Chemical Composition and Phytotoxic Effects of Essential Oils of *Salvia hierosolymitana* Boiss. and *Salvia multicaulis* Vahl. var. *simplicifolia* Boiss. Growing Wild in Lebanon

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**Abstract:** The chemical composition of the essential oils of *S. hierosolymitana* Boiss. and *S. multicaulis* Vahl. var. *simplicifolia* Boiss. collected in Lebanon was studied by means of GC and GC-MS analysis. In all 115 compounds were identified: 82 for *S. hierosolymitana* and 72 for *S. multicaulis* var. *simplicifolia*. The presence of carbonylic compounds (17%) characterizes the oil from *S. hierosolymitana*, while *S. multicaulis* var. *simplicifolia* oil is rich of monoterpenes (34.5%) and sesquiterpenes (46.9%). The effects of the essential oils on germination and initial radical elongation of *Raphanus sativus* L. (radish) and *Lepidium sativum* L. (garden cress) were studied, indicating in a different activity against radical elongation of the species tested.

**Keywords:** *Salvia hierosolymitana* Boiss.; *Salvia multicaulis* Vahl. var. *simplicifolia* Boiss.; essential oil; germination; radical elongation.
1. Introduction

Interactions between higher plants take place either by competition or by chemical inhibition or allelopathy. When the effect is due to the release of an effective phytotoxin, it is called allelopathy. Small quantities of toxins are responsible for massive reductions in plant growth. Allelopathy is one expression of the general phenomenon of chemical interaction and is probably of widespread significance in the functioning of natural communities. In fact, a number of plants have inhibitory effects on the growth of neighboring or successional plants by releasing allelopathic chemicals into the soil, either as exudates from living tissues or by decomposition of plant residues [1–3]. The study of compounds produced by plants, which inhibit or stimulate the germination and the development of other plants, is important for understanding the mechanisms of the ecological interaction. For this reason, our research group is carrying out a series of studies on the possible allelopathic properties of medicinal plants [4] that, being rich in active principles, are considered a primary source of potential allelochemicals. Aromatic species, in particular, are toxic almost without exception and the degree of toxicity seems to vary in effectiveness roughly with the intensity of aroma.

One of the best known and well-studied examples of allelopathy is the “Salvia phenomenon” [5]. The genus Salvia (Lamiaceae: subfamily Nepetoideae, tribe Mentheae) represents a cosmopolitan assemblage of nearly 1,000 species displaying a remarkable diversity in growth forms, secondary compounds, floral morphology and pollination biology. The first studies that demonstrated the presence of volatile growth inhibitors produced by Salvia species were carried out on Salvia leucophylla and S. apiana by Muller and co-workers [5]. The authors showed that when cucumber seedlings were placed in proximity to crushed leaves of the two Salvia species, their root growth was markedly inhibited, and this inhibition was increased as the amount of leaves was increased. Successively, the same authors suggested that the inhibition of growth of annual grassland species in and about colonies of Salvia and the gradually decreasing inhibition of herbs extending 9 m out into grassland from Salvia patches, were due to the production of volatile terpenes, particularly camphor and cineole [6–9].

In continuation of our studies on the possible phytotoxic activity of essential oils from plants collected in the Mediterranean area [4], we carried out in vitro experiments in order to verify the possible effects of the essential oils from S. hierosolymitana and S. multicaulis var. simplicifolia collected in Lebanon on germination and initial radical elongation of Raphanus sativus L. (radish) and Lepidium sativum L. (garden cress).

Salvia hierosolymitana Boiss., Jerusalem sage, is also known by the Hebrew name moriah and the Arabic name Quwaysah al quds, Lisân al’ijlah. This plant is characterized by the presence of triterpenoids and shows antinflammatory [10] and antioxidant properties [11]. The essential oil was previously studied by us [12].

