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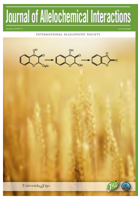

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Phytotoxic activity and GC-MS chemical characterization of apple mint foliar volatiles and essential oils

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ABSTRACT

Foliar volatiles and essential oils produced by apple mint [*Mentha rotundifolia* auct. non (L.) Huds.], a widely distributed Mediterranean species, were investigated for their phytotoxic activities on seed germination and/or root growth of the sensitive test species *Lactuca sativa* and four of the most common weeds largely found in several agro-ecosystems (*Amaranthus retroflexus, Chenopodium album, Echinochloa crusgalli* and *Eleusine indica*). The results pointed out that foliar volatiles (VOCs) were characterized by a high inhibitory effect on both germination and root growth of lettuce. Moreover, apple mint essential oils, extracted from fresh material through steam distillation, were characterized by a high phytotoxicity on weeds germination and root growth. The chemical characterization of the essential oils, carried out through GC-MS, highlighted that monoterpenes were the main chemicals followed by sesquiterpenes. Interestingly, the most abundant compounds were α-pinene, β-pinene and limonene, which are largely known to be strong phytotoxic allelochemicals.

Keywords: Apple mint, phytotoxicity, essential oils, terpenes, volatile organic compounds, root growth, seed germination, weeds.

Introduction

Weeds are one of the major constrains in agricultural systems limiting both crop yield and quality (Schmidt and Pannell, 1996). Nowadays, synthetic herbicides are an important and effective component of any weed control program (Derksen *et al*., 1994; Melander *et al*., 2005). However, they are responsible of important problems such as environmental pollution, health human hazards and weed resistance development (Melander *et al*., 2005). Therefore, the use of natural compounds could be considered as an eco-friendly and cost-effective alternative strategy to synthetic herbicides for weed control (Duke *et al*., 2000). In the plant kingdom, aromatic species represent an important source of natural compounds with herbicidal activity. They release a variety of volatiles, especially terpenoids, which affect neighboring growth and fitness, altering, in natural ecosystems, the composition of plant communities (Araniti *et al*., 2017b; Souza-Alonso *et al*., 2017). Furthermore, their essential oils, mainly composed by terpenoids, have been widely studied for their phytotoxic potential and their possible employment in weed management (Dudai *et al*., 1999; Tworkoski, 2002; Batish *et al*., 2008). Several essential oils isolated from species, belonging to the Labiatae family, have shown a high phytotoxicity on several common weeds (Tworkoski, 2002; Vasilakoglu *et al*., 2007; Araniti *et al*., 2013). For example, Araniti *et al*. (2013) demonstrated that both essential oils and volatiles released by *Calamintha nepeta* strongly affected germination and root growth of lettuce, radish and *Amaranthus retroflexus*, whereas *Echinochloa crusgalli* was slightly influenced. Recently, Souza-Alonso *et al*. (2017) demonstrated that the volatiles released from the alien species *Acacia longifolia* were able to affect several physiological parameters of different native species.

In the recent years, a great interest has been mainly focused on the identification of the mode of action of single molecules isolated from the essential oils. In particular, Graña *et al*. (2013) demonstrated that, in *Arabidopsis thaliana*, the monoterpene citral was able to affect the cell ultrastructure, the hormonal balance, the photosynthetic machinery as well as plant fitness. Similarly, Araniti *et al*. (2017a) observed that the sesquiterpene farnesene caused a left-handedness root phenotype altering auxin transport and distribution as well as microtubule organization

(Araniti *et al*., 2016). In addition, the sesquiterpene *trans*-caryophyllene affected the plant water status of adult plants of *A. thaliana* (Araniti *et al*., 2017c).

These findings highlighted that different terpenoids might have different targets and mode of actions; therefore, they could be used as a mixture in the development of new bio-herbicides characterized by a multi-mode of action. This could represent a possible strategy to avoid weed resistance that is largely increased when the herbicides are characterized by a single target and/or mode of action (Holt *et al*., 1993). Indeed, the essential oils, which are a mixture of biologically active multi-target compounds, might satisfy such requirements since the synergistic effects among their constituens have been largely proved (Araniti *et al*., 2012; Asplund, 1969; Vokou *et al*., 2003).

