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Allelopathic potential of some essential oils vis-à-vis three noxious weed species invading cereals

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Abstract

This investigation was performed to assess the allelopathic potential of essential oils extracted from seven medicinal and aromatic plants of the Tunisian flora: *Artemisia herba-alba* Asso., *Mentha pulegium* L., *Rosmarinus officinalis* L., *Salvia officinalis* L., *Lavandula officinalis* L., *Eucalyptus gomphocephala* DC. and *Foeniculum vulgare* Mill.; selected based on ethnobotanical data. To achieve the target, *in vitro* trials were carried out to test the inhibitory activity of these volatile oils against seed germination of three Mediterranean noxious weed species namely *Sinapis arvensis* L., *Rumex crispus* L. and *Phalaris minor Retz.*; invading most particularly grain crops. Accordingly, the same bioassays were conducted concurrently for two winter cereals wheat and barley as non-target species. The scrutiny of results revealed a differential response among weed species as well as a disparity across the essential oil activities. Indeed, solely oils from *A. herba-alba, M. pulegium* and *L. officinalis* drastically inhibited seed germination of *P. minor* and *S. arvensis*. Based on their phytotoxic potency (IC50), these most active essential oils could be subsequently ranked as *M. pulegium* > *L. officinalis* > *A. herba- alba*. In a second step, a phytochemical study using GC/FID and GC/MS was undertaken. The abundance of oxygenated monoterpenes might thereby explain the potent inhibitory activity of the oils. Moreover, it is worth noting that *A. herba-alba* essential oil exhibited a distinct chemical composition which would characterize the Tunisian Dorsale. Hence, these promising findings may solve some environmental issues related to pesticide pollution and hold the key to non-chemical weed management strategy.

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Introduction

The Mediterranean region, characterized by a great variety of climates and soils, is particularly propitious for the development of a concern numerous weed species. Situated on the southwest shore of the Mediterranean Sea, on the North African side, Tunisia is endowed with diverse crop patterns (cereals, vegetable crops, orchards, vineyard, etc.) due to irrigation possibilities and different climatic zones; hosting unluckily some noxious weeds. In fact, according to floristic investigations carried out across northern Tunisia, mainly in sub-humid and semi-arid agroclimatic regions, approximately 223 weed taxa have been reported to be amply subservient to arable crops (Careme, 1990). These taxa which are the most frequent and plentiful belong to 129 genera and 35 botanical families, 30 of which belong to Magnoliopsida class and 5 belong to Liliopsida class. Admittedly, weeds cause severe yield losses in arable and horticultural crops including impairment of some harvest products when infested by their parasite seeds (Tworkoski, 2002). The fight against weeds acquires therefore a growing economic importance. A common practice to tackle weed infestations is to use synthetic herbicides. Nevertheless, extensive use of chemicals in plant protection has given rise to concerns about pesticide residues causing potential damage to both environment and human health, and to increasing incidence of herbicide- resistant weed biotypes (Dudai et al., 1999, Singh et al., 2003, Batish et al. 2007, Dayan et al. 2009). Additionally, it should be noted that in organic farming systems, growers are not allowed to use synthetic herbicides. Alternative approaches for sustainable weed management such as crop rotation, competitive varieties, mechanical tillage, mulching, cover crops, etc. are being employed, but these practices can be costly and need some specific know how (Bund and Grundy, 2001, Tworkoski, 2002). Of late, the interest in essential oils has regained momentum. These naturally occurring products are produced particularly from medicinal and aromatic plants and provide a wide spectrum of bioactivities including medicinal, antimicrobial, antifungal, etc. (Singh and Maurya, 2005, Ghrabi-Gammar et al., 2009, Mahboubi and Haghi 2008, Ait-Ouazzou et al., 2012). Furthermore, volatile oils may contain so called allelochemicals which are involved in plant-plant interactions being able to inhibit seed germination and seedling growth by causing phytotoxicity. Accordingly, this ecological phenomenon of allelopathy has tremendously increased the interest in exploring essential oils for further use as potential bioherbicides in an integrated weed management system (Dudai et al., 1999, Duke et al., 2002, Singh et al., 2003).

In the current research work, we sought to assess the allelopathic potential of essential oils extracted from seven medicinal and aromatic plant species of Tunisian flora selected based on ethnobotanical data (Ben Haj Jilani et al., 2011) which were as follows: *Artemisia herba-alba* Asso. (White Wormwood), *Mentha pulegium* L. (Pennyroyal), *Rosmarinus officinalis* L. (Rosemary), *Salvia officinalis* L. (Sage), *Lavandula officinalis* L. (Lavender), *Eucalyptus gomphocephala* DC. (Tuart) and *Foeniculum vulgare* Mill. (Fennel); against seed germination of three common weed species growing up in the Mediterranean area such as *Sinapis arvensis* L., *Rumex crispus* L. and *Phalaris minor* Retz. commonly known as wild mustard, curly dock and minor canary grass, respectively. These noxious weeds have been reported to be a serious threat to agriculture causing damages most particularly to winter cereals (durum wheat: *Triticum durum* Desf. and barley: *Hordeum vulgare* L.) and row crops (Careme, 1990). Hence, we carried out *in vitro* trials in order to test the phytotoxic activity of the essential oils aforementioned on seed germination of peculiar weeds as target species but also on those of two host plants: wheat and barley. In the second step, we proceeded to determine the chemical composition of the oils so as to identify the most active allelochemicals that could be involved in the mechanisms of weed germination inhibition. Our aim is to screen available essential oils from plants for further use as natural herbicide. This may solve some environmental issues related to pesticide pollution and hold the key to non-chemical weed management strategy.

Materials and Methods

Procurement of Plant Material

Weeds and Cereals

All seeds were collected during June 2011. Seeds of weeds: *S. arvensis* (Brassicaceae), *R. crispus* (Polygonaceae) and *P. minor* (Poaceae) were collected locally from the garden of the National Agronomic Institute of Tunisia. Seeds of durum wheat (variety Om rabii) were collected from a plot at Center of Biotechnology of Borj Cedria- Tunis and those of barley (variety Manel) were provided by the Laboratory of Genetics and Plant breeding of the National Agronomic Institute of Tunisia. We selected these two cereals since it was easier to germinate their seeds under laboratory conditions with a consistent and higher germination rate.

Essential Oil Plants

The study focused on seven medicinal and aromatic plant species of Tunisian flora namely *Artemisia herba-alba, Mentha pulegium*, *Rosmarinus officinalis*, which are spontaneous; *Salvia officinalis*, *Lavandula officinalis*, and *Eucalyptus gomphocephala* which are cultivated and *Foeniculum vulgare* that was purchased commercially from local herbal shop in Tunis. These plants are known for their medicinal, antifungal or insecticidal properties. Information on their botanical family, collection site, harvest date, extraction plant part and its state (dried or fresh) are given in Table 1. To the best of our knowledge, there is no previous report on allelopathic effects of the essential oils extracted from these plant species on the studied weeds. Voucher specimens of essential oil plant species and weeds are kept at the herbarium of the Medicinal and Aromatic Plants Laboratory at the Tunisian National Agronomic Institute.

Table 1. Information on studied medicinal and aromatic plant species and their essential oil yields.

