Herbicidal value of essential oils from oregano-like flavour species

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ABSTRACT
Chemical composition and phytotoxicity of oregano, marjoram and Thymus mastichina essential oils against Portulaca oleracea L., Lolium multiflorum Lam. and Echinochloa crus-galli (L.) Beauv. has been investigated. Seventy-seven compounds reaching 97.3% and 99.4% were identified by gas chromatography–mass spectrometry. Carvacrol (60.42 ± 0.07%), p-cymene (15.52 ± 0.02%) and γ-terpinene (5.19 ± 0.02%) were the main compounds in oregano essential oil, whereas large amounts of 1,8-cineole (59.59 ± 0.85%, 49.49 ± 0.37%), linalool (13.05 ± 0.04%, 5.66 ± 0.01%) and α-terpineol (3.36 ± 0.10%, 5.59 ± 0.01%), followed by β-pinene (4.35 ± 0.39, 5.54 ± 0.01%) and α-pinene (4.11 ± 0.53, 4.28 ± 0.01%) were found, respectively, in marjoram and T. mastichina essential oils. Oregano essential oil completely inhibited seed germination and seedling growth at all concentrations assayed (0.125–1 µL mL⁻¹), whereas marjoram and T. mastichina essential oils only showed significant effects in hypocotyl and/or hypocotyl + radicle length depending on the weed and dose.

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Introduction
The commercial value of certain herbs and spices, widely employed in Mediterranean diets as well as their essential oils as natural ingredients in beverages, medicines and cosmetics, plays an important role in their adulteration. Variations in chemical composition of herbs
detected in a quite common way do not necessarily reveal adulteration and may be due to satisfy supply and demand (Sprink & Moyer, 2013), such as it occurs with the essential oils. In this case, these changes are due to aging, storage, as well as by the use of species with different common names according to place of origin. Thus, authentication or standardization of commercial essential oils by means of selected main compounds is a fundamental subject for consumers.

In this sense, sweet marjoram (Origanum majorana L.) is a species of remarkable economic and industrial importance because both its fresh and dried highly aromatic leaves and flowering tops are widely used as spice and condiment to flavour many foods, with sweet marjoram essential oil also employed in the food industry as flavouring in foodstuff and beverages, in perfumeries for its spicy and herbaceous notes or in pharmaceutical and industrial products due to their antimicrobial and antioxidant properties. However, marjoram is usually confused with other aromatic species, especially oregano (Origanum vulgare L.), the most traded and consumed spice, and well-known culinary herb commonly associated with pizzas and other Mediterranean dishes, and even with Thymus mastichina L., because this latter species is also known as Spanish marjoram. All species belong to the Lamiaceae family and their confusion may be due to the fact that all bear oregano-like flavour.

Oregano identity is complicated by both the large heterogeneity of Origanum genus and the grouping of different botanical genera, Origanum from the Mediterranean and Lippia from Mexico. The European Pharmacopoeia and the European Spice Association only allow O. vulgare L. ssp. hirtum and Origanum onites L. to be marketed as true oregano. However, the International Organization for Standardization (ISO7925) allows leaves of all Origanum genus, species and subspecies, except O. majorana, to be marketed as oregano (Black, Haughey, Chevallier, Galvin-King, & Elliott, 2016). European O. vulgare essential oil shows a great variability in both yield and chemical composition. Plants from the Mediterranean climate usually exhibit an active/efficient cymyl- and/or linalool pathway, whereas in plants from regions with Continental climate the essential oils are comparatively poor in monoterpenes and geranyl pyrophosphate (GPP) is mainly converted by the sabinyl pathway (Lukas, Schmider, & Novak, 2015).

In this way, high-quality plant material from Mediterranean area with high content of phenolic monoterpenes, mainly carvacrol and/or thymol and their biosynthetic precursors γ-terpinene and p-cymene with pungent oregano flavour, have a wide commercial potential. So, qualitative and quantitative analyses are needed to ensure quality, consumer safety (Salgueiro, Martins, & Correia, 2010) and fair trade of herbs and spices widely used as culinary seasoning and especially when this species or theirs essential oils are present in cosmetic products or pharmaceutical specialties by their pharmacological activity.

Several studies have described that essential oils obtained from Origanum spp. show a wide range of biological activities, such as antifungal, antibacterial, antioxidant, anti-inflammatory, insecticidal, cytotoxic and anti-acetylcholinesterase (Hajlaoui, Mighri, Aouni, Gharsallah, & Kadri, 2016; Revajová, Pistl, Levkut, Marcin, & Levkutová, 2010). In this sense, a supplementation of oregano essential oil increases proliferation of lymphocytes, suggesting higher immune defense ability of the body (Revajová et al., 2010). According to their antifungal activity, they have been demonstrated to be very active against numerous pathogens and spoilage fungi affecting worldwide crops and post-harvest products, such as Verticillium dahlia, Penicillium aurantiogriseum (Rus et al., 2016), as well as in humans and animals mycosis, like Candida glabrata isolated from patients (Khosravi
et al., 2011). In addition, oregano essential oil denotes a promising natural additive in foodstuff due to its capacity to prevent bacterial contamination and consequently improving food preservation (Huang, Lin, & Chuang, 2010; Revajová et al., 2010).

On the other hand, *Thymus mastichina* essential oil also has been reported by their antimicrobial activity against *Candida* spp, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus agalactiae* (Silva, Gomes, & Palmeira-de-Oliveira, 2014), as well as by their antioxidant, anti-inflammatory and anti-hyperglycaemic activities. In this way, *Thymus caespititius* and *T. mastichina* were the main scavengers of nitric oxide radicals between the six *Thymus* species assayed (Aazza et al., 2016).

