0.85 |
| | | 4-Cumylphenol | 35.28 | 0.74 |
| | | Isomenthone | 35.49 | 0.56 |

RT: Retention time (min).

### 3.3. The Specification of Effective Concentrations of the Most Inhibitory Plants

The determination of the effective concentration of plants on germination inhibition ($EC_{50}$ and $EC_{25}$) was performed on seven plants, including *S. lavandulifolia* (flowering branch), *S. ceratophylla* (leaf), *E. platyloba* (stem), *T. ammi* (fruit), *L. officinalis* (flower), *N. binaloudensis* (leaf), and *P. graveolens* (leaf). For all these plants, $EC_{50}$ and $EC_{25}$, based on the radicle and hypocotyl inhibition, were calculated (Table 3).

**Table 3.** The effective concentration of the most inhibitory plants on lettuce radicle and hypocotyl by dish pack method.

| Plant Scientific Name | Part of Use | Radicle Inhibition | | Hypocotyl Inhibition | |
|---|---|---|---|---|---|
| | | $EC_{25}$ | $EC_{50}$ | $EC_{25}$ | $EC_{50}$ |
| | | mg/well | | | |
| *Stachys lavandulifolia* | Flowering branch | 20.57 | 72.80 | 58.97 | 107.8 |
| *Salvia ceratophylla* | Leaf | 36.96 | 83.70 | 92.87 | 130.5 |
| *Echinophora platyloba* | Stem | 2.44 | 8.99 | 1.97 | 7.915 |
| *Trachyspermum ammi* | Fruit | 9.81 | 25.48 | 29.72 | 52.19 |
| *Lavandula officinalis* | Flower | 7.01 | 9.86 | 8.65 | 11.21 |
| *Nepeta binaloudensis* | Leaf | 6.76 | 7.85 | 2.64 | 7.923 |
| *Pelargonium graveolens* | Leaf | 0.53 | 5.31 | 2.72 | 13.71 |

### 3.4. Allelopathic Interaction of the Most Inhibitory Plants

The interaction effect of the strongest inhibitory plants was evaluated separately on radicle and hypocotyl growth based on the screening test results.

#### 3.4.1. Interaction Result

The results showed a different combination of synergistic, additive, and antagonistic effects. As shown in Table 4, 42 results were obtained from 21 combinations, which were investigated on radicle and hypocotyl growth separately. In the investigation of these interactions on radicle inhibition, 15 combinations showed antagonistic interactions, 4 combinations showed additive interactions, and 2 combinations showed synergistic interactions. The combination of *P. graveolens* (leaf) and *L. officinalis* (flower) had the most antagonistic interaction (28.13%). The most synergistic interaction (80.00%) was observed in the combination of the *T.* amm (fruit) and *N. binaloudensis* (leaf).

**Table 4.** Fractional Inhibitory Concentration (FIC) of combinations among the strongest inhibitory plants using $EC_{25}$ of lettuce radicle and hypocotyl.

| Plant Interactions | | FIC | |
|---|---|---|---|
| | | Radicle | Hypocotyl |
| *Echinophora platyloba* × | *Stachys lavandulifolia* | 0.52 $^{An}$ | 1.46 $^{S}$ |
| | *Salvia ceratophylla* | 0.15 $^{An}$ | 1.17 $^{Ad}$ |
| | *Pelargonium graveolens* | −0.09 $^{St}$ | 1.11 $^{Ad}$ |
| | *Trachyspermum ammi* | 0.33 $^{An}$ | 1.61 $^{S}$ |
| | *Nepeta binaloudensis* | 0.56 $^{An}$ | 1.30 $^{S}$ |
| | *Lavandula officinalis* | −0.12 $^{St}$ | 1.49 $^{S}$ |
| *Stachys lavandulifolia* × | *Salvia ceratophylla* | 0.27 $^{An}$ | 1.37 $^{S}$ |
| | *Pelargonium graveolens* | 0.73 $^{Ad}$ | 1.46 $^{S}$ |
| | *Trachyspermum ammi* | 0.72 $^{Ad}$ | 1.28 $^{S}$ |
| | *Nepeta binaloudensis* | −0.53 $^{St}$ | 1.56 $^{S}$ |
| | *Lavandula officinalis* | 0.18 $^{An}$ | 1.85 $^{S}$ |
| *Salvia ceratophylla* × | *Pelargonium graveolens* | 0.58 $^{An}$ | 1.05 $^{Ad}$ |
| | *Trachyspermum ammi* | 0.52 $^{An}$ | 1.24 $^{S}$ |
| | *Nepeta binaloudensis* | 0.97 $^{Ad}$ | 1.22 $^{S}$ |
| | *Lavandula officinalis* | −0.50 $^{St}$ | 1.36 $^{S}$ |
| *Pelargonium graveolens* × | *Trachyspermum ammi* | 1.03 $^{Ad}$ | 1.97 $^{S}$ |
| | *Nepeta binaloudensis* | −0.38 $^{St}$ | 1.46 $^{S}$ |
| | *Lavandula officinalis* | −0.56 $^{St}$ | 0.54 $^{An}$ |
| *Trachyspermum ammi* × | *Nepeta binaloudensis* | 1.60 $^{S}$ | 2.00 $^{S}$ |
| | *Lavandula officinalis* | −0.20 $^{St}$ | 1.47 $^{S}$ |
| *Nepeta binaloudensis* × | *Lavandula officinalis* | 1.50 $^{S}$ | 2.00 $^{S}$ |