Medicinal and aromatic plant species /Botanical Family	Collection site and date	Extraction plant part/ Plant material state (dried or fresh)	Essential Oil yields (%)
Lamiaceae			
Lavandula officinalis L.	Kef July 2010	Dried flowering twig tops	1.28
Mentha pulegium L.	Sejnane July 2010	Dried leafy and flowering twig tops	1.73
Rosmarinus officinalis L.	Kef July 2010	Dried leaves	1.53
Salvia officinalis L.	INAT April 2010	Dried leafy and flowering twig tops	0.72
Myrtaceae			
Eucalyptus gomphocephala DC.	Sejnane July 2010	Fresh leaves and fruits	0.51
Asteraceae			
Artemisia herba-alba Asso.	Kef December 2010	Dried flowering twig tops	0.26
Apiaceae			
Foeniculum vulgare Mill.	Purchased commercially	Dried seeds	0.81

Extraction of Essential Oils

For *E. gomphocephala*, we used freshly collected leaves and fruits whereas, for the other species, plant materials were naturally dried at room temperature and shade condition. To approximate field conditions, the essential oils were obtained by water/steam distillation (or wet steam distillation) method using a stainless steel apparatus. The plant material (one kilo) was introduced into the still tank and placed on a perforated grid keeping it above the water level and consequently, it was protected from direct heat. The heated water produced saturated and wet steam

which rose through the plant material vaporizing the essential oil with it. This mixture of water and volatile oil left the hot suspension, condensed and flowed into a separator called Florentine flask. Then, two products were obtained by decantation: – the essential oil and the condensed water containing water-soluble constituents of the essential oil, known as floral water or hydrosol. To produce "whole" oil, the aqueous layer was then cohobated. After 2 hours, the distillation process was stopped. For each plant species sample, the oil volume was recorded, collected in the phase separation flask, dried over anhydrous sulfate sodium and stored in tightly sealed dark vials at 4°C for further use in bioassay and composition determination. The oil yields were calculated on a dry weight basis and shown in Table 1. The extraction of the essential oils was carried out in the Medicinal and Aromatic Plants Laboratory at the National Agronomic Institute of Tunisia.

Bioassays of Essential Oils on Weed Germination

To test the inhibitory effect of the essential oils, we adopted a previous bioassay protocol (Azirak and Karman, 2008) to which we assigned some slight modifications. Firstly, weed and cereal seeds were treated with 70% ethanol added with few drops of Tween (0.1%). Subsequently, they were surface sterilized with a 5% sodium hypochlorite aqueous solution for 15 min and rinsed four times with sterilized distilled water. Finally, 10 seeds of each plant species were separately and equidistantly sown in sterilized Petri dishes of 9cm of diameter lined with a double layer of Whatman N°1 filter paper. For each essential oil, an oil-in-water emulsion was prepared at varying concentrations (C1= 100 ppm; C2= 1000 ppm; C3= 2000 ppm and C4= 3000 ppm). The essential oils utilized were 100% pure. Then, an equal volume (4 ml) of the test essential oil samples were introduced into each Petri dish. Similar volume of sterilized distilled water was used as control. Petri dishes were then sealed immediately with Parafilm® to reduce evaporation. For each seed species and treatment concentration, including control, three replicates were maintained in a randomized block design. All Petri-dishes were incubated for 15 days in a controlled growth chamber at 25 ± 2°C temperature except those of *Rumex crispus* which were kept in dark. Allelopathic behaviour was appraised by counting the number of daily germinated seeds until the control stabilized, reaching the maximum germination. Seeds were considered to be germinated when their emergent radical length was nearly 2 mm. It should be noted that an appropriate quantity of sterilized distilled water (control) or emulsion (essential oil trials) was added when the moisture content of the filter paper declined.

The final germination percentage was calculated by the following formula:

Germination percentage = Number of germinated seeds/Total number of seeds x 100

Chemical Characterization of Essential Oils

The studied essential oils were analyzed by GC/ FID and GC/MS, carried out in Tunisia at the National Institute of Research and Physico-chemical Analysis (INRAP) and the Center of Biotechnology of Borj Cedria (CBBC).The GC analysis was performed using an Agilent 6890 N gas chromatograph coupled with flame ionization. The separation was achieved using an Agilent 19091S-433 HP-5MS (5% Phenyl Methyl Siloxane) capillary column, 30 m × 0.25 mm, film thickness 0.25 µm. The applied temperature program was 50°C, held for 1 min, then raised up 310°C at a rate of 2°C/min and held at 310°C for 2 min. The injector temperature was set at 250°C with a flow rate of 49.7 ml/min. The carrier gas (Helium) was used with a flow rate of 1 ml/min. For each essential oil sample, a volume of 1.0 µl was injected using split mode with a split ratio of 1:50. The GC/MS analysis was carried out using an Agilent 7890A gas chromatograph on a capillary column of HP-5MS (5% Phenyl Methyl Siloxane) with the same dimensions as above. The temperature was programmed from 40°C to 205°C at the rate of 3°C/min and held at 205°C for 10 min. The carrier gas (He) flow rate was 1.6 ml/min). Each essential oil sample was diluted to 10% in diethyl ether and a volume of 1.0 µl was injected according a split mode (injector flow: 96 ml / min). The mass spectrometer source temperature was set at 230°C and the mass scan range was recorded from 50

to 550 amu (atomic mass units). For different essential oil compounds, the retention indices (Kovats index) were calculated from the retention times of a range of standard alkanes. Afterwards, the oil constituents were identified by comparison of mass spectra of each peak with those of authentic samples in a Wiley 8N08 spectral library.

Statistical Analysis

Statistical analysis of the variance was accomplished through the Statgraphics Centurion XV (version 1.15.02; StatPoint, Inc., Virginia, USA). Means were then compared with least significant difference (LSD) using *Fisher's* exact *test* (*F-test*). P values less than 0.05 (P = 0.05) were considered as statistically significant. Data processing of germination tests was performed using Microsoft Excel 2010 software. Therefore, curves of germination rate versus essential oil concentration were established. IC_{50} values corresponding to the concentration required to cause 50% inhibition of germination, were then derived by plotting the treatment concentration against germination rate on the *x* and *y* axes respectively.

Results

Essential oils emanating from different plant parts of *A. herba-alba*, *M. pulegium, R. officinalis*, *S. officinalis*, *L. officinalis*, *E. gomphocephala* and *F. vulgare* were obtained by wet steam distillation. Their respective resulting yields are set out in Table 1. *M. pulegium* has the highest yield (1.73%) followed in ascending order by *R. officinalis* (1.53%), *L. officinalis* (1.28%), *F. vulgare* (0.81%), *S. officinalis* (0.72%), *E. gomphocephala* (0.51%) and finally *A. herba-alba* (0.26%). For finding out the phytotoxicity of these essential oils, various concentrations were used to test their biological activity against seed germination of three common weed species *S. arvensis*, *R. crispus* and *P. minor*. Results from variance analysis showed that the two considered factors, essential oil and concentration treatments were significant for both weed species *S. arvensis* and *P. minor* whereas for *R. crispus*, only the concentration was significant. Thence, the examination of their interaction proved so negligible. Consequently, their effects on each weed seeds were statistically separately interpreted.

Effect of Essential Oils on Germination of Weed Seeds

One of the more significant *findings* to *emerge* from the results shown in Table 2 is that weed seeds revealed remarkable differences in their responses towards the studied essential oils. Indeed, *P. minor* was the most sensitive followed by *S. arvensis* and thus their germination was generally inhibited. In contrast, *R. crispus* was less affected and seemed to be very resistant since almost all seeds could germinate (97.5 - 100%) compared to the control. It is worth noting that there are also differences in essential oil potentials. In fact, while germination rate was 100% in the control group, only three essential oils of *L. officinalis*, *M. pulegium* and *A. herba-alba* displayed a prominent inhibitory effect against the germination of weed seeds. Among these essential oils, *M. pulegium*'s one gave the lowest germination rates 10.41 % and 28.33% respectively for *P. minor* and *S. arvensis*. The two other oils behaved almost similarly. As regards essential oils of *E. gomphocephala*, *S. officinalis*, *R. officinalis* and *F. vulgare*, they were mostly inefficient in inhibiting germination.

Table 2. Effect of essential oils extracted from medicinal and aromatic plants on germination of weed seeds.