As the biological activity is closely related with the chemical composition of the essential oils, and these mixtures of natural compounds represent at the same time useful markers for the evaluation of quality and authenticity of the final products widely demanded in perfumery, cosmetic, food, beverage, agricultural and pharmaceutical industries, commercial essential oils need more quality control because in several companies only the common name appears in the label of these biological materials. Known main compounds can provide useful information to discriminate between species of different geographical origin and to reveal frauds if substituted by others of different botanical origin.

So, the aims of this work are firstly to standardize through gas chromatography–mass spectrometry (GC–MS) analysis the essential oils from oregano-like flavour species purchased from a Spanish company dedicated to the supply of raw materials (pharmaceuticals, cosmetics and food supplements), packaging and laboratory material in Pharmacy and Hospitals and from a Portugal company in order to establish the importance of including in the label, take into account the variability in chemical composition, not only the scientific or common name, but also the main components in order to ensure its use, and secondly to compare the phytotoxic activity of these related commercial essential oils against seed germination and seedling growth of *Portulaca oleracea*, a cosmopolitan annual weed of tropical and subtropical climates, *Lolium multiflorum*, a grass distributed along temperate climates affecting mostly cereals and *Echinochloa crus-galli*, an annual plant seriously influencing irrigation crops, especially rice.

**Materials and methods**

**Plant material**

Commercial samples of oregano essential oil (Batch 0042451) and natural marjoram (Batch 0042773) essence, purchased from Guinama TM (Valencia, Spain), and *T. mastichina* essential oil (Batch TM010711), supplied by Planalto Dourado TM (Freixedas, Portugal), were stored at 4°C until chemical analysis and phytotoxic studies.

**Weeds**

Mature seeds of annual weeds of *P. oleracea* L., *L. multiflorum* Lam. and *E. crus-galli* (L.) Beauv. were purchased from Herbiseed TM (website: www.herbiseed.com).

**Gas chromatography–mass spectrometry**

GC–MS analysis was carried out with a 5973N Agilent apparatus, equipped with a capillary column (95 dimethylpolysiloxane – 5% diphenyl), Agilent HP-5MS UI (30 m long
and 0.25 mm i.d. with 0.25 μm film thickness). The column temperature programme was 60°C for 5 min, with 3°C/min increases to 180°C, then 20°C/min increases to 280°C, which was maintained for 10 min. The carrier gas was Helium at a flow-rate of 1 mL/min. Split mode injection (ratio 1:30) was employed. Mass spectra were taken over the m/z 30–500 range with an ionizing voltage of 70 eV. The individual compounds were identified by MS and their identity was confirmed by comparison of their Kovat’s retention index calculated using co-chromatographed standard hydrocarbons relative to C₈–C₃₂ n-alkanes, and mass spectra with reference samples or with data already available in the NIST 2005 mass spectral library and in the literature (Adams, 2007).

**Herbicidal activity**

Sets of 20 seeds each with 5 replicates per treatment were homogenously distributed in Petri dishes (9 cm diameter) between two layers of filter paper (Whatman No.1) moistened with 4 mL of distilled water and with 0 (control), 0.125, 0.250, 0.5 and 1 µL mL⁻¹ of marjoran, oregano and T. mastichina essential oils. Petri dishes were sealed with parafilm and incubated in a germination chamber Equitec EGCS 301 3SHR model, according to previous assays (Blázquez & Carbó, 2015) alternating 30.0 ± 0.1°C 16 h in light and 20.0 ± 0.1°C 8 h in dark and with (E. crus-galli) and without (P. oleracea, L. multiflorum) humidity. To evaluate the herbicidal activity of the essential oils, the number of germinated seeds was counted and compared with those of untreated seedlings. Emergence of the radicle (≥1 mm) was used as an index of germination and seedling length (hypocotyl and/or radicle) data were recorded after 3, 5, 7, 10 and 14 days in each replicate.

**Statistical analysis**

Experiments were made with five replicates. Data were subjected to one-way analysis of variance with SPSS statistics 22 software. Tukey’s test was used when variances remained homogeneous (Levene’s test) and T3 Dunnett’s post hoc test was employed if not, assuming equal variances. Differences were considered to be significant at p ≤ .05.

**Results**

**Essential oil composition**

Seventy-seven compounds reaching between 97.3% and 99.4% of the total commercial oregano, marjoram and T. mastichina essential oils were identified by GC/MS analysis. Components are clustered (Table 1) in homologous series of monoterpane hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, aromatic compounds (phenylpropanoids) and others and listed according to Kovat’s retention index calculated in GC on an apolar HP-5MS column.

In oregano essential oil, highest quantities of monoterpane compounds (93.05%) were found. Both hydrocarbons (27.00%) and oxygenated monoterpenes (66.05%) with 9 and 10 identified compounds, respectively, were also qualitatively the principal phytochemical group. The oxygenated monoterpane carvacrol (60.42 ± 0.07%), followed by their biogenetic precursors, the monoterpane hydrocarbons p-cymene (15.52 ± 0.02%) and γ-terpinene (5.19 ± 0.02%), were the main compounds. Among the sesquiterpene hydrocarbons, large quantities of β-caryophyllene (5.66 ± 0.01%) with small amounts of
Table 1. Chemical composition of oregano, marjoram and *T. mastichina* essential oils.

