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Potential Use of Essential oils from Four Tunisian Species of Lamiaceae: Biological Alternative for Fungal and Weed Control

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Abstract: The chemical composition of the essential oils (EOs) of four Lamiaceae (*Thymus capitatus* Hoff. et Link., *Rosmarinus officinalis* L., *Origanum vulgare* L. and *Mentha pulegium* L.) growing wild in Tunisia was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Obtained results showed significant variations among the different species. The major constituents identified for each species were respectively carvacrol (69%) and δ-terpinene (17%) for *T. capitatus*, 1,8-cineole (41%) and α-pinene (24%) for *R. officinalis*, menthol (39%) and 1.8-cineole (17%) for *M. pulegium*, thymol (30%), p-cymene (30%) and δ-terpinene (27%) for *O. vulgare*. EO herbicidal effects were evaluated against three invasive weed species in most cultivated crops: *Sinapis arvensis* L., *Phalaris paradoxa* L. and *Lolium rigidum* Gaud. The study of herbicidal activity was carried out on seed germination and seedling vigor and growth. All tested EOs significantly inhibited the germination and growth of weeds in a dose dependent manner and their herbicidal activity could be attributed mainly to their high content in oxygenated monoterpenes. The antifungal ability of EOs was assessed by using disc agar diffusion against ten plant pathogenic fungi affecting crops and stored foods. The EOs displayed strong inhibitory effect on all tested fungi. Our results on EOs chemical composition and biological activities showed properties that could be valorized in managing biocontrol of weeds and plant fungi.

Keywords: *Mentha pulegium; Origanum vulgare; Rosmarinus officinalis; Thymus capitatus*; essential oil; herbicidal activity; antifungal activity.

1. Introduction

Pesticide use in agriculture has radically increased the efficiency of production. However, strong selection pressures are applied on the phytopathogenic fungi and weeds because of pesticides

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misuse. In addition, applying chemical treatment with same targets greatly favors the occurrence of resistant species, pesticide resistance issues being thus unavoidable [1, 2]. In particular, weeds have evolved resistance to 23 of the 26 known herbicide sites of action and to 160 different herbicides. Herbicide resistant weeds have been reported in 86 crops in 66 countries. Likewise, fungicide resistance is a major threat to disease control and is common among many plant pathogenic fungi [3].

In addition to resistance problems, use of synthetic pesticides involves environmental damage and toxicological concerns within plant and animal food products [4]. Such inefficient and unhealthy pesticides become thus obsolete and do not meet trade opportunities anymore. The detrimental effect produced on both Human and environment gives stimulation to search a safer and more environmentally friendly biological alternative to fungal and weed control. In this context, several research studies have focused on the potential of plant extracts including essential oils that have a broad spectrum of activities in the control of phytopathogens and weeds [4–6]. Therefore, our study focused on four Lamiaceae species commonly and naturally growing in Tunisia. Lamiaceae is a relatively common botanical family, members of which are found in the temperate regions worldwide, including approximately 220 genera and 3500 to 4000 species. Most species are herbaceous, annual or perennial, simple or composite with opposed or crossed leaves, which can be sessile or petiolate [7]. Many of the species are used as ornamentals and can potentially be used as medicinal or aromatic herbs in the industries such as the cosmetics, foods, hygienic products and perfumery. The secondary metabolites studied so far in this family are basically terpenoids and flavonoids, although alkaloids, iridoids and ursolic acid have been found [7].