Salvia multicaulis Vahl. var. simplicifolia Boiss. is an evergreen shrub growing to 0.3 m × 0.25 m, native to South-West Asia, particular Eastern, Central, and Southern Turkey. Most of the components isolated from the extracts of S. multicaulis were terpenoids [13]. The essential oil of S. multicaulis from plants grown in Iran [14–17] and Lebanon [18] has been also previously reported. The essential oils and extracts of S. multicaulis collected in Turkey showed antimicrobial and antioxidative activities [19].
Results and Discussion

The volatile components in the essential oils isolated from the two Salvia species and their percentage contribution are shown in Table 1, according to their elution order on a HP-5 MS column. The hydrodistillation yielded 0.32% and 0.13% of pale yellow oil (on a dry mass basis) for S. hierosolymitana and S. multicaulis. var. simplificolia, respectively. One-hundred and fifteen compounds in all were identified, 82 for S. hierosolymitana, accounting for 86.6% of the total oil and 72 for S. multicaulis var. simplificolia (90.8% of the oil).

In S. hierosolymitana the monoterpenoid fraction amounted to 17.7% of the oil and was characterized only by oxygenated monoterpenoids, amongst which the most abundant was α-thujone (3.0%). The sesquiterpenoid fraction (16.5%) was mainly composed of sesquiterpenoid hydrocarbons (11.5%), with β-caryophyllene (2.4%) being the main compound. Spathulenol (1.9%) was the most abundant of the six oxygenated sesquiterpenoids identified. Noteworthy was the content of carbonylic compounds (17%), amongst which the C-18 ketone hexahydrofarnesyl acetone (5.3%) and the C-13 ketone β-ionone (3.5%) were particularly abundant. Also considerable was the phytol content (4.8%). Other components of the oil were fatty acids (21.4%) and hydrocarbons (3.7%), which were the most represented compounds, the former mostly represented by hexadecanoic acid (12.5%). 4-Vinyl-guaiacol (4.0%) was the sole phenolic compound determined.

In the oil from S. multicaulis var. simplificolia the sesquiterpenoid fraction amounted to 46.9% of the total oil, while the monoterpenoid fraction was lower (34.5%). Differently from S. hierosolymitana, in which monoterpenoid hydrocarbons were completely absent, in S. multicaulis oil they accounted for the 10.6% and were particularly represented by α-pinene (5.5%). Also oxygenated monoterpenoids were abundant (23.9%) and in this fraction the main compounds were myrtenol (4.6%), sabinyl acetate (4.6%) and 1,8-cineole (3.1%). Among sesquiterpenoids, sesquiterpenoid hydrocarbons (38.6%) prevailed, particularly α-copaene (6.6%), β-caryophyllene (4.4%) and aromadendrene (3.9%). Another important difference in comparison with S. hierosolymitana essential oil was the paucity of carbonylic compounds (1.5%) and of fatty acids (1.2%). As stated before [18] the sample studied by us is different from the other Iranian samples studied by Ahmadi and Mirza [14] and Rustaiyan and co-workers [15]. Also the oils from Iran studied successively by Morteza-Semnani and co-workers [16] and Mohammadhosseini and co-workers [17] are different from our sample for the high content of camphor, 1,8-cineole and α-pinene that characterize them. The oil from Turkey presents the same features, being rich of α-pinene (21.9%), camphor (11%) and eucalyptol (20.1%) [19]. These differences might have been derived both from harvest time and local, climatic and seasonal factors or we may hypothesize that the Lebanese samples belong to a different chemotype than the Iranian and Turkish samples.

The oils from S. hierosolymitana and S. multicaulis var. simplificolia from Lebanon were previously analyzed by us [12,18]; for the present study the aerial parts of the plants have been collected again in the same place (Kadiska valley and near Tannourine, both in Lebanon, respectively) and the GC and GC-MS analysis have been repeated on the new samples. As we can see (Table 1), data show that results obtained in the present study are similar to those of the previously studies [12,18], even if the percentages of some components are slightly different, thus confirming that the chemical composition of an essential oil depends strictly on the collection period.
Table 1. Essential oil composition (%) of *Salvia hierosolymitana* (H) and *Salvia multicaulis* var. *simplicifolia* (M) growing wild in Lebanon.