| RI  | Compounds                        | Oregano          | Marjoram         | *T. mastichina* |
|-----|----------------------------------|------------------|------------------|-----------------|
| 931 | Monoterpene hydrocarbons         | 27.00 ± 0.10     | 12.44 ± 1.30     | 18.65 ± 0.32    |
| 939 | α-Thujene                        | –                | 0.09 ± 0.02      | 0.14 ± 0.10     |
| 953 | α-Pinene                         | 1.39 ± 0.01      | 4.11 ± 0.53      | 4.28 ± 0.01     |
| 978 | Camphene                         | 0.19 ± 0.00      | 0.81 ± 0.10      | 0.37 ± 0.00     |
| 980 | β-Pinene                         | –                | 0.88 ± 0.09      | 4.24 ± 0.01     |
| 994 | Myrcene                          | 1.95 ± 0.02      | 1.34 ± 0.12      | 1.76 ± 0.00     |
| 1002| α-Phellandrene                   | 0.22 ± 0.01      | –                | 0.05 ± 0.00     |
| 1004| *p*-Mentha-1(7),8-diene           | –                | 0.08 ± 0.01      | –               |
| 1018| α-Terpine             | 1.13 ± 0.01      | –                | 0.19 ± 0.01     |
| 1025| *p*-Cymene    | 15.52 ± 0.02     | –                | 1.43 ± 0.28     |
| 1029| Limonene                         | 1.12 ± 0.02      | –                | –               |
| 1052| trans-Ocimene                     | –                | 0.37 ± 0.02      | 0.19 ± 0.01     |
| 1060| γ-Terpine                         | 5.19 ± 0.02      | 0.41 ± 0.02      | 0.34 ± 0.01     |
| 1094| Terpinolene                       | –                | –                | 0.13 ± 0.00     |
| 1100| Oxygenated monoterpenes          | 66.05 ± 0.08     | 84.49 ± 1.12     | 67.71 ± 0.33    |
| 1106| cis-Sabinene hydrate             | –                | 0.11 ± 0.01      | 0.37 ± 0.01     |
| 1107| cis-Linalool oxide               | –                | 0.26 ± 0.01      | 0.04 ± 0.00     |
| 1108| trans-Linalool oxide             | –                | 0.36 ± 0.01      | –               |
| 1110| Linalool                         | 2.07 ± 0.01      | 13.50 ± 0.04     | 5.66 ± 0.01     |
| 1112| Dehydrolinalool                  | –                | 0.18 ± 0.01      | –               |
| 1116| α-Thujone                         | 0.05 ± 0.00      | –                | –               |
| 1119| α-Fenchol                         | –                | 0.03 ± 0.00      | –               |
| 1122| cis-p-Mentha-2-en-1-ol           | –                | –                | 0.06 ± 0.00     |
| 1133| 1-Terpineol                      | –                | 0.12 ± 0.01      | –               |
| 1140| trans-Pinocarveol                | –                | 0.06 ± 0.01      | 0.10 ± 0.01     |
| 1141| trans-p-Mentha-2-en-1-ol          | –                | –                | 0.04 ± 0.01     |
| 1146| Camphor                          | 0.05 ± 0.00      | 1.04 ± 0.01      | –               |
| 1163| Isoborneol                       | –                | 0.39 ± 0.01      | 0.69 ± 0.01     |
| 1167| δ-Terpinol                       | –                | 0.40 ± 0.03      | 1.86 ± 0.01     |
| 1169| Borneol                          | 0.48 ± 0.00      | 1.06 ± 0.01      | –               |
| 1178| Terpinen-4-ol                     | 0.44 ± 0.00      | 0.41 ± 0.01      | 0.03 ± 0.01     |
| 1187| p-Cymen-8-ol                      | –                | –                | 0.05 ± 0.01     |
| 1191| α-Terpineol                      | 0.12 ± 0.01      | 3.36 ± 0.10      | 5.59 ± 0.01     |
| 1199| γ-Terpineol                      | –                | 0.43 ± 0.01      | –               |
| 1203| trans-Dihydrocarvone             | –                | –                | 0.15 ± 0.01     |
| 1205| Verbenone                        | –                | 0.02 ± 0.01      | –               |
| 1247| Carvacrol methyl ether           | –                | –                | 0.63 ± 0.00     |
| 1258| Linalool acetate                 | –                | 2.71 ± 0.14      | 0.04 ± 0.01     |
| 1267| Geranial                         | 0.03 ± 0.00      | –                | –               |
| 1286| Isobornyl acetate                | –                | –                | 0.03 ± 0.00     |
| 1288| Bornyl acetate                   | –                | 0.20 ± 0.01      | –               |
| 1293| Thymol                           | 1.77 ± 0.01      | –                | –               |
| 1302| Carvacrol                        | 60.42 ± 0.07     | 0.02 ± 0.01      | 1.63 ± 0.01     |
| 1349| α-Terpinyl acetate               | –                | 0.20 ± 0.01      | –               |
| 1361| Neryl acetate                    | –                | 0.01 ± 0.00      | –               |
| 1477| Geranyl propanoate               | –                | 0.04 ± 0.00      | –               |
| 1338| Sesquiterpene hydrocarbons       | 5.76 ± 0.01      | 1.40 ± 0.12      | 8.51 ± 0.05     |
| 1376| δ-Elemene                        | –                | –                | 0.15 ± 0.01     |
| 1385| α-Copaene                        | 0.05 ± 0.00      | –                | 0.04 ± 0.01     |
| 1392| β-Elemene                        | –                | 0.04 ± 0.01      | 0.26 ± 0.01     |
| 1409| α-Gurjunene                      | –                | –                | 0.08 ± 0.01     |
| 1419| β-Caryophyllene                  | 5.66 ± 0.01      | 0.86 ± 0.07      | 0.62 ± 0.00     |
| 1439| Aromadendrene                    | –                | –                | 0.06 ± 0.00     |
| 1454| α-Humulene                       | 0.05 ± 0.00      | 0.08 ± 0.01      | 0.05 ± 0.01     |
| 1461| allo-Aromadendrene               | –                | 0.06 ± 0.01      | 0.28 ± 0.02     |
| 1481| Germacrene D                     | –                | 1.27 ± 0.01      | –               |
| 1486| β-Selinene                       | –                | –                | 0.03 ± 0.01     |
| 1492| Valencene                        | –                | –                | 0.08 ± 0.00     |

(Continued)
α-copaene (0.05%) and α-humulene (0.05%) were found. Caryophyllene oxide (0.46 ± 0.01%) and 1-octen-3-ol (0.17%) were, respectively, the only oxygenated sesquiterpene and low molecular weight aliphatic compound identified. Finally, phenylpropanoid compounds were not detected in commercial oregano essential oil analysed here.