Mentha rotundifolia auct. non (L.) Huds., also known as apple mint, is a naturalized perennial grass belonging to the *Labiateae* family, widely distributed in the Mediterranean area, where it forms large monospecific communities. Generally found along streams and humid areas, it is extremely appreciate for its antiemetic, antidiarrhaea, antihaemorrhoidal, and analgesic effects (Moreno *et al*., 2002). Moreover, recent studies pointed out that its essential oils are characterized by a high antimicrobial, antiviral as well as insecticidal and antioxidant activity (Brahmi *et al*., 2016; Civitelli *et al*., 2014). Despite these many pharmacological roles, few information is available concerning to the phytotoxic activity of essential oils and no information on the ability of foliar volatiles to affect plant growth.

Therefore, in the present study the phytotoxic effects of apple mint foliar volatiles on germination and root growth of *Lactuca sativa* L were evaluated. Moreover, the essential oils, extracted through steam distillation, were chemically characterized trough GC-MS and assayed on germination and root growth of the sensitive species *L. sativa* and four of the most common weeds of the Mediterranean area.

Materials and methods

Sample collection and essential oils extraction

The aboveground parts (leaves, green stems and flowers) of apple mint were sampled on May in Southern Italy (Reggio Calabria), at the beginning of the flowering stage. Immediately after collection, the aerial parts (4 kg) were subjected to hydro-distillation for 3 h using a Clevenger-type apparatus. Successively, the essential oils were dried under anhydrous sodium sulphate to remove traces of moisture and then stored at 4-8 °C until use.

GC-MS analysis

The essential oils were chemically characterized using a Thermo Fisher gas chromatograph apparatus (Trace 1310) coupled to a single quadrupole mass spectrometer (ISQ LT). The capillary column was a TG-5MS 30 m×0.25 mm×0.25 µm, the gas carrier was helium with a flow of 1 mL min^{-1}. Injector and source were set at the temperature of 200 °C and 260 °C, respectively. Samples were solubilized in ethanol, at the 1000 ppm concentration, and injected in a split mode with a split ratio of 60. The programmed temperature was as follow: isocratic for 7 min at 45 °C, from 45 °C to 80 °C with a rate of 10 °C×min, from 80 °C to 200 °C with a rate of 20 °C×min then isocratic for 3 min at 200 °C. Mass spectra were recorded in electronic impact (EI) mode at 70 eV, scanning the 45–500 m/z range. Compounds identification was carried out comparing the relative retention time and mass spectra of the molecules with those of the commercial libraries (NIST).

In-vitro volatile bioassay

The *in-vitro* volatile bioassay was carried out as previously described by Araniti *et al*. (2013). In particular, immediately after collection, 2.5, 5, 10 and 20 g of *M. rotundifolia* leaves were cut, folded and wrapped in cheesecloth. Each sample was suspended at the lid of a capped 500 mL flask containing previously sterilized seeds (15 per flask) or seedlings (5 per flask) of *L. sativa*. Seeds and seedlings were placed in a double layer of filter paper moistened with 4 mL of deionized water. Flasks with no leaves were used as control. The experiment was carried out in dark condition at 25 ± 1 °C temperature and 70% relative humidity. Germination was assessed at regular intervals, depending on species, until complete germination (1 mm of root extrusion) of control plants. Total germination index [G_T (%)] was evaluated as previously described by Chiapusio *et al*. (1997), whereas, after 48 h of treatment, total root length (TRL) was captured by scanner (Epson Expression 800, Regent Instruments, Quebec, Canada) and then analysed using the WinRhizo Pro System v. 2002a software (Instruments Règent Inc., Quebec, Canada).

Essential oils bioassays

Both germination and root growth bioassays were carried out on *L. sativa* and four common weeds, *Chenopodium album*, *Echinochloa crusgalli*, *Amaranthus retroflexus* and *Eleusine indica*. Pre-germination treatments were applied in order