Lamiaceae family is one of the most diverse and widespread plant families in terms of ethnomedicine and its medicinal value is mainly based on the volatile oils composition. It is well documented that some plants belonging to this family possess antimicrobial properties [8, 9]. Herbs of the Lamiaceae family, like rosemary, menthe, oregano and thyme are well-known for their essential oil content as well as for the content of phenolic compounds to which their biological activity was attributed. As previously mentioned, the increasing occurrence of pest's resistance represents a worldwide major concern for both human and environment [6]. Although several strategies have been proposed to overcome and control this situation, a powerful trend recommends the plant extracts to be investigated for their biological activity. Therefore, nowadays, there is a growing interest in the herbicidal and fungicidal screening of extracts and essential oils from plants in order to discover new biological antibiotic agents. Indeed, since the use of EOs in food and food products is being recognized as GRAS practice (Generally Recognized As Safe), it could be also applied with caution and authority agreement in cultivated fields as weed and fungi management [10]. In Tunisia, *Thymus capitatus*, *Rosmarinus officinalis*, *Origanum vulgare* and *Mentha pulegium* were the most widespread species belonging to Lamiaceae family and which are known for their richness in essential oils. According to the literature, there are many reports on the chemical composition of essential oils isolated from thyme, rosemary, menthe and oregano. However, knowing that the chemical composition of essential oils from aromatic plants depends on several factors such as the geographical origin, sampling season and genetic background of plant from which the oil was obtained; to the best of our knowledge, there is no reported study on their herbicidal or antifungal activities. So, the aims of this work were, in a first step, to analyze the chemical composition and determine the main constituents of essential oils extracted from *Thymus capitatus*, *Rosmarinus officinalis*, *Origanum vulgare* and *Mentha pulegium*; in a second step, to assess their herbicidal effects against the germination and seedling growth of *Sinapis arvensis*, *Lolium rigidum* and *Raphanus raphanistrum*; and finally, to highlight their potential antifungal activity against very harmful and common fungal pathogens to cultivated crops.

2. Materials and Methods

2.1. *Plant Material*

Aerial parts of *Thymus capitatus*, *Rosmarinus officinalis* were collected from the INRGREF arboretums (National Institute of Researches on Rural Engineering, Water and Forests) from the region of Korbous (north-east of Tunisia) whereas those of *Origanum vulgare* and *Mentha pulegium*

were collected from the region of Beja (north of Tunisia). Five samples collected from more than five different plants were harvested, then mixed for homogenization and used in three replicates for essential oil extractions. Each plant specimen was submitted to the herbarium division of the institute (INRGREF) and identification was confirmed in the Laboratory of Forest Ecology according to the flora of Tunisia [11].

2.2. Isolation of the essential oils

The essential oils were extracted by hydrodistillation of fresh plant material (100 g of each sample in 500 mL of distilled water) using a Clevenger-type apparatus for 3 hours according to the standard procedure described in the European Pharmacopoeia [12]. The oils were dried over using anhydrous sodium sulfate (a pinch/10 mL) and stored in sealed glass vials at 4°C before analysis. Yield was calculated based on dried weight of the sample (mean of three replications).

2.3. Gas Chromatography analysis

EO composition was investigated by GC and GC/MS. The analytical GC was carried out on an HP5890-series II gas chromatograph (Agilent Technologies, California, USA) equipped with Flame Ionization Detectors (FID) under the following conditions: the fused silica capillary column, apolar HP-5 and polar HP Innowax (30 m x 0.25 mm ID, film thickness of 0.25 mm). The oven temperature was held at 50°C for 1 minute then programmed at a rate of 5°C/minute to 240°C and held isothermal for 4 minutes. The carrier gas was nitrogen at a flow rate of 1.2 mL/minute; injector temperature: 250°C, detector: 280°C; the volume injected: 0.1 mL of 1% solution (diluted in hexane). The percentages of the constituents were calculated by electronic integration of FID peak areas without the use of response factor correction. GC/MS was performed in a Hewlett Packard 5972 MSD System. An HP-5 MS capillary column (30 m x 0.25 mm ID, film thickness of 0.25 mm) was directly coupled to the mass spectrometry. The carrier gas was helium, with a flow rate of 1.2 mL/minute. Oven temperature was programmed (50°C for 1 minute, then 50-240°C at 5°C/minute) and subsequently held isothermal for 4 minutes. Injector port: 250°C, detector: 280°C, split ratio: 1:50. Volume injected: 0.1 mL of 1% solution (diluted in hexane); mass spectrometer: HP5972 recording at 70 eV; scan time: 1.5 s; mass range: 40-300 amu. Software adopted to handle mass spectra and chromatograms was ChemStation. The identification of the compounds was based on mass spectra (compared with Wiley 275.L, 6th edition mass spectral library). Further confirmation was done from Retention Index data generated from a series of alkanes retention indices (relatives to C9-C28 on the HP-5 column) [13].