| R<sub>i</sub><sup>a</sup> | R<sub>i</sub><sup>b</sup> | Compound | Identification<sup>c</sup> | H<sup>d</sup> | M<sup>d</sup> |
|-----------------|-----------------|-----------|-----------------|---------|---------|
| **Monoterpenoid hydrocarbons** | | | | 10.6 |
| 938 | 1032 | α-Pinene | 1, 2, 3 | 5.5 |
| 953 | 1076 | Camphene | 1, 2, 3 | 0.9 |
| 973 | 1132 | Sabinene | 1, 2 | 0.4 |
| 980 | 1118 | β-Pinene | 1, 2, 3 | 0.9 |
| 993 | 1174 | Myrcene | 1, 2 | 0.3 |
| 1025 | 1280 | p-Cymene | 1, 2 | 2.3 |
| 1030 | 1203 | Limonene | 1, 2, 3 | 0.3 |
| **Oxygenated monoterpenoids** | | | | 17.7 | 23.9 |
| 1034 | 1213 | 1,8-Cineole | 1, 2, 3 | 0.8 | 3.1 |
| 1074 | 1482 | cis-Linalool oxide (furanoid) | 1, 2 | 0.4 |
| 1085 | 1455 | trans-Linalool oxide (furanoid) | 1, 2 | 0.6 |
| 1098 | 1553 | Linalool | 1, 2, 3 | 1.9 | 0.5 |
| 1105 | 1430 | α-Thujone | 1, 2 | 3.0 | 1.0 |
| 1115 | 1451 | β-Thujone | 1, 2 | 1.6 | 0.9 |
| 1137 | 1664 | trans-Pinocarveol | 1, 2 | 0.6 |
| 1145 | 1532 | Camphor | 1, 2, 3 | 0.9 | 0.8 |
| 1167 | 1719 | Borneol | 1, 2, 3 | | 1.2 |
| 1176 | 1611 | Terpinen-4-ol | 1, 2, 3 | 0.5 | 1.2 |
| 1189 | 1706 | α-Terpineol | 1, 2 | 1.1 |
| 1193 | 1648 | Myrtenal | 1, 2 | 0.7 | 1.8 |
| 1196 | 1804 | Myrtanol | 1, 2 | 0.6 | 4.6 |
| 1197 | 1597 | Safranal | 1, 2 | 0.4 |
| 1217 | 1845 | trans-Carveol | 1, 2 | | 0.2 |
| 1227 | 1698 | Myrtenyl acetate | 1, 2 | | 1.8 |
| 1235 | 1857 | Geraniol | 1, 2 | 0.2 |
| 1237 | 1665 | Pulegone | 1, 2 | 1.4 |
| 1240 | 1656 | Neral | 1, 2 | 0.9 |
| 1259 | 1665 | Linalyl acetate | 1, 2, 3 | 1.6 |
| 1284 | 1597 | Bornyl acetate | 1, 2, 3 | | 0.5 |
| 1295 | 1658 | Sabinyl acetate | 1, 2 | 0.6 | 4.6 |
| 1333 | 1709 | α-Terpinyl acetate | 1, 2 | | 0.5 |
| 1343 | 1948 | Piperitenone | 1, 2 | 0.5 | 0.6 |
| **Phenolic compounds** | | | | 4.0 | 3.8 |
| 1293 | 2198 | Thymol | 1, 2, 3 | | 1.7 |
| 1299 | 2239 | Carvacrol | 1, 2, 3 | | 0.6 |
| 1353 | 2186 | Eugenol | 1, 2, 3 | | 1.5 |
| 1312 | 2180 | 4-Vinylguaiacol | 1, 2 | 4.0 |
| **Sesquiterpenoid hydrocarbons** | | | | 11.5 | 38.6 |
| 1352 | 1466 | α-Cubebene | 1, 2 | | 0.3 |
| 1363 | 1492 | Cyclosativene | 1, 2 | 0.5 |
| 1372 | 1493 | Ylangene | 1, 2 | | 0.3 |
| 1377 | 1497 | α-Copaene | 1, 2 | 1.7 | 6.6 |
| 1385 | 1535 | β-Bourbonene | 1, 2 | 0.6 | 0.7 |
| 1387 | 1600 | β-Elemene | 1, 2 | | 0.8 |
| 1411 | 1568 | α-Cedrene | 1, 2 | 0.5 |
Table 1. Cont.