Medicinal and aromatic plant species	Germination rate (%)		
	Sinapis arvensis	***Rumex crispus***	***Phalaris minor***
Lavandula officinalis	46.66 [a]	99.6 [b]	41.65 [b]
Mentha pulegium	28.33 [a]	98.33 [b]	10.41 [a]
Eucalyptus gomphocephala	100 [b]	100 [c]	100.0 [c]
Salvia officinalis	91.66 [b]	100 [c]	58.33 [c]
Rosmarinus officinalis	89.16 [b]	100 [c]	79.17 [c]
Artemisia herba-alba	46.66 [a]	97.5 [a]	45.83 [b]
Foeniculum vulgare	85.83 [b]	100 [c]	83.33 [c]
Control	100	100	100

[a,b,c] *Within a column, means followed by common letters are not significantly different at P < 0.05, according to Fisher's test*

Effect of Essential Oil Concentrations on Germination of Weed Seeds

Table 3 summarizes the weed seed germination rates obtained after applying the different concentrations of the essential oils extracted from the studied medicinal plant species, compared with respective controls. The data show that the weed seeds responded differently to the diverse test concentrations. However, as common trend for all weed species is that the inhibition ability was enhanced while increasing the oil concentrations. The inhibitory effect on weed seed germination was therefore dose-dependent. Among the suggested concentrations, the highest ones C3 (2000 ppm) and C4 (3000 ppm) have generally controlled most weeds. Compared to control group, their effect was found to be statistically noteworthy and highly significant particularly for *P. minor* and *S. arvensis* seeds. Thus, germination rate declined by 42.86% and 40.48% respectively in response to C3, and by 52.38% and 50.0% respectively in response to C4. Nevertheless, a very low inhibition was marked in case of *R. crispus*.

Data depicted in Figure 1 clearly show the effect of each essential oil concentration on the germination of the different weed seeds. As can be seen in Figures 1A and 1B, almost all tested essential oils have exerted an inhibitory effect against *S. arvensis* and *P. minor* seed germination in a dose-dependent manner. The essential oils of *L. officinalis*, *M. pulegium* and *A. herba-alba* showed a strong allelopathic activity. *M. pulegium* was the most effective. It revealed potent inhibition ability recording therefore a total suppression of *P. minor* seed germination at the highest concentration C_4. These three effective essential oils as well as those of *R. officinalis* and *F. vulgare* started reducing *S. arvensis* seed emergence from the concentration C2 (Fig 1B). Whereas, in case of *P. minor*, the inhibitory effect of all essential oils, except those of *F. vulgare and E. gomphocephala*, appeared prominent already upon exposure to the lowest concentration C_1. On the other hand, we should point out that no inhibition occurred when *E. gomphocephala* oil was tested against *S. arvensis* even with increasing concentrations. *S. officinalis* oil was rather able to record a low inhibition rate of 33.33% only at the highest concentration C_4 (Fig 1A). As for weed species *R. crispus*, the different essential oils did not show any inhibitory activity at all applied concentrations. Only the three medicinal plant species found to be most effective *M. pulegium, A. herba-alba* and *L. officinalis* exhibited a slight decline of germination in response to C_2 and C_4 concentrations (Fig 1C). Hence, these results are supported by the statistical analyses previously presented in Table3.

Table 3. Variance analysis of the effect of the studied essential oil concentrations on germination of weed seeds.

Essential oil	**Germination rate of weed seeds (%)**		
concentrations (ppm)	***Sinapis arvensis***	***Rumex crispus***	***Phalaris minor***
C_1 = 100	99.52^{ns}	100.0^{ns}	73.81*
C_2 = 1.000	70.0*	99.04^{ns}	60.71*
C_3 = 2.000	59.52**	100.0^{ns}	57.14**
C_4 = 3.000	50.0**	98.09*	47.62**
Control	100.0	100.0	100.0

** Fisher's test is significant at P = 0.05; ** Fisher's test is highly significant at P = 0.05; ^{ns} Fisher's test is non-significant at P = 0.05*

To further understand the mechanism of action of these essential oils, we attempted to find out the IC_{50} values at which germination is inhibited by 50%. These IC_{50} values were calculated for *S. arvensis* and *P. minor* by interpolation from dose-responses curves illustrated in Figures 2 and 3, respectively. In contrast, this index could not be determined in case of *R. crispus* as the percentage of inhibition was very low or even equal to zero (Fig 1C).

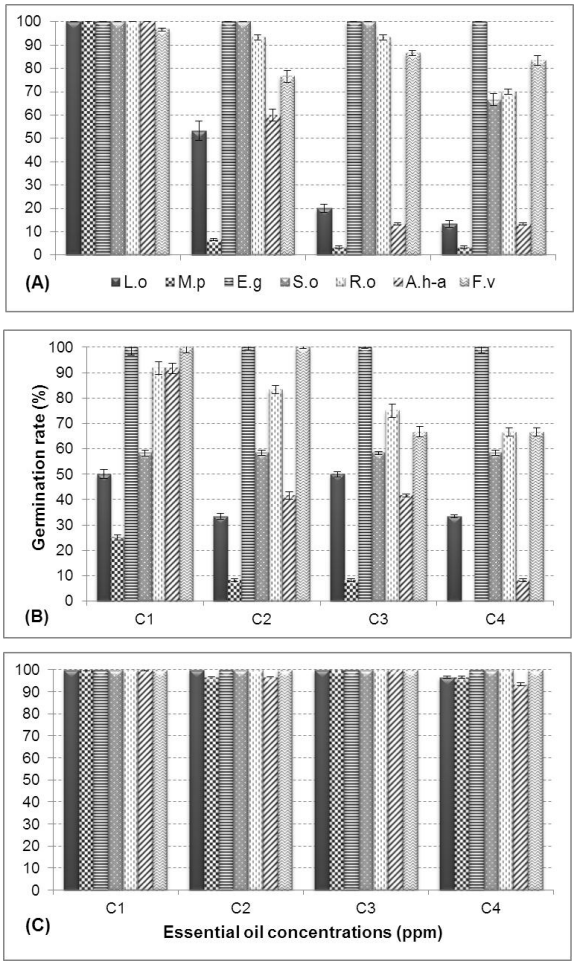

Fig.1 Effect of the essential oil concentrations on seed germination of (A) *S. arvensis*, (B) *P. minor* and (C) *R. crispus*. C_1=100 ppm; C_2=1.000 ppm; C_3=2.000 ppm; C_4=3.000 ppm. L.o : *L. officinalis*; M.p : *M. pulegium*; E.g : *E. gomphocephala*; S.o : *S. officinalis*; R.o : *R. officinalis*; A.h-a : *A. herba-alba*; F.v : *F. vulgare*.

Based on both curves, IC_{50} values could be ascertained only for the three most active essential oils and were ranged from 100 to 1200 ppm. In case of *S. arvensis*, IC_{50} were calculated to be 600 ppm, 1100 ppm and 1200 ppm for *M. pulegium*, *L. officinalis* and *A. herba-alba* respectively (Fig. 2). As regards *P. minor*, IC_{50} deduced for *A. herba-alba* was 850 ppm. Whereas, *L. officinalis* exhibited rather an unexplainable behavior and revealed two IC_{50} values 100 ppm and 2000 ppm corresponding respectively to C1 and C3 evaluated concentrations. In addition, *M. pulegium* showed an IC_{50} even lower than the minimal concentration tested (C1 = 100 ppm). Indeed, as seen in Figure 3, the germination rate recorded at C1 was already very low (25%) and it declined gradually until reaching zero at C4. However, for both weed species, the other essential oils extracted from *R. officinalis*, *S. officinalis*, *E. gomphocephala* and *F. vulgare* would require amounts beyond the maximal concentration tested (C4 = 3000 ppm).

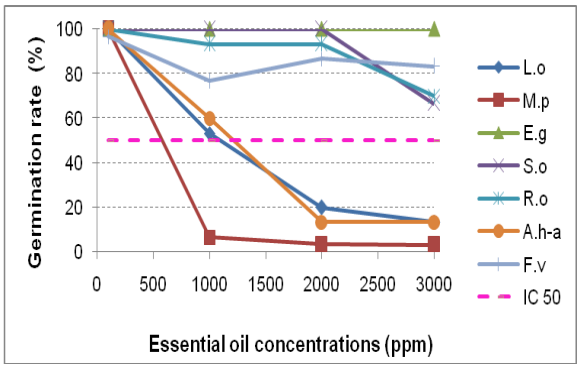

Fig.2 IC_{50} values deduced by interpolation from the curve of germination of *Sinapis arvensis* versus essential oil concentrations of *L. officinalis* (L.o); *M. pulegium* (M.p); *E. gomphocephala* (E.g) ; *S. officinalis* (S.o); *R. officinalis*(R.o); *A. herba-alba* (A.h-a) and *F. vulgare* (F.v).