Also large quantities of monoterpene compounds (96.93% and 86.36%) both hydrocarbons (12.44% and 18.65%) and oxygenated monoterpens (84.49% and 67.71%) were found in marjoram and T. mastichina essential oils. 1,8-cineol (59.59 ± 0.85%, 49.49 ± 0.37%), linalool (13.15 ± 0.04%, 5.66 ± 0.01%) and α-terpineol (3.36 ± 0.10%, 5.59 ± 0.01%), followed by the monoterpene hydrocarbons β-pinene (4.35 ± 0.39%, 5.54 ± 0.01%) and α-pinene (4.11 ± 0.53%, 4.28 ± 0.01%), were the main compounds.

No higher percentages than 0.14% were found between the 16 sesquiterpene compounds identified in marjoram essential oil; however, among the sesquiterpene fraction of T. mastichina essential oil with 17 sesquiterpene hydrocarbons (8.51%) and six oxygenated sesquiterpenes (2.41%) identified, germacrene D (1.27 ± 0.01%), bicyclogermacrene (2.70 ± 0.01%), β-bisabolene (2.09 ± 0.01%) and globulol (1.03 ± 0.01%) reached percentages higher than 1%. Finally, the aromatic compound eugenol (0.01%) only was detected in marjoram essential oil.

**Seed germination and seedling growth against P. oleracea, L. multiflorum and E. crus-galli**

The effect of oregano, marjoram and T. mastichina essential oils against seed germination and seedling growth of P. oleracea, L. multiflorum and E. crus-galli is shown in Tables 2 and 3 and Figures 1–3, respectively. Oregano essential oil was the most effective, completely
inhibiting the seed germination of the three weeds at all doses (0.125, 0.25, 0.50 and 1 µL mL\(^{-1}\)) applied (Table 2). Marjoram and *T. mastichina* essential oils did not show any effect against *P. oleracea*, *L. multiflorum* and *E. crus-galli* seed germination. No significant differences were found between control and all concentrations of marjoram and *T. mastichina* essential oil tested (Table 2). However, despite germination not being inhibited, the germinated seed did not develop normally compared with the control (Figures 1–3).

### Table 2. Effects of oregano, marjoram and *T. mastichina* essential oils on *P. oleracea*, *L. multiflorum* and *E. crus-galli* seed germination.

| Concentration (µL mL\(^{-1}\)) | Oregano | Marjoram | *T. mastichina* |
|---------------------------------|---------|----------|-----------------|
| **P. oleracea**                 |         |          |                 |
| Control                         | 73.00 ± 3.74 a | 74.00 ± 4.60 a | 73.00 ± 3.74 ab |
| 0.125                           | 0.00 ± 0.00 b  | 80.00 ± 1.60 a | 66.00 ± 5.10 a  |
| 0.25                            | 0.00 ± 0.00 b  | 76.00 ± 5.30 a | 81.00 ± 1.87 ab |
| 0.5                             | 0.00 ± 0.00 b  | 83.00 ± 4.40 a | 81.25 ± 5.54 ab*|
| 1                               | 0.00 ± 0.00 b  | 84.00 ± 4.50 a | 84.00 ± 3.67 b  |
| **L multiflorum**               |         |          |                 |
| Control                         | 73.00 ± 3.39 a | 73.00 ± 3.39 a | 73.00 ± 3.39 a  |
| 0.125                           | 0.00 ± 0.00 b  | 67.00 ± 5.83 a | 71.00 ± 2.45 a  |
| 0.25                            | 0.00 ± 0.00 b  | 65.00 ± 4.47 a | 63.00 ± 2.55 a  |
| 0.5                             | 0.00 ± 0.00 b  | 65.00 ± 4.18 a | 69.00 ± 3.67 a  |
| 1                               | 0.00 ± 0.00 b  | 61.00 ± 5.10 a | 54.00 ± 2.92 a  |
| **E crus-galli**                |         |          |                 |
| Control                         | 71.00 ± 4.30 a | 71.00 ± 4.30 a | 71.00 ± 4.30 a  |
| 0.125                           | 0.00 ± 0.00 b  | 75.00 ± 1.58 a | 79.00 ± 4.85 a  |
| 0.25                            | 0.00 ± 0.00 b  | 78.00 ± 5.61 a | 80.00 ± 3.54 a  |
| 0.5                             | 0.00 ± 0.00 b  | 61.00 ± 4.30 a | 73.00 ± 3.00 a  |
| 1                               | 0.00 ± 0.00 b  | 71.00 ± 6.78 a | 81.00 ± 7.48 a  |

Notes: Values are mean of five replications ± error deviation after 14 days of incubation. Means followed by different letters in the same column indicate that they are significantly different at \( p < .05 \) according to T3 Dunnett’s test and Tukey's test (* four replications).