| Compound                        | 1, 2, 3 | 2.4 | 4.4 |
|---------------------------------|---------|-----|-----|
| β-Caryophyllene                 | 1, 2, 3 | 2.4 | 4.4 |
| Aromadendrene                   | 1, 2    | 3.9 |
| (E)-β-Farnesene                 | 1, 2    | 1.2 |
| 1,2                                 | 1.2 |
| α-Humulene                       | 1, 2    | 0.9 |
| 1,2                                 | 0.5 |
| allo-Aromadendrene               | 1, 2    | 0.4 |
| 1,2                                 | 1.1 |
| β-Selinene                       | 1, 2    | 0.5 |
| 1,2                                 | 0.9 |
| Germacrene D                     | 1, 2    | 0.6 |
| 1,2                                 | 2.0 |
| γ-Murolene                       | 1, 2    | 0.4 |
| 1,2                                 | 2.0 |
| ar-Curcumene                     | 1, 2    | 1.1 |
| 1,2                                 | 0.9 |
| Viridiflorene                    | 1, 2    | 0.2 |
| 1,2                                 | 1.3 |
| γ-Cadinene                       | 1, 2    | 1.2 |
| 1,2                                 | 2.9 |
| 1S-cis-Calamenene                | 1, 2    | 0.3 |
| 1,2                                 | 1.5 |
| δ-Cadinene                       | 1, 2    | 0.1 |
| 1,2                                 | 3.0 |
| α-Calacorene                     | 1, 2    | 0.5 |
| 1,2                                 | 0.9 |
| Cadalene                         | 1, 2    | 0.1 |
| 1,2                                 | 1.5 |

Oxygenated sesquiterpenoids

| Compound                       | 1, 2, 3 | 5.0 | 8.3 |
|---------------------------------|---------|-----|-----|
| Ledol                           | 1, 2    | 0.6 |
| 1,2                                 | 0.6 |
| (E)-Nerolidol                   | 1, 2    | 1.4 |
| 1,2                                 | 1.4 |
| Spathulenol                     | 1, 2    | 1.9 |
| 1,2                                 | 0.7 |
| Caryophyllene oxide             | 1, 2, 3 | 0.8 |
| 1,2                                 | 0.8 |
| Globulol                        | 1, 2    | 0.2 |
| 1,2                                 | 0.4 |
| Viridiflorol                    | 1, 2    | 0.9 |
| 1,2                                 | 0.9 |
| Humulene epoxide II             | 1, 2    | 0.5 |
| 1,2                                 | 0.5 |
| epi-Globulol                    | 1, 2    | 0.6 |
| 1,2                                 | 0.6 |
| T-Cadinol                       | 1, 2    | 1.2 |
| 1,2                                 | 1.2 |
| T-Murolol                       | 1, 2    | 0.6 |
| 1,2                                 | 0.5 |
| Cubenol                         | 1, 2    | 0.6 |
| 1,2                                 | 0.6 |
| Torreyol                        | 1, 2    | 0.1 |
| 1,2                                 | 0.1 |
| α-Cadinol                       | 1, 2    | 0.5 |
| 1,2                                 | 0.8 |
| β-Eudesmol                      | 1, 2    | 0.4 |
| 1,2                                 | 0.4 |
| 14-Hydroxy-α-humulene           | 1, 2    | 17.0| 1.5 |