FIC values $\leq 0$ indicate stimulant effects ($^{St}$), between 0 and 0.7 indicate antagonistic effects ($^{An}$), values between 0.7 and 1.2 indicate additive effects ($^{Ad}$) and values greater than 1.2 indicate synergistic effects ($^{S}$).

For hypocotyl inhibition among 21 combinations, 1 combination showed antagonistic effects, 3 combinations showed additive effects, and most combinations (17 combinations) showed synergistic effects. The combination of *P. graveolens* (leaf) and *L. officinalis* (flower) was the only combination to show an antagonistic effect (26.91%). Also, the most synergistic effects (100.00%) were observed in two combinations of *N. binaloudensis* (leaf) with *T. ammi* (fruit) or *L. officinalis* (flower).

### 3.4.2. Isobologram Curves

Different types of allelopathic effects were shown in the isobologram curves (Figures 6 and 7). In the isobologram curves of root growth inhibition (Figure 3), 11 curves showed antagonistic effects between strong inhibitory plants, 3 curves showed synergistic effects, and 7 combinations showed growth stimulating effects. The highest number of antagonistic effects related to *S. ceratophylla* (leaf) (with five antagonistic effects) and *P. graveolens* (leaf) had the highest number of synergistic effects (two synergistic effects) with other plants. The additive effects were not observed in these compounds. In the isobolograms showing hypocotyl inhibition (Figure 4), one combination showed antagonistic effects (*L. officinalis* (flower) with *P. graveolens* (leaf)), two curves showed synergistic status between plants, and 18 other curves showed synergistic effects. *N. binaloudensis* (leaf) and *T. ammi* (fruit) showed the highest number of synergistic curves.

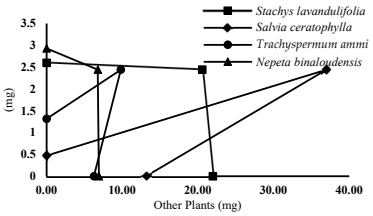
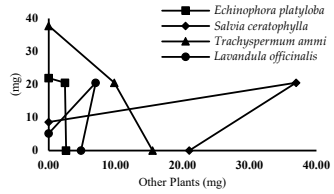
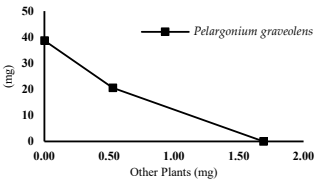
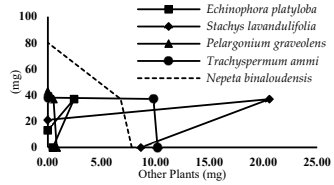
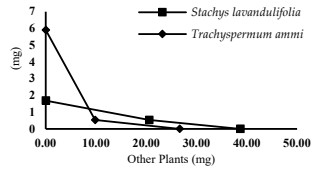
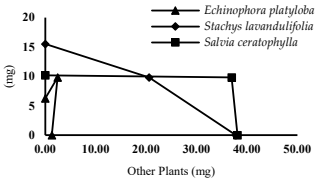
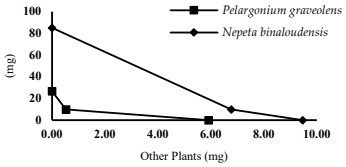
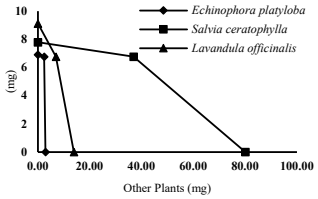
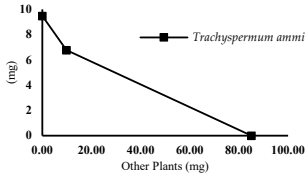
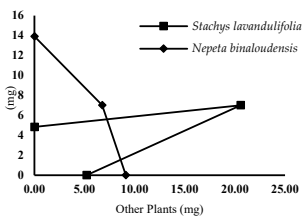
| | Additive Effects | Antagonistic Effects | Synergistic Effects | Stimulant Effects |
|---|---|---|---|---|
| *Echinophora platyloba* | Not observed |  | Not observed | *Pelargonium graveolens*<br>*Lavandula officinalis* |
| *Stachys lavandulifolia* | Not observed |  |  | *Nepeta binaloudensis* |
| *Salvia ceratophylla* | Not observed |  | Not observed | *Lavandula officinalis* |
| *Pelargonium graveolens* | Not observed |  |  | *Echinophora platyloba*<br>*Nepeta binaloudensis*<br>*Lavandula officinalis* |
| *Trachyspermum ammi* | Not observed |  |  | *Lavandula officinalis* |
| *Nepeta binaloudensis* | Not observed |  |  | *Stachys lavandulifolia*<br>*Pelargonium graveolens* |
| *Lavandula officinalis* | Not observed |  | Not observed | *Echinophora platyloba*<br>*Salvia ceratophylla*<br>*Pelargonium graveolens*<br>*Trachyspermum ammi* |