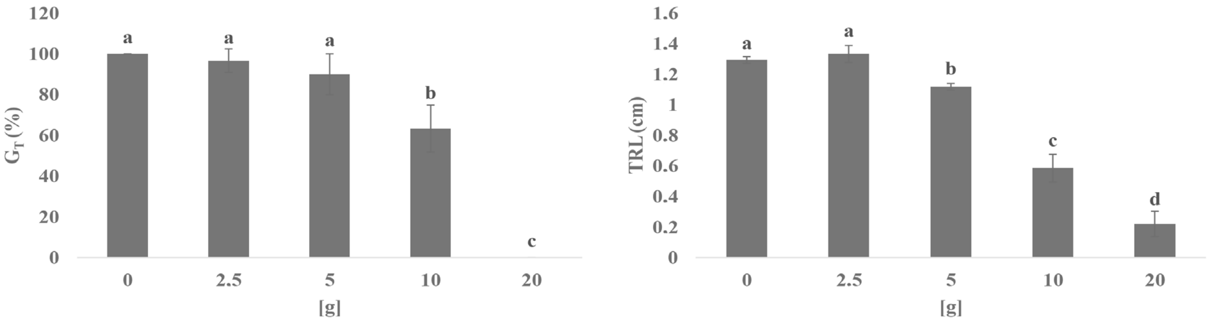

Figure 1.
Total Germination Index [G_T (%)] and Total Root Length (TRL, cm) parameters of lettuce seeds and seedlings, exposed to foliar VOCs released by 0, 2.5, 5, 10 and 20 g of apple mint fresh leaves. Different letters along the bars indicated statistically significant differences at $P < 0.05$ (Tukey's test). (N=4).

to break seed dormancy and synchronize weeds germination. Specifically, *A. retroflexus*, *C. album* and *E. crusgalli* seeds were soaked in water at 4 °C for 15 days, while no pre-treatments were applied for *E. indica* and lettuce. After sterilization with 15% (v/v) NaClO solution for 15 min, 15 seeds for each species were evenly distributed in Petri dishes (9 cm Ø) on a double filter paper layer wetted with 5 mL of 0, 8, 16, 32, 64, 125, 250, 500 µL L^{-1} of *M. rotundifolia* essential oils, previously solubilised in EtOH (0.1%). Petri dishes were then placed in dark conditions in a growth chamber at 25 °C (30 °C for *A. retroflexus*) and 70% relative humidity. Depending on the tested species, germinated seeds were counted at different times of exposure to essential oils and seeds were considered germinated as reported above. According to Chiapusio *et al*. (1997) at the end of these observations, total germination index [GT (%)], average speed of germination (S) and speed of accumulated germination (AS) were calculated.

To study the effects of the essential oils on root growth, five seedlings, selected for uniformity in size, were placed in sterile Petri dishes and treated, as previously described, for 48 h. At the end of the experiment, root growth was evaluated as described in the previous paragraph.

Experimental design and statistical analysis

All the experiments were carried out in a completely random design with 4 replications. Data were firstly evaluated for normality (Kolmogorov-Smirnov test) and then tested for homogeneity of variances (Levene's test). Successively, statistical differences among groups were estimated through one-way ANOVA followed by Tukey's test ($P \leq 0.05$).

The ED_{50} values of both germination and root growth of species treated with *M. rotundifolia* essential oils, were evaluated by nonlinear regression using a log-logistic function as previously reported by Araniti *et al*. (2012). To compare the phytotoxic effects of both volatiles and essential oils on the different test species the ED_{50} values were checked for normality (Kolmogorov-Smirnov test) and tested for homogeneity (Leven Median test) and then statistical differences among groups were evaluated through one-way ANOVA using the Tukey's test ($P < 0.05$).

Results and discussions

In-vitro volatile bioassay

The preliminary experiment was carried out in order to verify the biological activity of the VOCs released by *M. rotundifolia* on lettuce germination and/or root growth. The sensitivity to xenobiotics and/or allelochemicals of this test species has been largely documented (Pennacchio *et al*., 2005; Macias *et al*., 2000). The volatile bioassay, already tested on *Artemisia vulgaris* foliage (Barney *et al*., 2005) and *Calamintha nepeta* (Araniti *et al*., 2013), was designed to verify, in an enclosed environment, only the indirect contact between the VOCs released and the test species.

Interestingly, the results pointed out that the apple mint VOCs strongly affected both lettuce germination and root growth (Fig. 1).

The G_T (%) parameter was not significantly affected by apple mint VOCs released by 2.5 g and 5 g of leaves, while those released by 10 and 20 g caused 37% and 100% of inhibition of seeds germination,

Table 1.
Total germination index [G_T (%)], average speed of germination (S) and speed of accumulated germination (AS) of lettuce and weeds exposed to different concentrations of apple mint essential oils.