Carbonylic compounds

| Compound                       | 1, 2, 3 | 17.0 | 1.5 |
|---------------------------------|---------|-----|-----|
| (Z)-4-Heptenal                  | 1, 2    | t   |
| 1,2                                 | t   |
| Benzaldehyde                     | 1, 2, 3 | 0.7 |
| 1,2                                 | 0.7 |
| 1-Octen-3-one                   | 1, 2    | 0.1 |
| 1,2                                 | 0.1 |
| (E,E)-2,4-Heptadienal           | 1, 2    | 0.2 |
| 1,2                                 | 0.2 |
| Phenylacetaldehyde               | 1, 2, 3 | 1.0 |
| 1,2                                 | 1.0 |
| (E)-2-Octenal                   | 1, 2    | 0.5 |
| 1,2                                 | 0.5 |
| (E,Z)-2,6-Nonadienal            | 1, 2    | t   |
| 1,2                                 | t   |
| p-Methylaceto phenone           | 1, 2    | 0.5 |
| 1,2                                 | 0.5 |
| Decanal                         | 1, 2    | t   |
| 1,2                                 | t   |
| (E)-β-Damascenone               | 1, 2    | 2.2 |
| 1,2                                 | 0.2 |
| (E)-Geranyl acetone             | 1, 2    | 0.8 |
| 1,2                                 | 0.8 |
| (E)-β-Ionone                    | 1, 2, 3 | 3.5 |
Table 1. Cont.

|     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|
|     |     |     |     |     |     |
| 1580 | 1815 | Tridecan-2-one | 1, 2 | 0.3 |
| 1694 | 2031 | Pentadecan-2-one | 1, 2 | 0.7 |
| 1835 | 2131 | Hexahydrofarnesyl acetone | 1, 2 | 5.3 | 1.3 |
| 1918 | 2384 | \((E,E)\)-Farnesyl acetone | 1, 2 | 1.2 |

|     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|
|     |     |     |     |     |     |
| Hydrocarbons |     |     |     |     |
| 1179 | 1763 | Naphtalene | 1, 2, 3 | 0.3 |
| 1208 | 1567 | \(\alpha\)-Ionene | 1, 2 | 0.3 |
| 2400 | 2400 | Tetracosane | 1, 2, 3 | 0.1 |
| 2500 | 2500 | Pentacosane | 1, 2, 3 | 0.5 | 0.7 |
| 2600 | 2600 | Hexacosane | 1, 2, 3 | 0.2 |
| 2700 | 2700 | Heptacosane | 1, 2, 3 | 1.0 | 0.7 |
| 2800 | 2800 | Octacosane | 1, 2, 3 | t |
| 2900 | 2900 | Nonacosane | 1, 2 | 0.8 | 0.9 |
| 3000 | 3000 | Triacantane | 1, 2, 3 | t |
| 3100 | 3100 | Enatriacantane | 1, 2, 3 | 0.5 |
| 3200 | 3200 | Dotriacantane | 1, 2, 3 | t |

|     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|
|     |     |     |     |     |     |
| Fatty acids |     |     |     |     |
| 1957 | 2931 | Palmitic acid | 1, 2, 3 | 12.5 | 1.2 |
| 1568 | 2467 | Dodecanoic acid | 1, 2, 3 | 0.1 |
| 1768 | 2672 | Tetradecanoic acid | 1, 2, 3 | 1.5 |
| 1873 | 2740 | Pentadecanoic acid | 1, 2, 3 | 0.2 |
| 2099 | 3195 | \((Z,Z,Z)-9,12,15\)-Octadecatrienoic acid | 1, 2, 3 | 2.2 | t |
| 2104 | 3160 | \((Z,Z),9,12\)-Octadecadienoic acid | 1, 2, 3 | 3.3 |
| 2120 | 3157 | \((Z)-9\)-Octadecenoic acid | 1, 2, 3 | 1.6 |

|     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|
|     |     |     |     |     |     |
| Others |     |     |     |     |
| 1002 | 1243 | 2-Pentylfuran | 1, 2 | t |
| 1290 | 2471 | Indole | 1, 2, 3 | 0.7 |
| 1485 | 2354 | Dihydroactinidiolide | 1, 2 | 1.3 |
| 1672 | 2175 | Tetradecanol | 1, 2 | 0.2 |
| 1950 | 2622 | Phytol | 1, 2 | 4.8 | 0.6 |

\(a\): Kovats retention index on HP-5 MS column; \(b\): Kovats retention index on HP Innowax; 
\(c\): 1 = Kovats retention index, 2 = mass spectrum, 3 = coinjection with authentic compound; 
\(d\): t = trace, less than 0.05%.