### Table 3. Effects of oregano, marjoram and *T. mastichina* essential oils on seedling length (hypocotyl and radicle) of *P. oleracea*, *L. multiflorum* and *E. crus-galli*.

| Concentration (µL mL\(^{-1}\)) | Marjoram | *T. mastichina* |
|---------------------------------|----------|-----------------|
| **P. oleracea**                 |          |                 |
| Control                         | 9.80 ± 0.92 a | 10.00 ± 1.58 a  |
| 0.125                           | 6.40 ± 0.25 b  | 9.20 ± 0.74 a   |
| 0.25                            | 6.60 ± 0.60 b  | 8.80 ± 0.86 a   |
| 0.5                             | 6.80 ± 0.37 b  | 9.00 ± 1.58 a   |
| 1                               | 6.60 ± 0.25 b  | 7.00 ± 0.84 a   |
| **L. multiflorum**              |          |                 |
| Control                         | 48.50 ± 3.35 a | 39.20 ± 2.14 a  |
| 0.125                           | 28.79 ± 2.27 b  | 36.77 ± 3.33 a  |
| 0.25                            | 22.69 ± 1.44 b  | 31.57 ± 1.29 a  |
| 0.5                             | 23.64 ± 1.26 b  | 32.38 ± 0.67 a  |
| 1                               | 21.63 ± 1.25 b  | 31.88 ± 0.80 a  |
| **E crus-galli**                |          |                 |
| Control                         | 30.01 ± 1.47 a | 21.06 ± 1.54 ab |
| 0.125                           | 25.69 ± 3.10 abc | 26.06 ± 1.94 a  |
| 0.25                            | 27.26 ± 0.52 ab | 15.28 ± 1.37 bc |
| 0.5                             | 20.17 ± 2.16 bc | 15.08 ± 1.60 bc |
| 1                               | 17.98 ± 1.89 c  | 12.95 ± 2.44 c  |

Notes: Oregano germination was 0 in all treatments and there was no seedling length to measure. Values are mean of five replications ± error deviation after 14 days of incubation. Means followed by different letters in the same column indicate that they are significantly different at \( p < .05 \) according to T3 Dunnett’s test and Tukey's test.
Regarding seedling growth, due to strong oregano phytotoxic activity, no seedling length was measured in the three weeds. Marjoram essential oil significantly inhibited hypocotyl of *P. oleracea* and *L. multiflorum* at all doses assayed without any significant effect in radicle elongation (Table 3). With respect to *E. crus-galli*, noteworthy alterations in both hypocotyls and radicle were detected between control and the highest dose applied (Table 3).

**Figure 1.** Values of seedling length (mm) (mean ± SE) of *P. oleracea* control and treated with natural marjoram essence (a) and *T. mastichina* essential oil (b) at 0.125, 0.25, 0.5 and 1 µL mL⁻¹ measured over 14 days.

**Figure 2.** Values of seedling length (mm) (mean ± SE) of *L. multiflorum* control and treated with natural marjoram essence (a) and *T. mastichina* essential oil (b) at 0.125, 0.25, 0.5 and 1 µL mL⁻¹ measured over 14 days.

**Figure 3.** Values of seedling length (mm) (mean ± SE) of *E. crus-galli* control and treated with natural marjoram essence (a) and *T. mastichina* essential oil (b) at 0.125, 0.25, 0.5 and 1 µL mL⁻¹ measured over 14 days.
*T. mastichina* essential oil showed no significant differences between control and treated seedlings' length (hypocotyls and/or radical), even without differences between concentrations, against *P. oleracea* (Table 3). However, this essential oil was able to inhibit in a dose-dependent manner both hypocotil and radicle elongation of *L. multiflorum* and *E. crus-galli* with significant inhibitory effect especially between the control and the highest dose (1 µL mL⁻¹) (Table 3) assayed.

**Discussion**

Because oregano is the common name of different species used for culinary purposes, such as Greek oregano (*O. vulgare* L. ssp *hirtum*), Turkish oregano (*O. onites* L.), and also Spanish oregano (*Thymus capitatus* (L.) Hoffmanns & Link) and Mexican oregano (*Lippia graveolens* HBK) belonging to other genera, several differences are found in their essential oil composition. In general, phenolic compounds (thymol and carvacrol) and their biogenetic precursors γ-terpinene and p-cymene are the main compounds in oregano essential oils, but with great variability in the percentage depending on the geographical origin. In certain regions of India, *O. vulgare* produces an essential oil rich in p-cymene (6.7–9.8%), γ-terpinene (12.4–14.0%), thymol (29.7–35.1%) and carvacrol (12.4–20.9%) (Pande, Tewari, Singh, & Singh, 2012), whereas Turkish oregano essential oil together with the phenolic compounds thymol (15.66%) and carvacrol (24.52%) contain high amounts of linalool (50.53%) (Ozkan & Erdoğan, 2011). Spanish oregano is rich in carvacrol (61.21%), p-cymene (15.12%) and γ-terpinene (4.80%) (Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2007), and finally, Mexican oregano content also has carvacrol as the main compound (47.41%) followed by p-cymene (26.44%) and thymol (3.02%) (Rodríguez-García et al., 2016). Our results are similar to those reported for *O. vulgare* growing in the Mediterranean area (Viuda-Martos et al., 2007), complying with the values of the monographs, so the commercial oregano analysed here is *O. vulgare* with optimum quality compatible with public health.

Although oxygenated monoterpenes are the main fraction of commercial marjoram essence analysed here and also of sweet marjoram (*O. majorana* L.) harvested in Tunisia at four phenological stages (Sellami et al., 2009), great differences were found between the principal compounds. Terpinen-4-ol (29.13–32.57%), cis-sabinene hydrate (19.9–29.27%) and trans-sabinene hydrate (3.5–11.61%) were the main components of this fraction in *O. majorana* essential oils harvested in Tunisia, whereas 1,8-cineole (58.59 ± 0.85%), linalool (13.05 ± 0.04%) and α-terpineol (3.33 ± 0.10%) were the main compounds in commercial natural marjoram essence analysed here.