Concentration	G_T (%)	S	AS
	L. sativa		
0	97.3(±1.6)[a]	4.3(±0.1)[a]	8.9(±0.2)[a]
15	96.0(±2.7)[a]	3.7(±0.1)[ab]	6.6(±0.3)[b]
30	96.0(±2.7)[a]	3.6(±0.1)[b]	6.0(±0.4)[c]
60	2.7(±1.6)[b]	0.1(±0)[c]	0.3(±02)[c]
120	0.0(±0)[c]	0.0(±0)[c]	0.0(±0)[d]
240	0.0(±0)[c]	0.0(±0)[c]	0.0(±0)[d]
480	0.0(±0)[c]	0.0(±0)[c]	0.0(±0)[d]
	C. album		
0	96.0(±2.7)[a]	3.6(±0.08)[a]	10.4(±0.18)[a]
15	97.3(±1.6)[a]	3.5(±0.06)[a]	9.5(±0.25)[b]
30	93.3(±2.1)[a]	2.8(±0.04)[b]	6.7(±0.25)[c]
60	45.3(±2.5)[b]	1.1(±0.06)[c]	1.6(±0.15)[d]
120	0.0(±0.0)[c]	0.0(±0)[d]	0.0(±0)[e]
240	0.0(±0.0)[c]	0.0(±0)[d]	0.0(±0)[e]
480	0.0(±0.0)[c]	0.0(±0)[d]	0.0(±0)[e]
	A. retroflexus		
0	100.0(±0.0)[a]	4.6(±0.1)[a]	10.2(±0.2)[a]
15	100.0(±0.0)[a]	4.2(±0.0)[b]	8.5(±0.2)[b]
30	61.3(±4.9)[b]	2.3(±0.2)[c]	4.1(±0.4)[c]
60	24.0(±4.0)[c]	0.7(±0.1)[d]	0.8(±0.2)[d]
120	0.0(±0.0)[d]	0.0(±0.0)[e]	0.0(±0.0)[e]
240	0.0(±0.0)[d]	0.0(±0.0)[e]	0.0(±0.0)[e]
480	0.0(±0.0)[d]	0.0(±0.0)[e]	0.0(±0.0)[e]
	E. crus-galli		
0	94.7(±2.5)[a]	5.9(±0.2)[a]	11.6(±0.5)[a]
15	84.0(±3.4)[b]	4.7(±0.2)[b]	8.6(±0.5)[b]
30	70.7(±4.5)[c]	3.5(±0.2)[c]	5.8(±0.6)[c]
60	33.3(±3.0)[d]	1.5(±0.2)[d]	2.3(±0.3)[d]
120	0.0(±0.0)[e]	0.0(±0.0)[e]	0.0(±0.0)[e]
240	0.0(±0.0)[e]	0.0(±0.0)[e]	0.0(±0.0)[e]
480	0.0(±0.0)[e]	0.0(±0.0)[e]	0.0(±0.0)[e]
	E. indica		
0	96.0(±2.7)[a]	4.1(±0.1)[a]	8.5(±0.2)[a]
15	94.7(±3.9)[a]	4.1(±0.2)[a]	8.4(±0.5)[a]
30	82.7(±4.0)[b]	3.2(±0.1)[b]	5.9(±0.4)[b]
60	44.0(±4.5)[c]	1.7(±0.2)[c]	2.9(±0.3)[c]
120	2.7(±1.6)[d]	0.1(±0.0)[d]	0.1(±0.0)[d]
240	0.0(±0.0)[e]	0.0(±0.0)[d]	0.0(±0.0)[d]
480	0.0(±0.0)[e]	0.0(±0.0)[d]	0.0(±0.0)[d]

Different letters along the columns indicate statistically significant differences among treatments, $P < 0.05$ (Tukey's test). Values within the brackets indicated the standard error. (N = 4).

Table 2.
Total root length (TRL) of lettuce and weeds exposed to different concentrations of apple mint essential oils.