The two essential oils were evaluated for their phytotoxic activity against germination and initial radical elongation of radish and garden cress, two species frequently utilized in biological assays [4]. The oils affected the germination and the radical elongation of radish and garden cress in a different way. Radical elongation seemed to be more affected than germination. The germination of radish did not appear significantly sensitive to the two essential oils (Table 2). Moreover, at a dose of 0.625 \(\mu\)g/mL the essential oil of \(S.\) hierosolymitana significantly inhibited the germination of radish. At the lowest dose tested the essential oil of \(S.\) multicaulis var. simplificifolia significantly promoted the germination of radish. The germination of garden cress was weakly inhibited in response to 0.625 \(\mu\)g/mL of essential oil of \(S.\) multicaulis var. simplificifolia. Radical elongation of radish was inhibited significantly in response to 0.125 \(\mu\)g/mL and 1.25 \(\mu\)g/mL of \(S.\) multicaulis var. simplificifolia (Table 3). Radical elongation of garden cress was promoted in response to 0.625 \(\mu\)g/mL of essential oil.
of *S. multicaulis* var. *simplicifolia* (Table 3). The difference in biological activity of the two oils could be attributed to their different chemical composition and to the presence, in *S. multicaulis* var. *simplicifolia* oil, of a major amount of oxygenated terpenoids, reported as germination and seedling growth inhibitors [20].

**Table 2.** Biological activities of essential oils of *Salvia hierosolymitana* and *S. multicaulis* var. *simplicifolia* against germination of *Raphanus sativus* (radish) and *Lepidium sativum* (garden cress), 120 hrs after sowing. Results are shown as mean ± standard deviation (SD) of three experiments.

|               | *Salvia hierosolymitana* | *Salvia multicaulis var. simplicifolia* |
|---------------|--------------------------|-----------------------------------------|
| **Raphanus sativus** | Germinated seeds ± SD | Germinated seeds ± SD |
| Control       | 12.67 ± 1.51             | 10.67 ± 1.37                            |
| 0.062 μg/mL   | 13.33 ± 1.15             | 13.00 ± 1.00 ***                        |
| 0.125 μg/mL   | 13.33 ± 0.58             | 11.00 ± 2.00                            |
| 0.250 μg/mL   | 13.67 ± 0.58             | 12.33 ± 1.53                            |
| 0.625 μg/mL   | 8.33 ± 2.31*             | 12.67 ± 2.52                            |
| 1.25 μg/mL    | 11.67 ± 1.53             | 12.00 ± 1.00                            |
| 2.50 μg/mL    | 12.00 ± 0.00             | 11.33 ± 1.53                            |
| **Lepidium sativum** | Germinated seeds ± SD | Germinated seeds ± SD |
| Control       | 11.17 ± 2.04             | 11.17 ± 1.47                            |
| 0.062 μg/mL   | 11.33 ± 1.15             | 11.33 ± 2.08                            |
| 0.125 μg/mL   | 11.67 ± 4.16             | 8.33 ± 2.08 *                          |
| 0.250 μg/mL   | 12.00 ± 2.00             | 10.33 ± 2.52                            |
| 0.625 μg/mL   | 12.00 ± 1.73             | 12.00 ± 2.65                            |
| 1.25 μg/mL    | 11.67 ± 2.08             | 12.00 ± 1.73                            |
| 2.50 μg/mL    | 12.33 ± 0.58             | 10.67 ± 0.58                            |

Note: *p < 0.05; **p < 0.01; ***p < 0.001 vs positive control.