Several studies indicate that terpinen-4-ol is responsible for the antihypertensive (Seong, Hong, Hur, & Lee, 2013) and anticancer (Shapira, Pleban, Kazanov, Tiros, & Arber, 2016) properties. This compound only reached 0.41% in marjoram essence analysed here. So, an anti-anxiety (Kim, Seo, Min, Park, & Seol, 2014), antimicrobial with application as natural preservative in food packaging (Taqi, Askar, Mutihac, & Stamatin, 2013), anti-inflammatory, antiviral or inhibitory of nuclear factor (NF)-kB effect (Li et al., 2016) could be expected with marjoram essence due to high 1,8-cineole (59.59%) content. Our results are in agreement with the percentages obtained from 20 samples of *T. mastichina* essential oils (1,8-cineol 56.80–69.60%) from Spain (Delgado et al., 2014), and according with ISO quality standards (Table 4) of *T. mastichina* (Mendez-Tovar,
Novak, Sponza, Herrero, & Asensio-S-Manzanera, 2016). So, marjoram essence analysed here could be this last species, known as Spanish marjoram. These results corroborate the need to indicate on the label not only the common name of the species as it appears in marjoram essence purchased, but also the main compounds (terpinen-4-ol or 1,8-cineole), especially when this product is the raw material in perfumery, cosmetic, agricultural and pharmaceutical industries.

In commercial *T. mastichina* essential oil analysed here, 1,8-cineol (49.49 ± 0.37%), linalool (5.66 ± 0.01%) and α-terpineol (5.59 ± 0.01%) were the main compounds. Recent studies between 11 wild populations of *T. mastichina* collected in Spain (Mendez-Tovar et al., 2016) showed that 1,8-cineol (58.52–68.82%) was the main compound in all analysed samples, and linalool (1.16–10.24%) exhibited a large range of variation. Despite this fact it was the least environmentally influenced species between the analysed ones (Spanish marjoram, spike lavender and Spanish sage). It is an important fact taking into account that 90% of their production in the Iberian Peninsula is harvested from its natural habitat. On the other hand, limonene, camphor and borneol which are included in quality ranges from ISO for *T. mastichina* (Table 4) were not identified in commercial *T. mastichina* essential oil analysed here, despite limonene was described in the label. Although *T. mastichina* is known as Spanish marjoram, qualitative and quantitative differences were found between the two commercial essential oils analysed, which could affect the biological activities, so that we tested also the phytotoxicity of these essential oils against *P. oleracea*, *L. multiflorum* and *E. crus-galli*, important weeds in summer crops of the Mediterranean area.

According to phytotoxicity, 1-O-cis-cinnamoyl-β-D-glucopyranose, the most potent allelochemical isolated from *Spiraea thunbergii* Sieb., could be explained by its cis-cinnamic configuration which showed a 100 times higher inhibition of lettuce root-growth than trans-cinnamic acid, being also able to inhibit the root growth of *Avena sativa*, *Triticum aestivum*, and *Arabidopsis thaliana* (Nishikawa et al., 2013). However, no phenylpropanoids in oregano essential oil that justify the strong inhibitory effect against *P. oleracea*, *L. multiflorum* and *E. crus-galli* seed germination have been detected.

The herbicidal activity of oregano essential oil is not due to the high percentage in oxygenated monoterpenes fraction, because also higher percentages are found in both marjoram (84.49%) and *T. mastichina* (67.71%). The responsible compound was the oxygenated monoterpenic carvacrol (60.42% vs. 0.02 and 1.63%, respectively) instead of 1,8-cineole (0.62% vs. 59.59 and 49.94%). This is in agreement with previous studies

### Table 4. Quality ranges from the International Standard Organization for *T. mastichina* L. (ISO 4728:2003) (Mendez-Tovar et al., 2016), natural marjoram and *T. mastichina* essential oils composition.

| Compounds       | *T. mastichina* ISO 4728:2003 (%) | Marjoram (%) | *T. mastichina* (%) |
|-----------------|----------------------------------|--------------|---------------------|
| α-Pinene        | 1.0–4.5                          | 4.11         | 4.28                |
| β-Pinene        | 2.0–5.0                          | 4.35         | 5.54                |
| Limonene        | 1.0–6.0                          | –            | –                   |
| 1,8-Cineole     | 30.0–68.0                        | 59.59        | 49.94               |
| Linalool        | 3.0–48.0                         | 13.50        | 5.66                |
| Camphor         | 0.1–2.0                          | 1.04         | –                   |
| δ-Terpineol     | 0.2–2.0                          | 0.40         | 1.86                |
| Borneol         | 0.1–1.8                          | 1.06         | –                   |
| Terpinen-4-ol   | 0.2–1.2                          | 0.41         | 0.83                |
| Linalyl Acetate | 0.2–4.0                          | 2.71         | 0.04                |
| β-Caryophyllene | 0.5–1.5                          | 0.86         | 0.62                |