2.4. Seed germination and seedling growth experiments

Mature seeds of *Sinapis arvensis* L., *Lolium rigidum* Gaud. and *Phalaris canariensis* L. were collected from parent plants growing in cereal crop fields. To avoid possible inhibition of germination due to fungal or bacterial toxins, seeds were first sterilized with 15% sodium hypochlorite for 20 minutes and then rinsed with distilled water. Empty and undeveloped seeds were discarded by floating in tap water and the remaining seeds were sown in Petri dishes (90 mm diameter) containing two layers of Whatman filter paper impregnated with 8 mL of either the negative control solution (1% (v/v) of Tween 20 in H_2O), the positive control solution (commercial 2,4-D herbicide) and the essential oil solutions at the different concentrations. The essential oils were dissolved in and diluted to the desired doses (0.25, 0.5, 0.75, and 1 µL/mL) with a 1% (v/v) solution of Tween 20 in H_2O. Afterward, 20 seeds of each weed species were placed on the impregnated filter papers. The Petri dishes were closed with an adhesive tape to prevent escaping of volatile compounds and kept at 25°C in a growth chamber supplied with 12 hours of fluorescent light [14]. The number of germinated seeds and seedling lengths were measured after 15 days. All tests were arranged in a completely randomized design with three replications per treatment. The seedling vigor index (SVI) was calculated according to the formula of Abdul-Baki and Anderson [15] as SVI = Seedling length (mm) x Germination percentage (%).

2.5. Antifungal activity assays

Ten plant pathogenic fungi were obtained from the culture collection of the Tunisian National Institute of Agronomic Research (INRAT). Cultures of each fungal species were maintained on potato dextrose agar (PDA) for 2-3 months storage at 4°C; additionally, one mL glycerol (25%) stock was kept at -20°C for longer storage period. The fungal species used in this study were: *Fusarium culmorum*, *F. avenaceum*, *F. oxysporum*, *F. subglutinans*, *F. verticillioides*, *F. nygamai*, *Bipolaris sorokiniana*, *Botrytis cinerea*, *Microdochium nivale* and *Alternaria* sp.; they are the most common pathogens to cultivated crops, mainly cereals. For instance, infections by *Fusarium* are responsible for destroying crops or dramatically reducing production yields. In addition, the economic importance of *Fusarium* species is enhanced by their ability to synthesize harmful mycotoxins that may be transferred further within grains, food and stored products [16]. Antifungal activity was studied by using an *in vitro* contact assay which produces hyphal growth inhibition [17]. Essential oil was dissolved in 1 mL of Tween 20 (0.1% v/v) and then added into 20 mL PDA at 50°C to obtain a final concentration of 0.5 mg/mL. A mycelia disk of 5 mm in diameter, cut from the periphery of a 7 day-old culture, was inoculated in the center of each PDA plate, and then incubated at 24°C for 7 days. PDA plates treated with Tween 20 (0.1%) without essential oil were used as negative control. In addition, PDA plates treated with benomyl (0.8 mg/mL PDA), a commercial fungicide, were used as positive control. Tests were repeated in triplicate. Growth inhibition was calculated as the percentage of inhibition of radial growth relative to the control using the following formula: % Inhibition = ((C – T)/C) x 100. Where C is an average of three replicates of hyphal extension (mm) of controls, and T is an average of three replicates of hyphal extension (mm) of plates treated with essential oil.

2.6. Statistical analysis

Data of germination, seedling growth and antifungal activity assay were subjected to one-way analysis of variance (ANOVA) using the SPSS 17.0 software package (Statistical Package for the Social Sciences). Differences between means were tested through Student-Newman-Keuls (SNK) and values of $p \leq 0.05$ were considered significantly different [18]. To determine whether the EO constituents identified in our analysis are useful in reflecting the chemical relationships between Lamiaceae species, 11 compounds, detected in the oil samples with contents in the EOs higher than 8% in any species, were subjected to principal components analysis (PCA) using the same SPSS software.

3. Results and Discussion

3.1. Chemical composition of essential oils

The chemical composition of EOs, the percentage content of the individual components, the retention indices and the chemical class distribution of the oil compounds are summarized in Table 1. The hydrodistillation of aerial parts of *Mentha pulegium* yielded 1.84% with a yellowish color.