Concentration ($\mu L\ L^{-1}$)	*L. sativa*	*C. album*	*A. retroflexus*	*E. crus-galli*	*E. indica*
			TRL (cm)		
0	1.83(±0.04)^{a}	3.2(±0.15)^{a}	3.03(±0.0)^{a}	2.26(±0.1)^{a}	1.92(±0.07)^{a}
15	1.19(±0.10)^{b}	2.6(±0.20)^{b}	2.76(±0.1)^{b}	2.06(±0.1)^{b}	1.37(±0.8)^{b}
30	0.69(±0.05)^{c}	1.7(±0.06)^{c}	1.96(±0.0)^{c}	1.68(±0.1)^{c}	1.57(±0.08)^{b}
60	0.36(±0.4)^{d}	1.0(±0.04)^{d}	1.18(±0.1)^{d}	0.98(±0.1)^{d}	0.83(±0.03)^{c}
120	0.23(±0.3)^{e}	0.5(±0.03)^{e}	0.63(±0.1)^{e}	0.24(±0.0)^{e}	0.75(±0.11)^{d}
240	0.14(±0.02)^{f}	0.2(±0.006)^{f}	0.20(±0.0)^{f}	0.16(±0.0)^{f}	0.27(±0.03)^{e}
480	0.11(±0.0)^{g}	0.1(±0.007)^{g}	0.11(±0.0)^{g}	0.11(±0.0)^{f}	0.15(±0.01)^{f}

Different letters along the columns indicate statistically significant differences among treatments, $P < 0.05$ (Tukey's test). Values within the brackets indicated the standard error. (N = 4).

respectively (Fig. 1). Similar results were observed on lettuce seeds treated with *C. nepeta* and *D. viscosa* volatiles (Araniti *et al.*, 2013, 2017b) as well as by Muller (1964) on *Bromus rigidus* and *Festuca megalura* germination treated with *Salvia leucophylla* volatiles.

Concerning to the effects of *M. rotundifolia* VOCs on lettuce root growth, all the concentrations higher than 2.5 g caused a dose dependent inhibition of this parameter (Fig. 1). In particular, VOCs released by 5 g of leaves determined 14% reduction of root growth, whereas 10 and 20 g caused 55% and 83% inhibition, respectively (Fig. 1).

Similar effects were also observed by Araniti *et al.* (2013; 2017b) on lettuce roots treated with *C. nepeta* and *Dittrichia viscosa* volatiles, by Barney *et al*. (2005) and by Eom *et al* (2006) on *Lepidium sativum* treated with mugwort and *Calamintha nepeta* × *faassenii* VOCs, respectively.

Bioassay with essential oils

All the germination parameters showed, in all the assayed species, an inhibitory effect in response to the increasing doses of apple mint essential oils pointing out their strong phytotoxic potential.

In particular, the sensitive species *L. sativa* exhibited 97% of inhibition of total germination index [G_T (%)] when treated with 60 $\mu L\ L^{-1}$ of essential oils concentration, while this effect was less marked, in *C. album*, *A. retroflexus*, *E. crus-galli* and *E. indica,* which showed 53%, 76%, 65% and 55% of inhibition (Table 1), respectively. In all the species, higher concentrations caused 100% inhibition of the germination process (Table 1).

This different response was also confirmed by comparison of ED_{50} values, which provided the following hierarchy of sensitivity: *A. retroflexus* (36.3 $\mu L\ L^{-1}$) > *E. crusgalli* (47.16 $\mu L\ L^{-1}$) > *L. sativa* (55 $\mu L\ L^{-1}$) ≥ *E. indica* (56 $\mu L\ L^{-1}$) > *C. album* (196 $\mu L\ L^{-1}$) (Table 3).

Concerning to the speed of germination (S) and the accumulated germination (AS), which explains the dynamics of germination process, both parameters were sensitive to apple mint essential oils already at low concentrations (15 and 30 $\mu L\ L^{-1}$). In particular, the most sensitive were *A. retroflexus* and *E. crusgalli* (15 $\mu L\ L^{-1}$) followed by *L. sativa*, *C. album* and *E. indica* (30 $\mu L\ L^{-1}$) (Table 1).

The inhibition of germination as well as its delay play a significant ecological role in natural ecosystems increasing the competitive ability of the species being able to retard the growth of their competitors. McCalla and Norstadt (1974) defined the germination as the most sensitive vegetative stage to phytotoxins, since a short period of its inhibition or stimulation could strongly increase or reduce the ability of species to compete with others (Norstadt and McCalla, 1971; Rice, 1984). The ability to inhibit and/or delay the germination of neighboring species through the release of VOCs has been largely documented. Field studies demonstrated that *Salvia leucophylla* volatile monoterpenes caused a growth inhibition of grasses present in the surroundings (Muller and Muller, 1964). Moreover, Araniti *et al.* (2017b) as well as Souza-Alonso (2017) demonstrated that volatiles released by two invasive species, *Dittrichia viscosa* and *Acacia longifolia*, could strongly affect both germination and growth of several species.