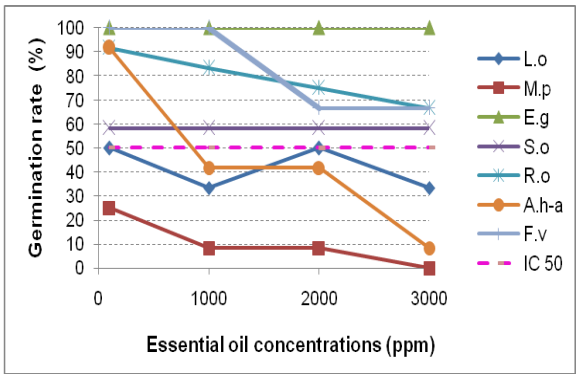

Fig.3 IC_{50} values deduced by interpolation from the curve of germination of *Phalaris minor* versus essential oil concentrations of *L. officinalis* (L.o); *M. pulegium* (M.p); *E. gomphocephala* (E.g) ; *S. officinalis* (S.o); *R. officinalis*(R.o); *A. herba-alba* (A.h-a) and *F. vulgare* (F.v).

To sum up all the results presented above, we can say that amongst the seven aforementioned essential oils subjected to *in vitro* bioassays in order to assess their allelopathic activity; those of *M. pulegium*, *A. herba-alba* and *L. officinalis* were selected since they revealed a noteworthy inhibition activity against the two weeds *P. minor* and *S. arvensis*. Both weedy

species are known to be noxious most particularly to cereal fields (Careme, 1990). Therefore, we sought to test the action of the selected essential oils against the germination of two winter cereals: durum wheat and barley as non-target species.

Effect of Selected Essential Oils on Germination of Cereals

Analyses of variance revealed a significant difference between the two considered factors, essential oil and concentration treatments, as well as their interaction at the probability threshold $P = 0.05$. According to Table 4, *A. herba-alba* oil was found to be somewhat less toxic to cereals favouring the highest germination rate: 76.67% and 59.17% for durum wheat and barley respectively. However, germination of cereals was affected when they were treated by *L. officinalis* essential oil and this reduction was more accentuated on exposure to *M. pulegium* oil. Furthermore, our results show that the three selected essential oils behaved similarly towards wheat. Whereas, in the case of barley *M. pulegium* oil was different in its action recording the lowest mean germination rate 46.67% against 54.17% and 59.17% compared to *L. officinalis* and *A. herba-alba* oils respectively.

Table 4. Effect of selected essential oils extracted from *Mentha pulegium*, *Artemisia herba-alba* and *Lavandula officinalis,* on germination of durum wheat and barley.

Selected Essential oil plant species	Germination rate (%)	
	Durum wheat (*Triticum durum*)	Barley (*Hordeum vulgare*)
Artemisia herba-alba	76,67 [a]	59,17[b]
Lavandula officinalis	48,33[a]	54,17[b]
Mentha pulegium	31,67 [a]	46,67[a]
Control	100	100

Within a column, means followed by common letters are not significantly different at P < 0.05, according to Fisher's test

The germination kinetics of cereal seeds versus the action of selected essential oils were plotted (Figures 4 and 5). The results show that the oils revealed a gradual decline in germination of both cereals, generally in a time and a dose-dependent manner. Compared to the control group, *A. herba-alba* oil clearly reduced the germination rate of wheat and barley but with a low inhibition rate and a germination delay of one day on average.

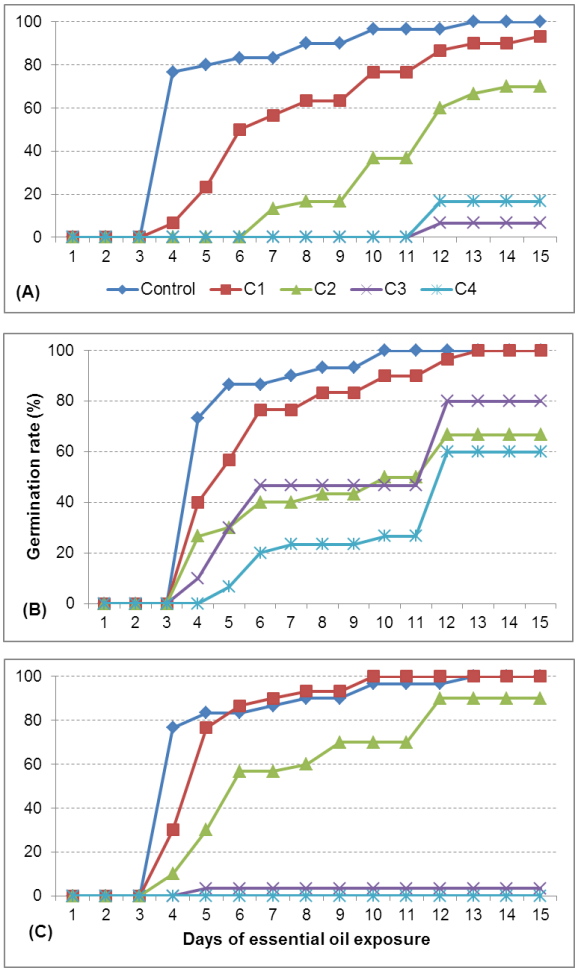

Fig.4 Kinetics of germination of durum wheat seeds versus the action of selected essential oils: (A) *M. pulegium*; (B) *A. herba-alba* and (C) *L. officinalis*. C_1=100 ppm; C_2=1.000 ppm; C_3=2.000 ppm; C_4=3.000 ppm.

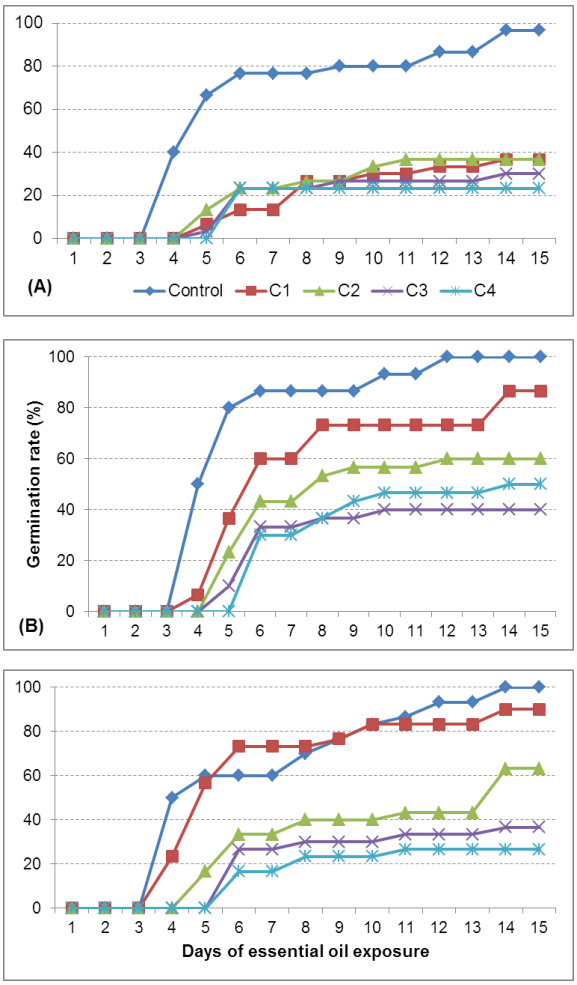

Fig.5 Kinetics of germination of barley seeds versus the action of selected essential oils: (A) *M. pulegium*; (B) *A. herba-alba* and (C) *L. officinalis*. C_1=100 ppm; C_2=1.000 ppm; C_3=2.000 ppm; C_4=3.000 ppm.

When applying *M. pulegium* oil, the cereal seed emergence became subsequently slow. Indeed, compared to the control, the first seed germinated after one day for barley. However in wheat it required on average one week to emit its first radical. Moreover, we should point out that on exposure to Lavender essential oil, germination rate was greatly reduced. In wheat, although seed emergence was delayed by only one day in comparison with control, it was found to be tremendously inhibited while increasing oil concentrations. In response to quiet low concentrations C_1 (500 ppm) and C_2 (1000 ppm), wheat test exhibited 100% and 90% of germination respectively. Further, at C_3 (2000 ppm) and C_4 (3000 ppm), the germination declined drastically to achieve almost a total inhibition. From these findings, we can deduce that the essential oil could not affect wheat emergence. Nevertheless, from some threshold concentration (C2 = 1000 ppm), it might lead to a contrary effect causing thereby severe negative allelopathy. Likewise, this phenomenon was also observed in barley but it was relatively less important.