24 compounds were identified, representing 97.9% of the EO which is characterized by a high amount of oxygenated monoterpenes (86.35%). The major components were menthol (39.2%), 1,8-cineole (17.1%), menthone (12.6%) and pulegone (11.7%). EOs of *M. pulegium* have been previously reported in Tunisia (8); data obtained from this study showed the richness of *M. pulegium* in pulegone (44.27%), menthone (19.05%) and piperitone (10.44%), which is in accordance with our results, yet with slight differences in percentage values. Rosemary EO yielded 0.6% and the major compounds found in the oil were 1,8-cineole (40.9%), α-pinene (24.2%), camphor (11.1%) and borneol (9.4%). Similar results obtained by Zaouali and Boussaid [19] showed the same major components of the essential oil from Tunisian *Rosmarinus officinalis* but with different levels. *Origanum vulgare* yielded 0.9% yellow EO and 18 constituents were identified, representing 97.85% of the oil totality.

Table 1. Essential oils composition of *Mentha pulegium*, *Rosmarinus officinalis*, *Origanum vulgare* and *Thymus capitatus.*

N°	Compounds	RI	*M. pulegium*	*R. officinalis*	*O. vulgare*	*T. capitatus*
1	tricyclene	926	0.38	0.10	-	-
2	α-thujene	931	0.21	-	0.40	0.64
3	**α-pinene**	939	1.11	**24.20**	2.60	1.05
4	α-fenchene	952	1.30	-	0.10	-
5	camphene	954	0.98	0.30	-	0.31
6	sabinene	976	0.12	0.10	0.17	0.14
7	β-pinene	980	0.47	1.40	-	-
8	β-myrcene	991	1.57	0.23	0.60	-
9	α-phellandrene	1005	0.83	-	0.16	-
10	**1.8-cineole**	1014	**17.1**	**40.90**	0.31	-
11	α-terpinene	1017	0.50	-	2.51	-
12	**p-cymene**	1024	-	-	**29.40**	**8.29**
13	β-ocimene	1026	-	0.28	-	-
14	limonene	1029	0.31	0.30	0.51	-
15	**δ-terpinene**	1062	-	-	**26.80**	**16.60**
16	α-terpinolene	1088	0.38	0.41	-	-
17	linalool	1098	0.28	0.19	-	-
18	**menthone**	1128	**12.60**	-	-	-
19	**camphor**	1146	-	**11.10**	-	-
20	**menthol**	1164	**39.20**	-	-	-
21	**borneol**	1169	-	**9.40**	-	-
22	terpinen-4-ol	1177	1.91	1.30	0.71	-
23	α-terpineol	1189	0.42	0.61	0.60	-
24	verbenone	1204	-	0.97	-	-
25	**pulegone**	1228	**11.7**	-	-	-
26	(*Z*)-pinocarveol	1139	2.30	0.14	0.81	-
27	piperitone	1252	0.84	0.10	-	-
28	**thymol**	1280	-	-	**29.60**	0.90
29	**carvacrol**	1283	-	-	1.45	**69.15**
30	iso- bornyl acetate	1285	-	1.31	-	0.58
31	α-terpenyl acetate	1349	-	0.90	-	-
32	(Z)-caryophyllene	1408	1.4	1.70	-	-
33	α-humulene	1454	-	0.80	-	-
34	germacrene D	1485	0.99	0.20	0.29	-
35	caryophyllene oxide	1588	0.80	2.10	0.83	0.20
Total identification (%)			97.70	99.04	97.85	97.86
Monoterpene hydrocarbons (%)			8.16	27.32	63.25	27.03
Oxygenated monoterpenes (%)			86.35	66.92	33.48	70.63
Sesquiterpene hydrocarbons (%)			2.39	2.70	0.29	0.00
Oxygenated sesquiterpenes (%)			0.80	2.10	0.83	0.20

Retention Index on apolar HP-5 MS column; Percentage calculated by GC-FID on apolar HP-5 MS column; -, not detected.