**Figure 6.** Isobologram curves of allelopathic interaction effects of plants on lettuce radicle growth inhibition.

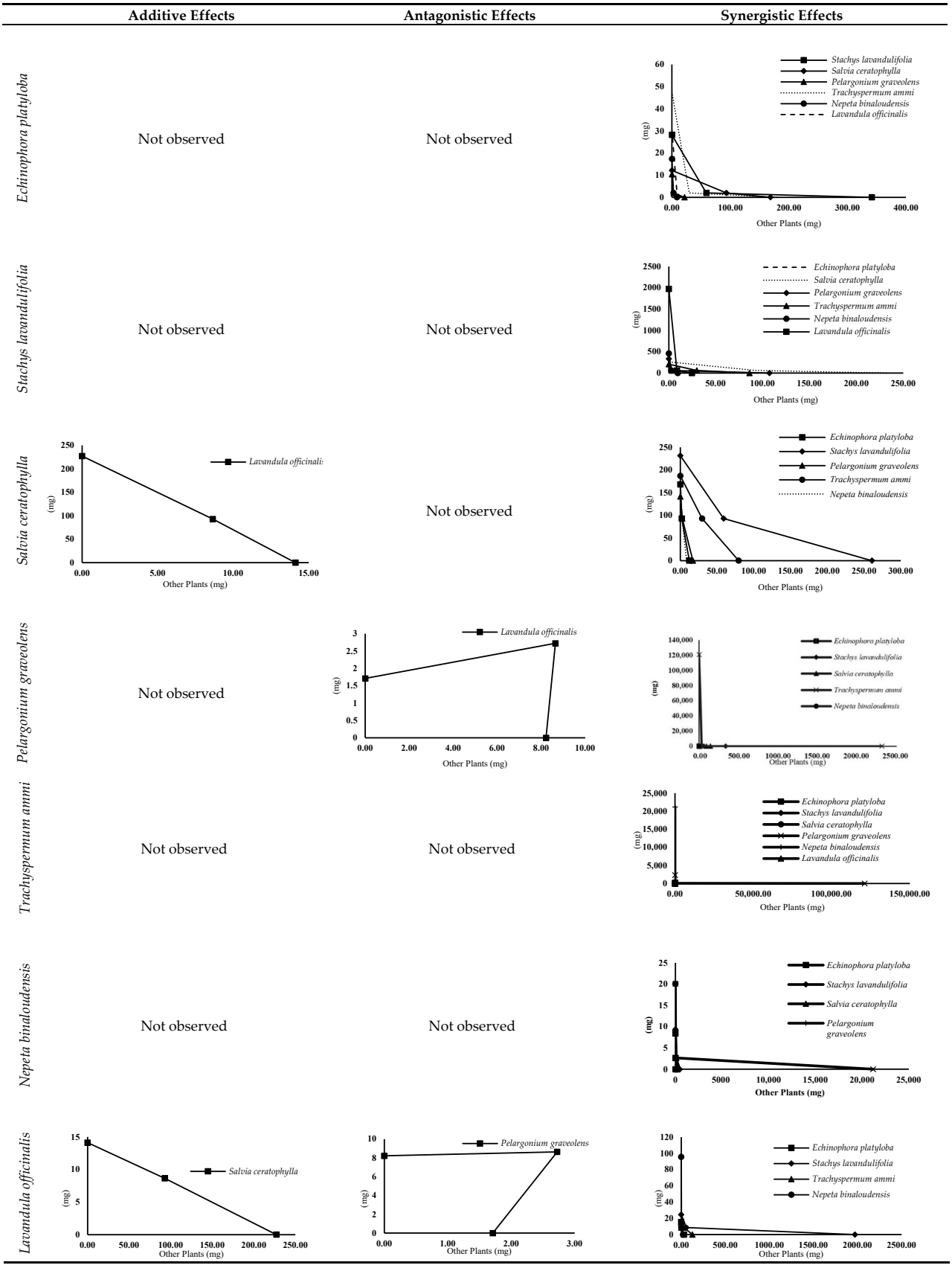



**Figure 7.** Isobologram curves of allelopathic interaction effects of plants on lettuce hypocotyl growth inhibition.

#### 3.4.3. Comparison of Evaluation Methods of Allelopathic Interactions

In order to compare two evaluation methods (FIC and Isobologram curves), the results are summarized in Table 5.

**Table 5.** Fractional Inhibitory Concentration (FIC) of combinations of the strongest inhibitory plants using $EC_{25}$ of lettuce radicle and hypocotyl.

| Plant Interactions | | FIC | | Isobologram | |
|---|---|---|---|---|---|
| | | Radicle | Hypocotyl | Radicle | Hypocotyl |
| *Echinophora platyloba* × | *Stachys lavandulifolia* | An | S | An | S |
| | *Salvia ceratophylla* | An | Ad | An | S |
| | *Pelargonium graveolens* | St | Ad | St | S |
| | *Trachyspermum ammi* | An | S | An | S |
| | *Nepeta binaloudensis* | An | S | An | S |
| | *Lavandula officinalis* | St | S | St | S |
| *Stachys lavandulifolia* × | *Salvia ceratophylla* | An | S | An | S |
| | *Pelargonium graveolens* | Ad | S | S | S |
| | *Trachyspermum ammi* | Ad | S | An | S |
| | *Nepeta binaloudensis* | St | S | St | S |
| | *Lavandula officinalis* | An | S | An | S |
| *Salvia ceratophylla* × | *Pelargonium graveolens* | An | Ad | An | S |
| | *Trachyspermum ammi* | An | S | An | S |
| | *Nepeta binaloudensis* | Ad | S | An | S |
| | *Lavandula officinalis* | St | S | St | Ad |
| *Pelargonium graveolens* × | *Trachyspermum ammi* | Ad | S | S | S |
| | *Nepeta binaloudensis* | St | S | St | S |
| | *Lavandula officinalis* | St | An | St | An |
| *Trachyspermum ammi* × | *Nepeta binaloudensis* | S | S | S | S |