These preliminary results seemed to be encouraging and brought about further element of research. Since the selected essential oils were strongly effective against target weeds, a phytochemical study was undertaken in a second step in order to identify the bioactive compounds derived from them and that might be responsible for their inhibitory action.

Chemical Composition of Essential Oils

The compounds of each essential oil sample were identified by a combined analysis using GC/FID and GC/MS. These compounds arranged in order of their retention indices are listed in Table 5. Thus, 81 terpene components were detected accounting for 92.01 % (*Artemisia herba-alba*) - 99.81 % (*Foeniculum vulgare*) of total essential oils and showing important qualitative and quantitative variations. Among them, monoterpenes were found to be the most abundant. They were largely represented by oxygenated monoterpenes and at a lesser degree by monoterpene hydrocarbons. In addition, some sesquiterpene hydrocarbons and oxygenated sesquiterpenes were detected in low amounts. For all studied medicinal plant essential oils, these diverse terpene classes and their respective percentages are reported in Table 5.

Table 5. Chemical composition of the essential oils extracted from the studied medicinal and aromatic plants.

Compound	Ri*	Percentage composition						
		L. officinalis	***M. pulegium***	***E. gomphocephala***	***S. officinalis***	***R. officinalis***	***A. herba-alba***	***F. vulgare***
cis-Salvene	847	---	---	---	0.27	---	---	---
Santolinatriene	908	---	---	---	---	---	0.33	---
α-Thujene	937	0.22	---	0.06	0.89	0.20	0.15	---
α-Pinene	942	1.01	1.70	0.26	2.69	**12.36**	2.87	1.40
Verbenene	967	---	---	0.09	---	---	0.48	---
Sabinene	976	0.39	2.10	0.08	---	---	2.98	0.47
β-Pinene	980	---	---	0.88	**5.77**	**4.97**	1.95	---
1-Octen-3-ol	982	0.59	---	---	0.12	0.19	---	---
3-Octanone	987	---	---	---	---	0.07	---	---
β-Myrcene	992	0.46	---	0.73	2.04	1.51	---	0.28
3-Octanol	993	---	0.48	---	---	---	---	---
α-Phellandrene	1003	---	---	0.33	0.16	0.39	0.40	---
δ-3-Carene	1011	0.28	---	---	---	0.37	---	---
α-Terpinene	1016	---	---	0.10	0.78	1.02	0.87	---
Chrysanthenone	1019	---	---	---	---	---	**22.48**	---
o-Cymene	1020	---	---	---	1.49	---	1.58	---
m-Cymene	1024	0.27	---	0.67	---	---	---	---
p-Cymene	1028	0.76	---	---	---	2.41	---	0.49
Limonene	1030	---	2.87	---	---	---	---	**11.23**
β-Phellandrene	1033	---	---	---	---	---	0.68	---
1,8-Cineole	1037	11.11	---	**63.70**	**17.15**	**33.57**	**5.62**	---
(Z)-β-Ocimene	1038	3.58	---	---	---	---	---	---
cis-Ocimene	1042	---	---	---	0.31	---	---	
(E)-β-Ocimene	1051	0.40	---	0.09	---	0.11	---	0.38
γ-Terpinene	1064	0.27	---	0.46	1.34	1.58	0.97	0.38
Fenchol	1067	---	---	---	---	0.09	---	---
cis-Linalooloxide	1074	0.40	---	---	---	---	---	---
α-Terpinolene	1088	0.42	---	0.17	0.46	---	0.37	---
Fenchone	1094	---	---	---	---	---	---	**5.80**
trans-Sabinene hydrate	1095	---	---	---	0.22	---	0.12	---
β-Linalool	1099	**37.97**	---	---	---	1.05	---	---
α-Thujone	1105	---	---	---	**6.87**	---	**9.22**	---
β-Fenchol	1108	---	---	0.07	0.30	0.77	---	---
β-Thujone	1115	---	---	---	**19.03**	---	**10.38**	---
trans-Pinocarveol	1141	---	---	0.01	---	---	0.58	---
Camphor	1146	**12.60**	---	---	**12.68**	**13.77**	**13.66**	0.23

Compound	Ri*	Percentage composition						
		L. officinalis	***M. pulegium***	***E. gomphocephala***	***S. officinalis***	***R. officinalis***	***A. herba-alba***	***F. vulgare***
cis-Isopulegone	1148	---	2.10	---	---	---	---	---
Menthone	1154	---	**15.42**	---	---	---	---	---
Pinocamphone	1160	---	---	---	0.49	---	---	---
cis-Chrysanthenol	1163	---	---	---	---	---	0.16	---
Pinocarvone	1164	---	---	0.45	---	---	---	---
Neomenthol	1165	---	1.90	---	---	---	---	---
Borneol	1168	**7.97**	---	0.07	1.74	**4.55**	1.25	---
Lavandulol	1170	1.48	---	---	---	---	---	---
Terpinen-4-ol	1176	**8.24**	---	---	---	---	0.82	---
Cryptone	1186	0.63	---	---	---	---	---	---
α-Terpineol	1191	1.02	---	0.74	---	3.86	---	---
Myrtenal	1193	---	---	---	3.83	**4.67**	**7.54**	---
Estragole	1195	---	---	---	---	---	---	**77.26**
Myrtenol	1197	---	---	---	0.12	---	---	---
Verbenone	1213	---	---	---	---	---	0.22	---
Carvone	1242	---	---	---	---	---	---	0.25
Pulegone	1243	---	**69.48**	---	---	---	---	---
Piperitenone	1250	---	1.42	---	---	---	---	---
p-Anisaldehyde	1252	---	---	---	---	---	---	0.24
p-Anethole	1262	---	---	---	---	---	---	1.40
Bornylacetate	1286	0.32	---	---	0.16	---	0.13	---
p-Thymol	1292	---	---	---	---	0.38	---	---
Lavandulylacetate	1294	1.21	---	---	---	---	---	---
Carvacrol	1300	0.48	---	---	---	---	---	---
Cyclofenchene	1370	2.25	---	---	---	---	---	---
α-Ylangene	1376	---	---	---	---	0.09	---	---
α-Copaene	1378	---	---	---	0.12	0.44	0.40	
Jasmone	1392	---	---	---	---	---	0.45	---
α-Gurjunene	1407	---	---	0.09	---	---	---	---
δ-Gurjunene	1410	---	---	0.84	---	---	---	---
β-Caryophyllene	1419	0.49	---	---	**6.37**	**5.77**	0.18	---
α-Humulene	1455	---	---	---	3.70	0.92	---	---
trans-(β)-Farnesene	1459	1.46	---	---	---	---	---	---
Allo-Aromadendrene	1462	---	---	1.16	0.16	0.09	---	---
γ-Gurjunene	1473	---	---	0.15	**5.85**	---	---	---
γ-Muurolene	1476	---	---	---	---	0.39	---	---
Germacrene D	1481	0.32	0.36	---	---	---	3.68	---

Compound	Ri*	Percentage composition						
		L. officinalis	***M. pulegium***	***E. gomphocephala***	***S. officinalis***	***R. officinalis***	***A. herba-alba***	***F. vulgare***
β-Selinene	1487	---	---	0.08	---	---	---	---
Bicyclogermacrene	1491	---	---	---	---	---	1.02	---
γ-Selinene	1503	---	---	0.06	---	---	---	---
β-Bisabolene	1509	---	---	---	---	0.15	---	---
δ-Cadinene	1522	---	---	---	0.40	0.89	0.35	---
α-Fenchene	1526	---	---	---	---	---	0.12	---
Caryophylleneoxide	1582	0.24	---	---	0.60	0.15	---	---
Manool	2056	---	---	---	1.51	---	---	---
Monoterpene Hydrocarbons		10.30	6.67	29.57	15.92	24.92	13.39	14.63
Oxygenated Monoterpenes		83.41	90.31	66.22	62.57	62.70	72.55	83.55
Sesquiterpene Hydrocarbons		2.27	0.36	2.38	16.86	8.74	5.63	0
Oxygenated Sesquiterpenes		0.24	0	0	0.60	0.15	0	0
Others		0.59	0	0	0.12	0.26	0.45	1.64
Total identified		96.81	97.81	98.18	97.59	96.77	92.01	99.81

**Ri= Retention index relative to C8-C30 n-alkanes on the HP-5 MS capillary column*
---: absent
Bold type indicates major component.