Total root length (TRL) was more sensitive to essential oils treatment than germination, being significantly reduced already at the lowest concentration, in all the species (Table 2).

Among all the species, *L. sativa* was the most sensitive pointing out 22 $\mu L\ L^{-1}$ ED_{50} value while *E.*

Table 3.
ED_{50} values evaluated on G_T (%) and TRL (cm) parameters of lettuce and weeds exposed to different concentrations of apple mint essential oils.

	L. sativa	*C. album*	*A. retroflexus*	*E. crusgalli*	*E. indica*
			ED_{50} (µL/L)		
G_T %	55.0(±1.81)^c	195.98(±2.62)^d	36.3(±1.44)^a	47.16(±2.22)^b	56.26(±2.14)^c
TRL (cm)	22.08(±1.8)^a	35.5(±2.7)^b	46.05(±2.0)^c	51.25(±3.2)^{cd}	64.52(±10.7)^d

Different letters along the rows indicated significant differences at P < 0.05 (Tukey's test). Values within the brackets indicated the standard error. (N=4). All the dose-response curves pointed out a significance level of P < 0.001.

indica was the least sensitive showing 64 µL L^{-1} ED_{50} (Table 3). On the other hand, *C. album, A. retroflexus* and *E. crusgalli* pointed out ED_{50} values of 35 µL L^{-1}, 46 µL L^{-1} and 51 µL L^{-1}, respectively (Table 3).

As observed by several authors (Araniti *et al*., 2012; Chon *et al*., 2002), these results confirmed that root growth exhibited a stronger sensitivity to allelochemicals than seed germination. Moreover, the results confirmed that lettuce seemed to be the most responsiveness species to phytotoxic molecules, whereas *E. indica* was the less sensitivity, as previously reported by several authors (Mudge *et al*., 1984; Lee and Ngim, 2000; Ng *et al*., 2004).Different letters along the columns indicate statistically significant differences among treatments, P < 0.05 (Tukey's test). Values within the brackets indicated the standard error. (N = 4).

GC-MS analysis

The essential oils of apple mint were characterized by the presence of 33 compounds being the terpenoids the major constituents. In particular, 16 monoterpenes, 4 monoterpenic alcohols and 13 sesquiterpenes were identified (Table 4).

Among monoterpenes, α- and β-Pinene as well as α-Limonene, *trans-α*-Ocimene and γ-Terpinene were the most abundant compounds representing almost the 58.7% of the total chromatogram (Table 4). Concerning to the sesquiterpenes the most abundant were β-Germacrene and D-(-)-Germacrene with an abundance of the 3.1% and 1.24%, respectively. All the other sesquiterpenes were characterized by a concentration ≤ than 0.7% (Table 4).

As previously reported by several authors, both monoterpenes and sesquiterpenes are largely known for their phytotoxic potential and their different modes of action (Dayan *et al*., 2000; Duke, 2003). In particular, monoterpenes strongly affect cellular membranes (Lorber and Muller, 1976) and mitochondrial respiration (Abrahim *et al*., 2000). Recently, Graña *et al*. (2013) reported that the monoterpene citral was able to affect root morphology and ultrastructure as well as hormone homeostasis and plant fitness (Graña *et al*., 2016). Among the most abundant monoterpenes found in apple mint essential oils, limonene was known to cause visible leaf injuries as well as growth reduction, increment of chlorophyll fluorescence and reduction of both net photosynthesis and stomatal conductance in cabbage and carrot (Ibrahim *et al*., 2004). Moreover, Vaid *et al*. (2011) observed that seeds and seedlings of *Amaranthus viridis* exposed to the vapors, produced by 0.7 µL limonene, were characterized by a strong reduction in germination and root growth as well as an inhibition of seedling growth, chlorophyll content and respiration.