**Table 3.** Biological activities of essential oils of *Salvia hierosolymitana* and *S. multicaulis* var. *simplicifolia* against radical elongation of *Raphanus sativus* (radish) and *Lepidium sativum* (garden cress), 120 hrs after sowing. Data are expressed in cm. Results are shown as mean±standard deviation (SD) of three experiments.

|               | *Salvia hierosolymitana* | *Salvia multicaulis var. simplicifolia* |
|---------------|--------------------------|-----------------------------------------|
| **Raphanus sativus** | Radicle length ± SD (cm) | Radicle length ± SD (cm) |
| Control       | 3.14 ± 2.24              | 3.89 ± 2.39                            |
| 0.062 μg/mL   | 2.60 ± 1.59              | 3.34 ± 2.03                            |
| 0.125 μg/mL   | 2.80 ± 2.01              | 2.62 ± 1.77***                         |
| 0.250 μg/mL   | 3.37 ± 2.00              | 3.18 ± 1.31                            |
| 0.625 μg/mL   | 2.71 ± 1.81              | 4.20 ± 2.62                            |
| 1.25 μg/mL    | 3.04 ± 1.80              | 2.13 ± 1.97***                         |
| 2.50 μg/mL    | 2.80 ± 2.07              | 3.29 ± 2.02                            |
Table 3. Cont.

| Lepidium sativum | Radicle length ± SD (cm) | Radicle length ± SD (cm) |
|------------------|--------------------------|--------------------------|
| Control          | 3.47 ± 1.78              | 3.21 ± 1.60              |
| 0.062 μg/mL      | 3.08 ± 1.58              | 2.64 ± 1.67              |
| 0.125 μg/mL      | 3.31 ± 2.36              | 4.05 ± 2.51              |
| 0.250 μg/mL      | 3.19 ± 1.67              | 3.61 ± 1.63              |
| 0.625 μg/mL      | 3.25 ± 1.80              | 4.12 ± 1.34**            |
| 1.25 μg/mL       | 3.18 ± 1.60              | 3.83 ± 1.85              |
| 2.50 μg/mL       | 2.99 ± 1.51              | 2.97 ± 1.82              |

Note: *p < 0.05; **p < 0.01; ***p < 0.001 vs positive control.

Our data agree with the literature on inhibitory activity exerted by essential oils of Salvia species on seed germination and radical elongation and in general on vegetation pattern. A dramatic example of zones free of annual herbs, influenced by terpenoids, was demonstrated by Muller [9] in California chaparral, in the areas surrounding patches of Salvia leucophylla L. (Labiatae) and Artemisia californica Lee. Volatile monoterpenoids emanating from leaves of Salvia leucophylla L. are responsible for anatomical and physiological changes occurring in herb seedlings which were exposed to vapors [21]. Camphor and 1,8-cineole, the main compounds of the oil of Salvia leucophylla, are potent inhibitors of oxygen uptake by mitochondrial suspensions [9].

Although the mode of inhibitory action of essential oils against germination still remains unclear, volatile oils and monoterpenoids inhibit cell division and induce structural breaks and decomposition in roots [22–25]. Both monoterpenoids and sesquiterpenoids appear to be involved in these allelopathic interactions. Several monoterpenoids are potent inhibitors of seed germination and seedling growth. These include 1,4 and 1,8-cineole [22], citronellal, citronellol, linalool [25,26], α-pinene [24,27], and limonene [27].

Recently, sesquiterpenoids (β-maaliene, α-isocomene, β-isocomene, δ-cadinene, 5-hydroxy-calamenene, and 5-methoxycalamenene) were shown to inhibit the seedling growth of associated native vegetation, and thus possibly help in successful invasion in the introduced sites [28].

Experimental

Plant material

Aerial parts of S. hierosolymitana Boiss were gathered at the full flowering stage from plants growing wild in the Kadiska valley (North Lebanon), on rocky soil, in June 2008, while aerial parts of S. multicaulis var. simplicifolia were collected near Tannourine (Lebanon), 1,700 a. s. l., in May 2008. Voucher specimens (leg. and det. N. Arnold s.n., confirm. Th. Raus) were deposited in the Herbarium of the Botanischer Garten, Berlin Universität, Germany.

Isolation of the volatile components

Fifteen grams of each air-dried sample were ground in a Waring blender and then subjected to hydrodistillation for 3 hours according to the standard procedure described in the European
Pharmacopoeia [29]. The oils were solubilised in \( n \)-hexane, filtered over anhydrous sodium sulphate and stored under \( N_2 \) at +4 °C in the dark until tested and analyzed.