Note: %: peak area percentage.
Angelini et al., 2003) with thymol, carvacrol and 1,8-cineole, in which the two phenolic compounds were more injurious to lettuce and common purslane, than 1,8-cineole at the same concentration. A recent study about the phytotoxic activity of 19 main compounds of essential oils against germination and root length of rigid ryegrass (Lolium rigidum), also corroborated that carvacrol, carvone, thymol, trans-anethole and linalool were the most phytotoxic components (Vasilakoglou, Dhima, Paschalidis, & Ritzoulis, 2013). However, in a study with 27 monoterpenes, both hydrocarbons and oxygenated ones, against seed germination and primary radicle growth of radish (Raphanus sativus L.) and garden cress (Lepidium sativum L.), only 1,8-cineole, inhibited their radicle elongation at the lowest concentrations (10^-5M, 10^-6M) applied (De Martino, Mancini, Almeida, & De Feo, 2010), showing also essential oils with high percentages of 1,8-cineole remarkable interference with germination and seedling growth of certain weeds like silver leaf nightshade (Solanum elaeagnifolium Cav.) that was inhibited by Eucalyptus spp., such as Eucalyptus salubris, Eucalyptus dundasii and Eucalyptus spathulata oils with 57.6%, 65.5% and 52.9% of 1,8-cineole, respectively (Zhang, An, Wu, Liu, & Stanton, 2012). However, our results showed that marjoram and T. mastichina essential oils with high 1,8-cineole content were not able to significantly inhibit P. oleracea radicle elongation at all concentrations (0.125, 0.25, 0.50 and 1 µL mL^-1) tested (Table 3). Although it is generally preferable to apply herbicides before crops’ emergence, weeds can arise to attack harvests after their germination so a post-control is also required. In this sense, 1,8-cineole and other cineole derivatives have also showed a dose-dependent post-emergence herbicidal activity against radish and annual ryegrass root and shoot growth (Barton, Clarke, Dell, & Knight, 2014).

On the other hand, linalool, the second main compound in T. mastichina and marjoram essential oils with 5.66 ± 0.01% and 13.50 ± 0.04%, respectively, has also been reported as the responsible oxygenated monoterpane of the herbicidal properties of a chemotype (90% linalool) of Zataria multiflora essential oil, against spontaneous barley, common rye, common amaranth and bermuda grass (Saharkhiz, Smaeili, & Merikhi, 2010). Our results corroborate that herbicidal activity of essential oils may be due to both main compounds since the phytotoxic effect of 1,8-cineole against different annual weeds (Chenopodium album, P. oleracea and E. crus-galli) and crops (R. sativus, Capsicum annum and Lactuca sativa) was smaller than other aromatic monoterpenes such as thymol and carvacrol (Angelini et al., 2003) and synergistic/antagonistic interaction between their different components, because minor variations in the essential oil constituents affect significantly hypocotyl seedling growth of P. oleracea (Table 3). So, for a given weed is possible to develop selective bioherbicides, least injurious to the crops as well as promising alternatives appropriate for uncultivated fields, with more phytotoxic components affecting both crops and weeds.

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No potential conflict of interest was reported by the authors.
References

Aazza, S., El-Guendouz, S., Miguel, M. G., Antunes, M. D., Faleiro, M. L., Correia, A. I., & Figueiredo, A. C. (2016). Antioxidant, anti-inflammatory and anti-hyperglycaemic activities of essential oils from *Thymbra capitata*, *Thymus albicans*, *Thymus caespititius*, *Thymus carnosus*, *Thymus lotocephalus* and *Thymus mastichina* from Portugal. *Natural Product Communications*, 11, 1029–1038.

Adams, R. P. (2007). *Identification of essential oil components by gas chromatography/mass spectrometry*. Carol Stream, IL: Allured Publishing.

Angelini, L. G., Carpanese, G., Cioni, P. L., Morelli, I., Macchia, M., & Flamini, G. (2003). Essential oils from Mediterranean Lamiaceae as weed germination inhibitors. *Journal of Agricultural and Food Chemistry*, 51, 6158–6164.

Barton, A. F. M., Clarke, B. R., Dell, B., & Knight, A. R. (2014). Post-emergent herbicidal activity of cineole derivatives. *Journal of Pest Science*, 87, 531–541.

Black, C., Haughey, S. A., Chevallier, O. P., Galvin-King, P., & Elliott, C. T. (2016). A comprehensive strategy to detect the fraudulent adulteration of herbs: The oregano approach. *Food Chemistry*, 210, 551–557.

Blázquez, M. A., & Carbó, E. (2015). Control of *Portulaca oleracea* by boldo and lemon essential oils in different soils. *Industrial Crops and Products*, 76, 515–521.

De Martino, L., Mancini, E., Almeida, L. F. R., & De Feo, V. (2010). The antigerminative activity of twenty-seven monoterpenes. *Molecules*, 15, 6630–6637.

Delgado, T., Marinero, P., Asensio-S.-Manzanera, M. C., Asensio, C., Herrero, B., Pereira, J. A., & Ramlhosa, E. (2014). Antioxidant activity of twenty wild Spanish *Thymus mastichina* L. populations and its relation with their chemical composition. *LWT-Food Science and Technology*, 57, 412–418.

Hajlaoui, H., Mighri, H., Aouni, M., Gharsallah, N., & Kadri, A. (2016). Chemical composition and in vitro evaluation of antioxidant, antimicrobial, cytotoxicity and anti-acetylcholinesterase properties of Tunisian *Origanum majorana* L. essential oil. *Microbial Pathogenesis*, 95, 86–94.

Huang, T. C., Lin, Y. L., & Chuang, K. P. (2010). Carvacrol has the priming effects of reactive oxygen species (ROS) production in C6 glioma cells. *Food and Agricultural Immunology*, 21, 47–55.

Khosravi, A. R., Shokri, H., Kermani, S., Dakhili, M., Madani, M., & Parsa, S. (2011). Antifungal properties of *Artemisia sieberi* and *Origanum vulgare* essential oils against *Candida glabrata* isolates obtained from patients with vulvovaginal candidiasis. *Journal de Mycologie Médicale/Journal of Medical Mycology*, 21, 93–99.