As well as limonene, also α- and β-Pinene were known for their high phytotoxicity. Indeeed, β-Pinene (at concentrations ≥ 0.04 mg mL^{-1}) significantly reduced the root and coleoptile length of rice, total chlorophyll content, enhanced the accumulation of macromolecules (proteins and carbohydrates) and inhibited the activities of hydrolyzing enzymes such as proteases, α-amylases, and β-amylases (Chowhan *et al*., 2011). Recently, Chowhan *et al*. (2013) observed, in weeds (*E. crusgalli, Phalaris minor* and *Cassia occidentalis*) treated with the same molecule, lipid peroxidation and loss of membrane integrity concluding that membrane could be the primary target of β-Pinene. Finally, Areco *et al*. (2014), evaluating the effects of different pinene stereoisomers on germination process, observed that the α-Pinene stereoisomers were not able to affect the germination of *Zea mays* whereas β-Pinene was highly active. On the other hand, in growth bioassay, all of the pinene isomers strongly affected plant growth, even if β-Pinene was still more phytotoxic than α-Pinene.

Although sesquiterpene concentration was extremely lower compared to that of monoterpenes, they could play a pivotal role in determining the

Table 4.
Chemical composition of natural compounds isolated, through distillation, in the essential oils of *Mentha rotundifolia*. Rf (retention factor), RAP% (Relative area percentage, peak area relative to total peak area %); Tr values < 0.1% are denoted as traces.

Rf	Molecule	RAP %	Class
9.90	β-Thujene	1.22	Monoterpene
10.08	α-Pinene	10.68	Monoterpene
10.5	Camphene	1.94	Monoterpene
11.15	(+)-Sabinene	0.52	Monoterpene
11.2	β-Pinene	16.07	Monoterpene
11.54	β-Myrcene	0.53	Monoterpene
11.88	3-Carene	0.48	Monoterpene
12.13	Cymene	1.83	Monoterpene
12.2	α-Limonene	15.02	Monoterpene
12.34	*trans*-α-Ocimene	9.98	Monoterpene
12.5	(e)-β-Ocimene	1.17	Monoterpene
12.65	γ-Terpinene	7.01	Monoterpene
12.78	*cis*-Sabinene hydrate	0.42	Monoterpene
13.09	(+)-2-Carene	2.09	Monoterpene
13.22	*trans*-4-Thujanol	0.41	Monoterpene alcohol
13.54	Neo-allo-Ocimene	0.23	Monoterpene
13.74	l-Camphor	0.24	Monoterpene
13.98	Borneol	1.62	Monoterpene alcohol
14.08	1-Terpinen-4-ol	2.54	Monoterpene alcohol
14.19	(-)-α-Terpineol	0.71	Monoterpene alcohol
15.75	α-Copaene	0.1	Sesquiterpene
15.85	β-Elemene	0.09	Sesquiterpene
16.09	β-Caryophyllene	0.73	Sesquiterpene
16.15	β-Copaene	0.08	Sesquiterpene
16.21	(e)-β-Farnesene	0.21	Sesquiterpene
16.33	α-Caryophyllene	0.45	Sesquiterpene
16.39	Aromadendrene	0.13	Sesquiterpene
16.41	β-Curcumene	0.21	Sesquiterpene
16.51	D-(-)-Germacrene	1.24	Sesquiterpene
16.61	Bicyclogermacrene	0.11	Sesquiterpene
16.71	γ-Cadinene	0.08	Sesquiterpene
16.75	δ-Cadinene	0.17	Sesquiterpene
17.06	β-Germacrene	3.1	Sesquiterpene

phytotoxic effects of apple mint essential oils. In fact, it has been proven that synergistic interactions among pure essential oils components played an important role in defining their phytotoxicity (Asplund, 1969; Vokou *et al*., 2003; Araniti *et al*., 2013). Further, the mixture of several terpenoids at non-phytotoxic concentration could become extremely phytotoxic (Araniti *et al*., 2013) and, sometimes, chemical traces could strongly increase the phytotoxicity of predominant compounds (Araniti *et al*., 2012).

Conclusions

The results pointed out that the VOCs released by apple mint were able to affect both germination and root growth of lettuce suggesting that its essential oils, which are a concentrated mixture of these chemicals, could be characterized by a high phytotoxic potential. This hypothesis was also confirmed by the experiments carried out on four of the most common weeds largely found in several agro-ecosystems.

Further, the chemical characterization of apple mint essential oils pointed out a large presence of monoterpenes and sesquiterpenes, which are largely known in literature for their inhibitory activity and their different modes of action. Among all the monoterpenes α-Pinene as well as β-Pinene and limonene were the most abundant. Interestingly, these compounds were known to be extremely phytotoxic suggesting that they could play a pivotal role in the inhibitory effects observed.

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