Therefore, 32 compounds were identified in *A. herba-alba* oil accounting for 92.01%. They were a mixture of oxygenated monoterpenes (72.55%), monoterpenic hydrocarbons (13.39%) and sesquiterpene hydrocarbons (5.63%). The main components were: chrysanthenone (22.48%), camphor (13.66 %), β-thujone (10.38%), α-thujone (9.22 %), myrtenal (7.54%), 1,8-cineole (5.62 %), germacrene D (3.68%), sabinene (2.98 %) and α-pinene (2.87 %). As for *M. pulegium* essential oil, it showed concurrently the higher and the lower percentages of oxygenated monoterpenes (90.31%) and monoterpene hydrocarbons (6.67%), respectively. Besides, only one sesquiterpene hydrocarbon: germacrene D (0.36%) was detected. Nine monoterpene components were therefore identified including mainly pulegone (69.48%), menthone (15.42%), limonene (2.87%), sabinene (2.1%), *cis*-isopulegone (2.1%), neomenthol (1.9%), α-pinene (1.7%) and piperitenone (1.42%). Likewise, *L. officinalis* essential oil was characterized by dominant monoterpenic fractions composed of 83.41% of oxygenated monoterpenes and 10.3% of monoterpene hydrocarbons. They grouped essentially β-linalool (37.97%), camphor (12.6%), 1,8 cineole (11.11%), terpinen-4-ol (8.24%) and borneol (7.97%). Sesquiterpenes were also found at relatively very low ratios: 2.27% and 0.24% of sesquiterpene hydrocarbons and of oxygenated sesquiterpenes, respectively.

Discussion

To develop eco-friendly approaches for sustainable weed management, a particular emphasis was given to naturally occurring products such as essential oils. This study was carried out in order to assess *in vitro* the allelopathic potential of essential oils extracted from seven medicinal and aromatic plants against three common prolific and competitive weeds causing damages to cereals. As the results, solely three essential oils from *M. pulegium, L. officinalis* and *A. herba-alba* were effective showing a potent inhibitory action on seed germination of two weeds *P. minor* and *S. arvensis*. These findings amount to those of

earlier studies wherein volatile essential oils emanating from diverse aromatic plants, including *Mentha*, *Lavandula* and *Artemisia* species; as well as their components were reported to possess a phytotoxic effect against a wide range of weed species (Dudai et al., 1999, Tworkoski, 2002, Angelini et al. 2003, Kaur et al. 2010, Rolim de Almeida, 2010).

Additionally, our results indicate that the weeds differed distinctly in their response towards the essential oils. The germination of *P. minor* and *S. arvensis* was significantly inhibited whereas *R. crispus* was mostly unaffected. This differential response to phytotoxic substances seemed to be species-dependent ensuring thereby some selectivity among target species (Gange et al., 1992, Clark et al., 2004, Chowhan et al., 2012).

In numerous previous studies dealing with plant toxicity topic, researchers forwarded some hypotheses in order to explain conclusively the sources of variability in plant responses versus toxicants. However, these hypotheses must be speculative since phytotoxicant substances and plant species tested here are different. Indeed, Wang and Freemark (1995) asserted that the sensitivity of a test organism to a toxic substance is a complex issue involving types of toxicants, environmental conditions, test methods and other factors.

Under laboratory conditions, this sensitivity is thought to be closely related to inherent physiological and biochemical characteristics of each species (Kobayashi, 2004). As it is well known, the seed germination is a physiological process beginning with water imbibition and culminating in the emergence of the radicle out of the seed coat. Mainly, this latter protects effectively the embryo from harmful external factors and since it has a wide range of anatomic forms; it plays a crucial role in controlling water uptake during imbibitions (Wierzbicka and Obidzinska, 1998). Accordingly, seed structure, in particular morphological seed coat structure can have selective permeability and may therefore explain the differential sensitivity obtained in plant toxicity testing (Hanley and Whiting, 2005). Based on this hypothesis, seed coats of the weed species tested in our study were found to be selectively permeable to the different essential oils. Thus, we can conclude that *P. minor* and *S. arvensis* whose germination was greatly altered are likely to have a pervious seed coat structure. In contrast, *R. crispus* displayed some tolerance since essential oils did not affect its germination suggesting that they could not pass through its seed coat; bearing in mind that what is thought to be as the "seed" is actually an achene with presumably a hardened pericarp enclosing the solitary seed without adhering to it.

The variability in plant responses could be also attributed to plant life cycle. Thus, Gange et al. (1992) reported the toxic effect caused by pesticides on the seeds of a range of annual weed species whereas, they recorded no inhibition of germination in any perennial ones including, inter alia, *Rumex crispus* which was tested in our study. They argued that this susceptibility or tolerance of seeds might be related to the nature of their storage products that could be subdivided according to Hodgson and Mackey (1986) into lipid- and carbohydrate-containing types; considering that the action of the herbicides has been shown to be internal in the seed, affecting vital enzymes activity such as amylase, ATPase, lipase and protease generally required for metabolic pathways during germination (Mayer, 1977 in Gange et al., 1992). Furthermore, Hodgson and Mackey (1986) alluded to the putative role of the nutritive tissue endosperm in the regulation of seed size assuming that annual plant species might be expected to produce smaller seeds than perennials. Consequently, as it was suggested earlier by Williams and Hoagland (1982); the seed size might also explain the observed variations in plant response to phytotoxicant exposures.

It must be stressed that the results of our study support these assertions. In fact, contrary to *P. minor* and *S. arvensis* which are annuals; there was scarcely any inhibition of germination in perennial weed species *R. crispus*. In addition, *P. minor* having the smallest seed tested was the most sensitive species. This latter finding is found to be congruent with the

ones set up by Chowhan et al. (2012). As regards seed reserves, we cannot rule on it since we have no evidence to support the hypothesis advanced above but we could only conclude that the tested weed seeds were provided with different endosperm structures.

In addition to all foregoing explanations, Boutin and Rogers (2000) asserted that sensitivity of plant species to chemical treatments could be also influenced by taxonomic groupings to which they belonged. Indeed, at genus and family levels species responded similarly to herbicides, as they have similar physiologies. Conversely, they conceded that this similarity between species quickly decreased as the taxonomic level increased from family to order and class. Concerning the former possibility, we are not able to approve or refute it since our studied sample was very restricted, composed only of three weedy species representing each one genus and one botanical family. It would be therefore recommended to expand the sample size to include a larger number and diversity of weed species. Nevertheless, at class taxonomic level, our results show that, based on IC_{50} values at which germination is inhibited by 50% (Fig. 2, Fig. 3), Ph*alaris minor* (monocotyledonous) was the most susceptible to the essential oils compared to *Sinapis. arvensis* (Dicotyledonous). This was in sharp contrast to earlier findings wherein Barnes and Putnam (1987) reported that Dicotyledonous species were more sensitive to phytotoxic compounds than the Monocotyledonous ones. We suggest that this disparity in results could be ascribed mainly to the combination patterns phytotoxicant-weed species chosen to be tested in the different studies. Thus, as it was concluded by Boutin and Rogers (2000); there is no inference that a given species can be labeled as consistently sensitive or insensitive to herbicides.

On the other hand, the present investigation shows that used at higher concentration trials (C_3 = 2000 ppm and C_4 = 3000 ppm); the selected essential oils of *M. pulegium, L. officinalis* and *A. herba-alba* inhibited almost totally the seed germination of the studied weed species. However, at these concentrations, the most active essential oils were found to be strongly toxic to tested agronomic crops: wheat (*Triticum durum*) and barley (*Hordeum vulgare*) as well. At lower concentrations, both cereals displayed a delay in germination. It should be noted that the inhibition of seed germination of wheat (*T. durum or T. aestivum*) and barley (*H. vulgare*) in response to volatile oils from various aromatic plants was also previously reported by Dudai et al. (1999) and Grosso (2010).