Monoterpene hydrocarbons (63.25%) and oxygenated monoterpenes (33.48%) constituted major portion of oregano oil. Thymol (29.6%), *p*-cymene (29.4%) and δ-terpinene (26.8%) were considered to be the major components of oregano oil. In previous studies, Mechergui et al. [20], when studying the chemical composition of EO of oregano collected from different Tunisian regions, obtained results

displaying richness in p-cymene, thymol, δ-terpinene and carvacrol which is consistent with our results. EO yield of *Thymus capitatus* was 2.85% with ten characterized compounds representing 97.86% of the totality with 27.03% hydrocarbonated monoterpenes and 70.63% oxygenated monoterpenes. The chemical composition of this oil consisted mainly in carvacrol (69.15%), δ-terpinene (16.6%) and *p*-cymene (8.29%). The chemical composition of Tunisian *T. capitatus* EO was previously investigated and it was shown that carvacrol, δ-terpinene and *p*-cymene were the major components of the oil which is in agreement with our results [9]. Principal component analysis of Lamiaceae EOs led us to the extraction of two components represented by axis 1 and 2 drawn on the factorial map (Figure 1). *M. pulegium* forms an isolated group highly correlated with the vertical axis and characterized by its richness in menthol, menthone and pulegone. While *R. officinalis* forms also a separated group that is correlated to the horizontal axis, with 1,8-cineole, α-pinene, camphor and borneol as principal components defining its chemotype. The last and third group is formed by *T. capitatus* and *O. vulgare* EOs that share somehow common components like δ-terpinene and p-cymene.

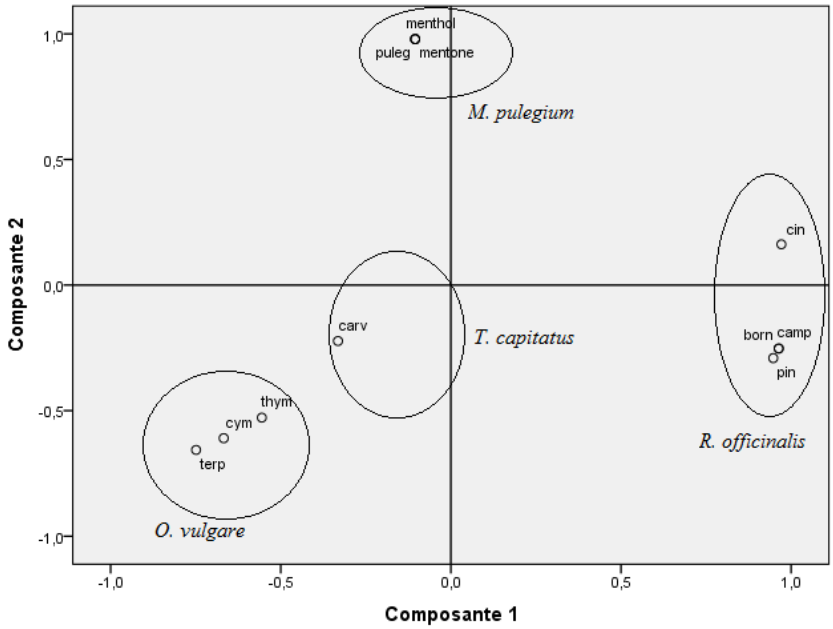

Figure 1. Principal component analysis of 11 compounds for the EOs of four Lamiaceae species

3.2. Herbicidal effects of essential oils on weeds germination and seedlings growth

The phytotoxic effects of tested oils were studied on seed germination and seedling growth of *Sinapis arvensis*, *Lolium rigidum* and *Phalaris canariensis*, important weeds in Tunisia and very common to most cultivated crops. Tables 2-5 show that essential oils strongly inhibited the germination and seedling growth of tested weeds in a dose dependent manner with the effect being significantly more effective on *S. arvensis* in comparison with *P. canariensis* and *L. rigidum*. Our study of EOs inhibitory effect on seed germination shows interesting results even better than those obtained by the commercial herbicide (positive control), *M. pulegium* EO displaying the best phytotoxic effects (Table 2). Indeed, at low concentrations of EOs (0.5 µL/mL for *S. arvensis* and 0.75 µL/mL for *P. canariensis* and *L. rigidum*), the germination and seedling growth of weeds were almost reduced. For higher concentrations (0.75 µL/mL for *S. arvensis* and 1 µL/mL for *P. canariensis*), the germination and seedling growth were completely inhibited. Moreover, tables 3 and 4 show that inhibitory effects of EOs affect both roots and aerial parts of weeds. In addition, in the same way,

seedling vigor of weeds (Table 5) is greatly affected by EOs treatment which in fields, minimize their competitiveness and should potentially help cultivated crops to better take advantage from soil nutrients and water. In addition to time of exposure, it is well recognized that the toxicity is function of the dose [21]. Allelopathic potential of Thyme and Rosemary EOs against some cultivated legumes and weed seeds have been reported by Angelini et al. [22]. In recent reports, Amri and co-workers [23–27] have shown the herbicidal effects of EOs belonging to Pinaceae, Cupressaceae, Anacardiaceae and Myrtaceae families. It has been shown that the herbicidal effects of EOs resulted from the combined reactions of several compounds including addition, synergetic and antagonistic effects [28].