| | *Lavandula officinalis* | St | S | St | S |
| *Nepeta binaloudensis* × | *Lavandula officinalis* | S | S | An | S |

(St): stimulant effects, (An): antagonistic effects, (Ad): additive effects, and (S): synergistic effects.

As you can see in Table 5, the analysis of two methods in more than one case provides similar results. Since in the FIC method, the comparison is based on numbers, it seems that it is more accurate, but more research is needed to ensure this claim.

## 4. Discussion

The results obtained from this research, in many items, confirm the allelopathic effects of the medicinal plants and their volatile compounds seen in other research. In the lamiaceae family, *L. officinalis*, *S. ceratophylla*, *S. lavandulifolia*, and *N. binaloudensis* were reported as strong inhibitory plants. In several field observations, *Nepeta* species prevent the germination of other plant species in their surroundings [29]. In another report on the allelopathic effect of *N. binaloudensis,* the germination and growth of sunflower seeds had been inhibited by the aqueous extract of its roots and leaves. The inhibitory effect of *L. officinalis* on the germination of lettuce seeds was observed by the plant box method [30]. Also, a high suppression (83–95%) of radicle elongation was observed in the flowers of *Lavandula vera* [16]. A study conducted on the allelopathic effects of volatile compounds of different medicinal plants, *N. binaloudensis*, *L. angustifolia*, *P. graveolens*, *T. ammi*, and *salvia* species, on factors such as germination percentage, average germination time, radicle and hypocotyl length, vigor index, and dormancy incubation, observed similar results in lettuce seeds [6].