Nonetheless, it must be borne in mind that auspicious herbicides are designed to be less injurious to agricultural crops and concurrently more toxic to the weedy species invading these crops (Clark et al., 2004, Azirak and Karaman, 2008). Hence, it is worthwhile to consider the IC_{50} effective concentrations resulting in 50% germination reduction. IC_{50} index indicates the threshold below which no obvious symptom appeared whereas germination rate decreased with increasing dose above it. From its values, we can therefore deduce the amount of each essential oil required for further weed control. Some authors attested though that this 50% inhibition of seed germination, obtained from laboratory testing, is not compulsorily a meaningful ecological effect (Bishop and Perry, 1981 in Wang and Freemark, 1995) since essential oils are highly volatile and thus less lasting in the environment. Therefore, additional research under natural field conditions would be required.

Likewise, the current study shows a disparity across the essential oil potencies. Indeed, while volatile oils from *A. herba-alba, L. officinalis* and *M. pulegium* exhibited strong phytotoxic potential inhibiting thereby seed germination of weed species *P. minor* and *S. arvensis*; those of *R. officinalis, S. officinalis, E. gomphocephala* and *F. vulgare* were mostly inefficient.

M. pulegium and *L. officinalis* gave the highest essential oil yields: 1.73% and 1.28% respectively. They belong to the family of Lamiaceae consisting of 24 genera and 93 species in Tunisian flora (Pottier-Alapetite, 1981). Both species are known for their antimicrobial and multiple pharmacological features (Mahboubi and Haghi, 2008, Stanojevic et al., 2011).

Additionally, many species among Lamiaceae family including *Lavandula* and *Mentha* species were reported to secrete phytotoxicants that hinder germination and seedling growth of different weeds (Dudai et al., 1999, Angelini et al., 2003, Azirak and Karaman, 2008).

Despite its too low yield (0.26%), *A. herba-alba* (Asteraceae) inhibited significantly weed seed germination. It should be stressed that the allelopathic effect of this species on annual plants developed in its immediate vicinity was previously reported in the Negev desert (Israel) and central Spain (Escudero et al., 2000). This asteraceous species is a medicinal and aromatic dwarf shrub growing wild on sandy and loamy steppes of the Mediterranean basin and is particular in North Africa (Pottier-Alapetite, 1981). In Tunisia, owing to its countless virtues (anthelmintic, emmenagogue, stomachic, intestinal antiseptic, tonic, antidiabetic, etc.); *A. herba-alba* known by its colloquial name "*Shih*"; is considered as a panacea in the traditional Tunisian pharmacopoeia (Le Floc'h, 1983, Ben Haj Jilani et al., 2011).

Interestingly, the low yield of white wormwood essential oil did not belittle its bioactive value. Earlier studies revealed that essential oil yields could vary widely among and even within species. In the latter case, fluctuations may be attributed to the concomitance of a multitude of parameters such as sites (geographical origin), plant parts, phenological stages correlated with the oil cell ontogeny, harvesting time, drying, storage and extraction processing, seasons, climate effects and plant genotype (Figueiredo et al., 2008, Muhammad Hazwan et al., 2010; Stanojevic et al.,–2011). In the former, the essential oil biosynthesis was found to be related to specialized secretory histological structures (glandular trichomes, cell walls, secretory cavities, vacuoles, resin ducts, etc.) which vary across botanical families (Fischer et al., 1994, Figueiredo et al., 2008).

With reference to *R.officinalis*, *S. officinalis*, *E. gomphocephala* and *F. vulgare,* our finding cannot totally invalidate the effectiveness of their essential oils since they already exhibited slight decline of weed germination at C4 concentration (Fig 1A, Fig 1B). In this regard, Dudai (1999) asserted that when a given essential oil inhibited partially germination, it does not infer that germinated seeds will develop into a normal seedling. Whence, increasing concentrations might likely provoke a significant inhibitory effect. The present result was corroborated by previous studies of Azirak and Karman (2008) who noted that the essential oils from *Foeniculum vulgare, Rosmarinus officinalis* and *Salvia officinalis* were also inefficient in inhibiting seed germination of *Sinapis arvensis* and *Rumex nepalensis*. Nevertheless, these same oils were reported to hinder seed emergence of some either weed or crop species (Angelini et al., 2003, Roilm de Almeida et al., 2010). This differential sensitivity across essential oils was highlighted earlier and could be attributed either to concentration treatments or to essential oil/weed interaction or also to chemical composition that will be discussed later on.

From the calculated IC_{50} doses, the one of *M. pulegium* was the lowest for both weeds *P. minor* and *S. arvensis* (<100 ppm and 600 ppm respectively) indicating its remarkable phytotoxicity. Our results seem to be congruent with those of Dudai et al. (1999) who marked the highest inhibitory effect of *Mentha piperita* essential oil compared to those of *Lavandula officinalis* and some *Artemisia* species. Based on their phytotoxic potency (IC50), the essential oils selected as most active can be subsequently ranked as *M. pulegium* > *L. officinalis* > *A. herba- alba*.

Presumably, the strong phytoxicity of these volatile oils must be accounted for, as asserted by Duke et al. (2002) and Dayan et al. (2009) on the presence of putative allelochemicals therein. Regardless of the chemical diversity, these allelochemicals can be substantially classified into phenolics and terpenoids (Singh et al., 2003). Among these latter, volatile monoterpenes and sesquiterpenes are reported to be the main occurring components in essential oils; displaying therefore a myriad of biological activities

including most particularly allelopathic one (Singh et al., 2003, Dayan et al. 2009).

Our phytochemical analysis showed that the tested essential oils were made up of mainly monoterpenes with low amounts of sesquiterpenes. Moreover, in agreement with available literature, the chemical composition varied with tested medicinal plant species and consequently led to differential inhibitory activities

Indeed, *M. pulegium* essential oil was dominated by pulegone (69.48%). This monoterpene ketone, being the major ingredient of the Pennyroyal commercial essential oil, was reported to be potentially lethal (Baser et al., 1998). This occurrence may undoubtedly explain why our tested essential oil totally impaired weed germination. Lawrence (1978) characterized three chemotypes of *M. pulegium* wild plants according to their major oil components. Therefore, our essential oil belongs to the pulegone chemotype. Similar *M. pulegium* chemotypes have been also described in Tunisia (Mkaddem et al., 2007, Hajlaoui et al., 2009) and elsewhere in Morocco (Ait-Ouazzou et al., 2012) and Greece (Cook et al., 2007). However, it is interesting to notice that our oil was clearly distinguishable by its relevant abundance of menthone (15.42%) not mentioned beforehand. This ketone was reported to have biological activities (Iscan et al., 2002). Despite their common Tunisian origin which is Sejenane; our oil revealed also higher pulegone amount (69.48%) than the one studied by Mkaddem et al. (2007) who recorded only 44.4%. Moreover, some components including sabinene, 3-octanol, cis-isopulegone and piperitenone not previously found in both aformentioned Tunisian oils (Mkaddem et al., 2007, Hajlaoui et al., 2009), were detected in our sample at relatively low amounts; while we did not perceive several other constituents such as isomenthone, piperitone, verbenone, borneol, menthol, methyl acetate, linalyl acetate, methyl eugeno, etc. These differences observed in oil chemical profiles are most likely due to a concomitance of many factors previously discussed, to which we can add the method of isolation of the essential oil, and the analysis conditions (Stanojevic et al., 2011).

As for Lavender essential oil obtained from dried flowering twig tops, it exhibited a typical composition of the genus *Lavandula* reported in available reference works with the main components linalool, camphor, 1,8 cineole and borneol. However, linalyl acetate existing in previously studied oils (Rolim de Almeida, 2010, Stanojevic et al., 2011) was not detected in our sample. Concurrently, the higher amount of linalool (37.972 %) and relatively increased contents of other terpenes in particular camphor (12.6), 1,8 cineole (11.11 %) and borneol (7.97 %) were reported to provide to the oil its pharmacological activities (Stanojevic et al., 2011). Besides, this latter oxygenated monoterpene (borneol) was found to possess allelopathic activity causing total impairment of weed germination (Fischer et al., 1994, Angelini et al., 2003). In fact, lavender is best known in the Mediterranean area and used as versatile medicinal plant. Moreover, borneol, camphor and 1,8 cineole have been previously described as potent monoterpenes causing inhibition of seed germination and plant growth (Fischer et al., 1994). This evidence seems to be therefore especially important in explaining somehow the pronounced inhibitory effect showed by *L. officinalis* essential oil in our investigation.