Table 2. Inhibitory effects of essential oils against weeds germination (%).

Weeds	**Doses (µL/mL)**	***M. pulegium***	***R. officinalis***	***T. capitatus***	***O. vulgare***
S. arvensis	C-	96.7a	96.7a	96.7a	96.7a
	0.25	53.3b	83.3b	66.7b	53.3b
	0.5	16.7c	53.3c	30.0c	20.0c
	0.75	0d	40.0d	6.7d	10.0cd
	1	0d	0f	0d	0e
	C+	20.0c	20.0e	20.0c	20.0c
P. canariensis	C-	93.3a	93.3a	93.3a	93.3a
	0.25	70.0b	56.7b	56.7b	70.0b
	0.5	43.3c	36.7c	36.7c	53.3c
	0.75	16.7d	30.0c	26.7d	33.3d
	1	6.7d	20.0d	16.7d	13.3e
	C+	17.5d	17.5d	17.5d	17.5e
L. rigidum	C-	83.3a	83.3a	83.3a	83.3a
	0.25	76.7a	76.7a	70.0b	53.3b
	0.5	53.3b	53.3b	43.3c	43.3c
	0.75	33.3c	20.0c	33.3c	26.7cd
	1	13.3d	16.7c	6.7e	16.7d
	C+	10.0d	10.0c	10.0d	10.0d

Means in the same column by the same letter are not significantly different of the test Student-Newman-Keuls ($p \leq 0.05$). (Mean of three replicates). C-: negative control, C+: positive control.

Looking to the chemical composition of the oils, more than 22 compounds are known to have herbicidal activity; α-pinene, β-pinene, camphene, β-myrcene, limonene, α-phellandrene, α-terpinene and γ-terpinene are 8 hydrocarbonated monoterpenes that are present in our oils, indeed, these compounds have been reported to have herbicidal activities [28, 29]. 1.8-cineole, menthone, camphor, menthol, borneol, pulegone, piperitone, linalool, α-terpineol, terpinyl acetate and bornyl acetate are 11 oxygenated monoterpenes; thymol and carvacrol are two phenolic monoterpenes, these compounds are present in tested oils with different percentages and they are known for their potential herbicidal activity [28, 29]. Although the exact mechanisms of EO action on germination and seedling growth inhibition remain unclear, however, a number of effects and hypothesis have been reported by many authors. Menthol would be responsible for the strongest phytotoxic activity displayed by *M. pulegium* EO. Regarding 1,8-cineole, major component of *R. officinalis* and *M. pulegium* EOs, the phytotoxicity of this molecule seems to be due to its epoxide moiety by inhibiting all stages of cell mitosis [4]. Monoterpenes like α-pinene, major compound of *R. officinalis* EO, provoke structural breakdown and decomposition of cell membrane via oxidative effect and suppression of respiratory control. Another proposed mechanism of action is due to the lipophilic nature of most monoterpenes that could alter the packing, fluidity, and/or physical arrangement of phospholipids in the membrane [4]. In general, the majority of reports agree that EOs have phytotoxic effects that may cause anatomical and physiological changes in plant seedlings leading to accumulation of lipid globules in the cytoplasm, reduction in some organelles such as mitochondria, possibly due to inhibition of DNA synthesis, affecting mitotic activity or disruption of membranes surrounding mitochondria and nuclei [30,31].

Table 3. Inhibitory effects of essential oils on roots growth of weeds (cm).