Kim, K. Y., Seo, H. J., Min, S. S., Park, M., & Seol, G. H. (2014). The effect of 1,8-cineole inhalation on preoperative anxiety: A randomized clinical trial. *Evidence-Based Complementary and Alternative Medicine*, 2014, 1–7.

Li, Y., Lai, Y., Wang, Y., Liu, N., Zhang, F., & Xu, P. (2016). 1,8-Cineol protect against influenza-virus-induced pneumonia in mice. *Inflammation*, 39, 1582–1593.

Lukas, B., Schmiederer, C., & Novak, J. (2015). Essential oil diversity of European *Origanum vulgare* L. (Lamiaceae). *Phytochemistry*, 119, 32–40.

Mendez-Tovar, I., Novak, J., Sponza, S., Herrero, B., & Asensio-S-Manzanera, M. C. (2016). Variability in essential oil composition of wild populations of Labiatae species collected in Spain. *Industrial Crops and Products*, 79, 18–28.

Nishikawa, K., Fukuda, H., Abe, M., Nakanishi, K., Tazawa, Y., Yamaguchi, C., … Shindo, M. (2013). Design and synthesis of conformationally constrained analogues of cis-cinnamic acid and evaluation of their plant growth inhibitory activity. *Phytochemistry*, 96, 223–234.

Ozkan, A., & Erdoğan, A. (2011). A comparative evaluation of antioxidant and anticancer activity of essential oil from *Origanum onites* (Lamiaceae) and its two major phenolic components. *Turkish Journal of Biology*, 35, 735–742.

Pande, C., Tewari, G., Singh, S., & Singh, C. (2012). Chemical markers in *Origanum vulgare* L. from Kumaon Himalayas: A chemosystematic study. *Natural Product Research*, 26, 140–145.
Revajová, V., Pistl, J., Levkut, M., Marcin, A., & Levkutová, M. (2010). Influence of oregano and salvia extracts on lymphocyte subpopulation and functional activity of blood phagocytes and lymphocytes in chickens. *Food and Agricultural Immunology, 21*, 307–316.

Rodríguez-García, I., Cruz-Valenzuela, M. R., Silva-Espinoza, B. A., González-Aguilar, G. A., Moctezuma, E., Gutiérrez-Pacheco, M. M., … Ayala-Zavala, J. F. (2016). Oregano (*Lippia graveolens*) essential oil added within pectin edible coatings prevents fungal decay and increases the antioxidant capacity of treated tomatoes. *Journal of the Science of Food and Agriculture, 96*, 3772–3778.

Rus, C. F., Alexa, E., Sumalan, R. M., Galuscan, A., Dumitrache, A.,imbrea, I. M., … Pop, G. (2016). Antifungal activity and chemical composition of *Origanum vulgare* L. essential oil. *Revista de Chimie, 67*, 2287–2289.

Saharkhiz, M. J., Smaeili, S., & Merikhi, M. (2010). Essential oil analysis and phytotoxic activity of two ecotypes of *Zataria multiflora* Boiss. growing in India. *Natural Product Research, 24*, 1598–1609.

Salgueiro, L., Martins, A. P., & Correia, H. (2010). Raw materials: The importance of quality and safety. A review. *Flavour and Fragrance Journal, 25*, 253–271.

Sellami, I. H., Maamouri, E., Chahed, T., Wannes, W. A., Kchouk, M. E., & Marzouk, B. (2009). Effect of growth stage on the content and composition of the essential oil and phenolic fraction of sweet marjoram (*Origanum majorana* L.). *Industrial Crops and Products, 30*, 395–402.

Seong, K., Hong, J. H., Hur, M. H., & Lee, M. S. (2013). Two-week aroma inhalation effects on blood pressure in young men with essential hypertension. *European Journal of Integrative Medicine, 5*, 254–260.

Shapira, S., Pleban, S., Kazanov, D., Tirossh, P., & Arber, N. (2016). Terpinen-4-ol: A novel and promising therapeutic agent for human gastrointestinal cancers. *PloS One, 11*, 1–13.

Silva, L., Gomes, A., & Palmeira-de-Oliveira, A. (2014, September). *Antimicrobial activity and composition of essential oils from Thymus mastichina L. collected in Beira Interior (Portugal).* Poster session presented at the meeting of 62nd International Congress and Annual Meeting of the Society of Medicinal Plant and Natural Product Research. doi:10.1055/s-0034-1394964

Sprink, J., & Moyer, D. C. (2013). Understanding and combating food fraud. *Food Technology, 67*, 30–35.

Taqi, A., Askar, K. A., Muthiac, L., & Stamatin, I. (2013). Effect of *Laurus nobilis* L. oil, *Nigella sativa* L. oil and oleic acid on the antimicrobial and physical properties of subsistence agriculture: The case of cassava/pectin based edible films. *Food and Agricultural Immunology, 24*, 241–254.

Vasilakoglou, L., Dhima, K., Paschalidis, K., & Ritzoulis, C. (2013). Herbicidal potential on *Lolium rigidum* of nineteen major essential oil components and their synergy. *Journal of Essential Oil Research, 25*, 1–10.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., & Pérez-Álvarez, J. A. (2007). Chemical composition of the essential oils obtained from some spices widely used in Mediterranean region. *Acta Chimica Slovenica, 54*, 921–926.

Zhang, J., An, M., Wu, H., Liu, D. L., & Stanton, R. (2012). Chemical composition of essential oils of four *Eucalyptus* species and their phytotoxicity on silver leaf nightshade (*Solanum elaeagnifolium* Cav.) in Australia. *Plant Growth Regulation, 68*, 231–237.