In another report on investigating plant growth inhibitory activities, Salvia officinalis had 100% inhibition of lettuce radicle and hypocotyl growth [7]. In addition, investigating the activity of the essential oil and methanolic extract of *E. platyloba* showed very strong activity against bacteria [31]. In a study to investigate the antioxidant activity of aqueous and ethanolic extracts of *S. lavandulifolia*, both types of extracts showed good potential with high phenolic content [32].

The use of plants as phytochemicals has been widely seen in recent years due to the presence of chemicals and the development of cross-resistance to the lack of use of synthetic insecticides [33]. The formulations obtained from different species of the genus *Nepeta* with a large amount of essential oil and flavonoids showed high antimicrobial, antifungal, and insecticidal properties [34,35]. In another study on the essential oil *Nepeta* cataria against *Spodoptera littoralis* larvae, a high insecticidal activity of this plant was seen ($LC_{50}$) value $\leq$ 10.0 mL/$m^3$) [36]. In a previously conducted study, the effect of *P. graveolens* has been investigated as an insecticidal property in killing larvae and preventing egg laying [37,38]. In our study, the leaf of this plant showed many inhibitory effects.

Forasmuch as Thymol, Carvacrol, P-Cymene, and 1,8-Cineole were very high in our headspace experiments, they are probably the main factor responsible for the inhibitory effects. In the report, it was shown that monoterpenes such as 1,8 cineole, thymol, geraniol, menthol, and camphor strongly inhibit the radicle growth of *Z. mays* L. seedlings [39]. In another study, the effects of insecticides and insect repellants of 1,8 cineole were confirmed [40,41].

Another important point in this experiment was the combination of the most inhibitory plants to check the allelopathic properties that were observed as synergistic, additive, and antagonistic effects. Before this, the synergistic effects in antibacterial and antioxidant activity of the combination of *Coriandrum sativum* with *Cuminum cyminum* essential oils was reported [42]. Also, the synergistic antimicrobial effects of volatile compounds, such as eugenol with menthol and linalool and carvacrol with thymol, have been reported [43]. In another study, the synergistic antifungal effects were shown between the essential oils of *Mentha spicata* with *Melaleuca alternifolia* and *Thymus vulgaris* with *Cinnamomum verum* and *Origanum majorana* [24].

## 5. Conclusions

Medicinal plants, especially aromatic ones, have been used in traditional medicine in Iran and have potential allelopathic activity. They are good candidates for finding new allelochemicals to be used in agriculture as bio-herbicides. Among the investigated plants, those with high inhibitory effects were introduced and could be used to control, and even destroy, weeds. In this research, the combination of the *N. binaloudensis* (leaf) with *T. ammi* (fruit) and *N. binaloudensis* (leaf) with *L. officinalis* (flower) had great synergistic effects. For more accurate investigations in the future, the interaction of the main compounds of these plants can be performed in vitro as well as in field conditions. Also, their interaction effects can provide a new field for the bioherbicide research and such effects can play a significant role in determining the effective dose of each compound. Therefore, applying these compounds in agriculture will help us to reduce the use of chemical pesticides, and ultimately contribute to the health of our community.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12123001/s1, Figure S1. Allelopathic effects of plant species of Lamiaceae on radicle and hypocotyl of lettuce. Figure S2. Allelopathic effects of plant species of Asteraceae on radicle and hypocotyl of lettuce. Figure S3. Allelopathic effects of plant species of Apiaceae on radicle and hypocotyl of lettuce. Figure S4. Allelopathic effects of plant species of Euphorbiaceae on radicle and hypocotyl of lettuce. Figure S5. Allelopathic effects of plant species of Solanaceae on radicle and hypocotyl of lettuce. Figure S6. Allelopathic effects of plant species of Amaranthaceae on radicle and hypocotyl of lettuce. Figure S7. Allelopathic effects of plant species of Malvaceae on radicle and hypocotyl of lettuce. Figure S8. Allelopathic effects of plant species of Verbenaceae on radicle and hypocotyl of lettuce. Figure S9. Allelopathic effects of plant species of Hypericaceae on radicle and hypocotyl of lettuce.

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