Weeds	Doses (µL/mL)	*M. pulegium*	*R. officinalis*	*T. capitatus*	*O. vulgare*
S. arvensis	C-	12.8a	12.8a	12.8a	12.8a
	0.25	7.7b	10.4b	8.9b	9.6b
	0.5	6.4c	9.5b	6.1c	6.9c
	0.75	0e	4.6c	2.4d	2.5d
	1	0e	2.0d	0e	0e
	C+	3.2d	3.2c	3.2d	3.2d
P. canariensis	C-	11.8a	11.8a	11.8a	11.8a
	0.25	11.1a	9.7b	8.9b	8.9b
	0.5	7.3b	6.9c	6.6c	6.9c
	0.75	4.0c	3.5d	3.9d	4.8d
	1	1.6d	1.9e	2.5e	2.3e
	C+	4.0c	4.0d	4.0d	4.0d
L. rigidum	C-	8.9a	8.9a	8.9a	8.9a
	0.25	7.6ab	7.7a	7.6ab	7.1b
	0.5	6.9b	5.5b	6.6b	6.2c
	0.75	4.6c	3.9c	4.6c	3.4d
	1	1.9d	2.3d	1.4d	1.1e
	C+	4.5c	4.5c	4.5c	4.5c

Means in the same column by the same letter are not significantly different of the test Student-Newman-Keuls ($p \leq 0.05$). (Mean of three replicates). C-: negative control, C+: positive control.

Table 4. Inhibitory effects of essential oils on shoots growth of weeds (cm).

Weeds	Doses (µL/mL)	*M. pulegium*	*R. officinalis*	*T. capitatus*	*O. vulgare*
S. arvensis	C-	12.5a	12.5a	12.5a	12.5a
	0.25	8.1b	10.3b	8.6b	10.5a
	0.5	3.5c	7.9c	5.5c	6.2b
	0.75	0d	4.2d	3.4d	3.6c
	1	0d	2.6e	0e	0d
	C+	2.5c	2.5de	2.5d	2.5c
P. canariensis	C-	10.5a	10.5a	10.5a	10.5a
	0.25	8.5ab	7.2b	9.7a	8.3b
	0.5	6.3b	5.5c	6.7b	6.1c
	0.75	3.1c	4.2c	6.6b	3.9d
	1	1.9c	2.0d	5.3b	2.1e
	C+	2.0c	2.0d	2.0c	2.0de
L. rigidum	C-	10.8a	10.8a	10.8a	10.8a
	0.25	9.2b	7.9b	8.6b	7.2b
	0.5	8.1b	5.4c	5.9c	4.6c
	0.75	3.6c	1.9d	3.3d	2.7d
	1	1.6d	1.3e	1.3e	1.5d
	C+	4.5c	4.5c	4.5c	4.5c

Means in the same column by the same letter are not significantly different of the test Student-Newman-Keuls ($p \leq 0.05$). (Mean of three replicates). C-: negative control, C+: positive control.

3.3. Antifungal activity of essential oils

EOs isolated from aerial parts of *Thymus capitatus*, *Rosmarinus officinalis*, *Origanum vulgare* and *Mentha pulegium* were tested for their antifungal activity against ten plant pathogenic fungal species. According to the results shown in table 6, all EOs displayed significant inhibition of fungal growth, also indicating that the antifungal activity is variable depending on the fungal strain and tested oils. All the tested fungi have been their growth reduced by at least 83% using one of the Lamiaceae EOs, *Thymus* EO displaying the strongest inhibitory effect. Noteworthy, Lamiaceae EOs could inhibit growth of *Alternaria* sp. clearly better than the commercial fungicide (positive control) (Table 6). Several species belonging to *Lamiaceae* family have shown an antimicrobial activity. Bounatirou et al.

[9] have reported EO antibacterial and antioxidant activities of Tunisian *T. capitatus* collected from different provenances. EOs of *Thymus* and *Origanum* species contain mainly aromatic monoterpenes, carvacrol, thymol, and p-cymene and their activity are very often attributed to these compounds [32, 33]. In another report, Ouraini et al. [34] have shown the antifungal activity of *T. saturejoides* L. and *M. pulegium* L. EOs, wich is in agreement with our obtained data.

Table 5. Inhibitory effects of essential oils on seedling vigor index (SVI).