The resulting essential oil from *Artemisia herba-alba* seems to be particular, showing a different chemical profile compared to previously described oils of the same species originating from Middle East such as Israel, the Sinai Desert and Egypt, southern Europe mainly southern Spain (Vernin et al., 1995, Salido et al., 2004) and northern Africa including Morocco (Paolini et al., 2010), Algeria (Vernin et al., 1995) and Tunisia (Neffati et al., 2008, Mighri et al., 2010a; Mighri et al., 2010b, Kadri et al., 2011). In fact, this taxon was reported to be endowed with a prominent chemodiversity of its volatile oil. Based upon available data, two main oil types were generally recognized; those being marked by the predominance of one component among camphor, α- or β-thujone, chrysanthenone, chrysanthenyl acetate or davanone;

and those characterized by the co-dominance of two or more of these constituents. In a like manner, the aforementioned literature review dealing with *A. herba-alba* growing in Tunisia; showed that wormwood essential oils were distinguished by either their richness in one element such as *α*-thujone, *β*-thujone, 1,8-cineole, camphor, chrysanthenone or trans-sabinyl acetate; or the occurrence of two or more of these compounds in substantial quantities.

Accordingly, the scrutiny of all available information, allowed us to clearly reveal an important qualitative and quantitative differences in our *A. herba-alba* essential oil sample. Firstly, it should be noted that the current study elucidated for the first time the presence of 07 new components in Tunisian *A. herba-alba* oil. We could mention *o*-cymene, *β*-phellandrene, verbenene, *α*-terpinolene, *cis*-chrysanthenol, trans-sabinene hydrate and *α*-fenchene. To our knowledge, excepting *cis*-chrysanthenol, the six other compounds were not reported elsewhere. Hence, our volatile oil exhibited a distinct chemical composition being in sharp contrast to earlier studies. It was characterized by unusual high amount of chrysanthenone (22.48%). Other major components such as camphor (13.66%), β-thujone (10.38%), α-thujone (9.22%), myrtenal (7.54%) and 1,8-cineole (5.62%) were also detected. In Tunisia, only one *A. herba-alba* oil sample originating from the south was reported to have chrysanthenone at a concentration of 17% (Haouari and Ferchichi, 2009). Nevertheless, this oil was devoid of camphor, thujones as well as cineole and the oxygenated monoterpene (chrysanthenone) was rather dominated by a sesquiterpene component (davanone) (20.14%). Considerable amounts of chrysanthenone were described in essential oils from Spain, Morocco (Salido et al., 2004) and Algeria (Vernin et al., 1995). In addition, the presence of myrtenal is worth mentioning. Indeed, this oxygenated monoterpene which is not a common component in *A. herba-alba* essential oils was not previously reported at such appreciable amount (7.54%). As already discussed, such variability in volatile fractions might arise from many differences substantially those related to geographical and ecological factors. In fact, all previously studied Tunisian *A. herba- alba* oils were obtained from plants growing either in the south (Neffati et al., 2008, Mighri et al., 2010a, Mighri et al., 2010b) or in the Center (Kadri et al., 2011). Nevertheless, our essential oil was extracted from *A. herba-alba* plants harvested in a restricted area in the Tunisian Dorsale; characterized by a semi-arid climate where the species grows wild and plentifully in cultigen plant communities. It should be noted that this species is threatened due to sustained overexploitation using devastating practices which may lead to its exhaustion; particularly by overgrazing, extension of agriculture to the detriment of pasture, harvesting, etc. Hence, appropriate actions for efficient and sustainable management of this natural resource should be necessarily implemented.

Regardless its activity, all components might be contributing factors in the potent inhibitory effect of *A. herba-alba* essential oil. Interestingly, the major component chrysanthenone, has been shown to have antimicrobial activity (Griffin et al., 1998). α-thujone occurring in many other *Artemisia* species was considered as the main active ingredient of absinthe oil and found to be neurotoxic principle (Höld et al., 2000). Camphor, β-pinene, borneol, and α-phellandrene were reported to produce allelopathic actions (Fischer et al., 1994, Angelini et al., 2003); 1,8-cineole has been identified as one of the most potent allelochemicals released by *Artemisia* species (Duke et al., 2002); bicyclogermacrene and mixture of α-pinene and sabinene were known to possess a strong antimicrobial activity (Glisic et al., 2007). In addition, the oxygenated monoterpene myrtenal was reported to have a significant antioxidant and anticancer activity (Babu et al., 2012). Admittedly, this particular chemical composition has allowed our *A. herba-alba* oil to acquire a potent phytotoxic activity.

Ultimately, the abundance of oxygenated monoterpenes which were reported to be significantly more active than hydrocarbon monoterpenes (Rolim de Almeida et al., 2010); might thereby explain the potent inhibitory activity of *M. pulegium*, *L.*

officinalis and *A. herba-alba* essential oils. Nevertheless, while compromising weed seed germination, phytotoxic activities of the oils are difficult to correlate to a specific compound owing to their complexity and variability. In fact, dominant compounds could not be the only putative phytotoxicants; but all components including even the less abundant ones could rather contribute to overall inhibitory activity of the oils. Hence, like other allelochemicals, volatile monoterpenes might be acting in additive or synergistic manner (Kaur et al., 2010).

Despite their richness in monoterpenes, essential oils of *R. officinalis*, *S. officinalis* and *F. vulgare* and *E. gomphocephala* were found to be devoid of activity. This finding is in sharp contrast to previous data. Roilm de Almeida et al. (2010) have shown the inhibitory effect exerted by *S. officinalis* and *F. vulgare* oils against seed germination of some crop species *Raphanus sativus* L., *Lepidium sativum* L. and *Lactuca sativa*. Compared to ours, both volatile oils showed disparate chemical compositions. In fact, the authors identified *trans*-thujone (37.9%) and *cis*-anethole (76.3%) as the main compounds of sage and fennel respectively. Whereas, we detected *β*-thujone (19.03%) and estragole (77.26%) as their respective major components. These noticeable differences in oil compositions related to processing, phenological and environmental factors, etc. are likely to affect essential oil potentials, since these parameters contribute to both the profile and relative concentrations of active components (Singh and Maurya, 2005). While in the contrary, Angelini et al. (2003) reported that one Italian rosemary oil, having the same typical chemotype with a high content of 1,8- cineole (47%) as our Tunisian studied oil (33,57%); inhibited drastically the seed emergence of some annual weeds. Accordingly, this difference perceived across both rosemary oil bioactivities could be attributed either to concentration trials or to essential oil/weed interaction. Indeed, Dudai (1999) and Tworkoski (2002) observed that the same volatile oil requires disparate doses to inhibit different weed seed germination. For their part, Boutin and Rogers (2000) confirmed the existence of significant interaction between chemical family and plant family.

Conclusion

The above results provide plausible evidence that medicinal and aromatic plants *Mentha pulegium*, *Lavandula officinalis* and *Artemisia herba-alba* which have been selected following an ethnobotanical approach; are potentially promising resources of naturally occurring herbicides. Indeed, their essential oils inhibited strongly the seed germination of two noxious weeds *Phalaris minor* and *Sinapis arvensis* causing deleterious damages on cereals. From an ecological standpoint, it is worthwhile to make use of these natural products for non-chemical weed management in organic farming systems. Accordingly, these findings allow for broadening the scope of investigation on large-scale under natural field conditions in order to provide additional insights into the interaction of environmental factors including volatilization of allelochemicals, leaching, soil adsorbent power, root secretion, microbial decomposition and chemical transformation. On the other hand, it should be noted that the notable abundance of chrysanthenone and the occurrence for the first time of myrtenal at appreciable content in *A. herba-alba* oil is worth mentioning. This typical essential oil may be peculiar to the Tunisian Dorsale.

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