**Gas chromatography**

Analytical gas chromatography was carried out on a Perkin-Elmer Sigma-115 gas chromatograph equipped with a FID and a data handling processor. The separation was achieved using a HP-5 MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Column temperature: 40 °C, with 5 min initial hold, and then to 270 °C at 2 °C/min, 260 °C (20 min); injection mode splitless (1 µL of a 1:1,000 \( n \)-pentane solution). Injector and detector temperatures were 250 °C and 290 °C, respectively. Analysis was also run by using a fused silica HP Innowax polyethyleneglycol capillary column (50 m × 0.20 mm, 0.25 µm film thickness). In both cases, helium was used as carrier gas (1.0 mL/min).

**Gas chromatography–Mass spectrometry**

Analysis was performed on an Agilent 6850 Ser. II apparatus, fitted with a fused silica DB-5 capillary column (30 m × 0.25 mm i.d.; 0.33 µm film thickness), coupled to an Agilent Mass Selective Detector MSD 5973; ionization energy voltage 70 eV; electron multiplier voltage energy 2000 V. Mass spectra were scanned in the range 40–500 amu, scan time 5 scans/s. Gas chromatographic conditions were as reported in the previous paragraph; transfer line temperature, 295 °C.

**Identification of components**

Most constituents were identified by gas chromatography by comparison of their Kovats retention indices (Ri) with either those of the literature [30,31] or with those of authentic compounds available in our laboratories. The Kovats retention indices were determined in relation to a homologous series of \( n \)-alkanes (C\(_8\)-C\(_{28}\)) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with either those stored in NIST 02 and Wiley 275 libraries or with mass spectra from the literature [30,32] and a home made library. Components relative concentrations were obtained by peak area normalization. No response factors were calculated.

**Biological assay**

A bioassay based on germination and subsequent radical growth was used to study phytotoxic effects of the essential oils of *S. hierosolymitana* and *S. multicaulis* var. *simplicifolia* on seeds of *Raphanus sativus* L. cv. “Saxa” (radish), and *Lepidium sativum* L. (cress). Seeds of *Lepidium sativum* L. and *Raphanus sativus* L. were purchased from Blumen srl, Piacenza, Italy. The seeds were surface-sterilized in 95% ethanol for 15 s and sown in Petri dishes (Ø = 90 mm), containing five layers of Whatman filter paper, impregnated with distilled water (7 mL, control) or tested solution of the essential oil (7 mL) at the different assayed doses. The germination conditions were 20 ± 1 °C, with natural photoperiod. The essential oils, in water–acetone mixture (99.5:0.5), were assayed at the doses of 2.5, 1.25, 0.625, 0.25, 0.125 and 0.062 µg/mL. Controls performed with water–acetone mixture alone showed no appreciable differences in comparison with controls in water alone. Seed germination
was observed directly in Petri dishes, each 24 h. Seed was considered germinated when the protrusion of the radicle became evident [33]. After 120 hrs (on the fifth day), the effects on radical elongation were measured. Each determination was repeated three times, using Petri dishes containing 15 seeds each. Data are expressed as the mean ± SD of both germination and radical elongation [34]. The Student’s t test of independence was applied [35].

Conclusions

Aromatic plants are considered a primary source of potential allelochemicals and are toxic almost without exception. Previous studies show that Salvia species produce volatile growth inhibitors, particularly oxygenated monoterpenoids. Our in vitro experiments on the essential oils from S. hierosolymitana and S. multicaulis var. simplicifolia collected in Lebanon on germination and initial radical elongation of radish and garden cress, show that the essential oil of S. multicaulis var. simplicifolia was more active, whereas S. hierosolymitana oil didn’t show such activity. The phytotoxic activity of S. multicaulis var. simplicifolia is probably due to the presence of a substantial amount of oxygenated terpenoids, along with the presence of α-pinene (5.5%) and p-cymene (2.3%). Our in vitro studies can contribute to explain the importance of volatile compounds as chemical mediators in biochemical interactions among higher plants and can suggest models for lead compounds in the development of new pesticides [36].

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Sample Availability: Samples of the compounds are available from the authors

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