Weeds	Doses (µL/mL)	*M. pulegium*	*R. officinalis*	*T. capitatus*	*O. vulgare*
S. arvensis	C-	24541	24541	24541	24541
	0.25	8421	17243	11672	10713
	0.5	1653	9274	3480	2620
	0.75	0	3520	388	610
	1	0	0	0	0
	C+	1140	1140	1140	1140
P. canariensis	C-	20806	20806	20806	20806
	0.25	13720	9582	10603	12040
	0.5	5889	4551	4881	6929
	0.75	1186	2310	2803	2897
	1	234	1170	1295	585
	C+	1575	1575	1575	1575
L. rigidum	C-	16410	16410	16410	16410
	0.25	12886	11965	11340	7569
	0.5	7995	5810	5456	4676
	0.75	2731	1180	2631	1623
	1	479	601	181	434
	C+	900	900	900	900

Means in the same column by the same letter are not significantly different of the test Student-Newman-Keuls ($p \leq 0.05$). (Mean of three replicates). C-: negative control, C+: positive control.

Mahboubi and Kazempour [35] have shown the antimicrobial potential of thirteen EOs (among them *Ocimum basilicum* L., *T. capitatus*, *M. pulegium*, *M. piperita* and *R. officinalis*) against microorganisms including bacteria, fungi and yeast. The antifungal activity of the oils in this study can be attributed to the high proportions of oxygenated monoterpenes such as carvacrol, thymol, pulegone and 1,8-cineole; however, other major or trace components in the oil could give rise to the antifungal activity of the oil. There are also possible synergistic and antagonistic interactions among the components. The mode of action of EOs was investigated by many authors who suggested that the antimicrobial activity is produced by interactions provoked by terpenes in the enzymatic systems related to energy production and in the synthesis of structural components of the microbial cells [36]. Both thymol and carvacrol isomers and their precursor p-cymene were shown to depolarize microbial cell membranes, increasing thus membrane permeability and disrupting ion homeostasis leading to metabolic activity disruption [37, 38].

Table 6. Inhibitory effects of essential oils on fungal growth

	M. pulegium	*R. officinalis*	*T. capitatus*	*O. vulgare*	C+
F. avenaceum	35.2 aB	21.83 abA	89.9 abcD	77.4 bcC	100 aE
F. culmorum	60.8 cdB	51.1 cA	99.3 dD	90.3 deC	100 aD
F. oxysporum	52.4 bcB	33.3 bA	89.5 abcD	80.3 bcC	100 aE
F. subglutinans	61.3 cdB	28.0 abA	88.8 abcC	94.4 eC	100 aD
F. verticillioides	56.9 bcdB	15.9 aA	83.3 aD	67.3 aC	100 aE
F. nygamai	55.9 bcdA	55.7 cA	95.5 cdC	82.1 bcdB	100 aD
Botrytis cinerea	47.8 abA	59.1 cB	100 dD	86.6 cdeC	100 aD
Microdochium nivale	58.5 bcdB	26.6 abA	86.1 abC	85.4 cdeC	100 aD
Alternaria sp	61.1 cdB	28.1 abA	92.3 bcdD	79.6 bcC	24.1 bA
Bipolaris sorokiniana	66.9 dB	25.1 abA	84.7 abC	75.8 bBC	100 aD

(%).Means in the same column/line by the same lowercase/uppercase letter are not significantly different of the test Student-Newman-Keuls ($p \leq 0.05$). (Mean of three replicates). C+: positive control (benomyl fungicide).

Other reports suggested that EO components cross the cell membrane, interacting with the enzymes and proteins of the membrane such as the H^+/ATPase pumping membrane, so producing a flux of protons toward the cell, exterior which induces changes in the cells and, ultimately, their death. Besides, several authors [39–41] reported that the antimicrobial activity is related to ability of terpenes to affect not only permeability but also other functions of cell membranes, these compounds might cross the cell membranes, thus penetrating into the interior of the cell and interacting with critical intracellular sites.

4. Conclusion

EOs obtained by hydrodistillation from selected Lamiaceae species were mainly composed of oxygenated monoterpenes and have shown promising activities. Our results showed that tested oils exhibited strong phytotoxic and antifungal effects that may represent useful and valuable leads for the development of new pesticides. Moreover, given their chemical composition, mixture of these Lamiaceae EOs would probably enhance their biological activities. Based on our preliminary results, the essential oils of four tested species could be suggested as alternative herbicides and fungicides. However, for a better and optimized formulation of a potential bio-pesticide, further studies are required to determine the biological standardization, quality control, cost, applicability, safety and phytotoxicity against cultivated crops.

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