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ESSENTIAL OIL CHEMICAL CHARACTERIZATION AND ALLELOPATHIC POTENTIAL OF *ARTEMISIA CAMPESTRIS* L GROWING IN TUNISIA

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ABSTRACT

The present study investigated the chemical composition and allelopathic potential of volatile oil from *Artemisia campestris* originating from Tunisia against four species: *Daucus carota*, *Cicer arietinum*, *Phaseolus vulgaris* and *Triticum sativum*. The essential oil was extracted using the hydrodistillation method and its composition was analyzed by gas chromatography coupled to mass spectrometry (GC-MS). Furthermore, the essential oil was tested for its inhibitory effect on seed germination of the four precited species.
According to the main results, *Artemisia campestris* essential oil was rich in monoterpenoids and a total of 27 components were identified, accounting for 95.5% of the whole oil. Furthermore, β-pinene (35.0%) and 1, 8-cineole (14.4%) were the two major constituents. The volatile oil was evaluated for its allelopathic potential against the four fore-mentioned species. We noted a significant reduction in germination for all the tested species at 1000 ppm and 2000 ppm concentrations. However, an increase was noticed at the lowest concentration (100 ppm). Moreover, there was a delay in germination for *D. carota* seeds and this delay was proportional to the essential oil concentration. It is worthy to notice that *Artemisia campestris* essential oil exhibited allelopathic properties against the tested species and particularly against *D. carota.*

Keywords: *Artemisia campestris*, essential oil, β-pinene, allelopathy, bioherbicide

INTRODUCTION

Weeds constitute a real threat to the agricultural fields because they reduce the quality and the quantity of the agricultural product and also are responsible for a huge economic loss to crops. There are several ways of weed control including prevention, mechanical, cultural, physical, biological and chemical methods **(Qasem, 1992)**. These methods can be used individually or may be integrated for a successful weed management program **(Qasem, 2003; Singh et al., 2006)**.
Herbicides are not only create perceived hazardous impacts on agricultural products and consequently on human health but are also responsible of enhancing environmental pollution **(Batishet al., 2007)**. Additionally, the risk of weed resistance development and high cost-benefit ratio are other disadvantages of synthetic herbicides and pesticides usage **(Kordali, 2009)**. One of the causes of herbicide tolerance/resistance in weeds is the continuous application of the same herbicide or herbicides of the same mechanism of action year after another in the same field **(Caseleyet al., 1991; Krikorian, 1997)**.
These problems promoted efforts to produce new bioherbicides with environmental coexistence and desirable action. Thus, a large number of natural products are being tested as possible bioherbicides. Therefore in recent years, new approaches such as plant allelopathic effects have been considered to suppress weeds in agricultural systems **(Dayan et al., 2009)**. Allelopathy has been described as positive or negative interference in the process by which products of secondary metabolism of a particular plant are liberated into the environment, and these substances (allelochemicals) can benefit or harm the receptor plants in natural and agricultural systems. Currently, allelopathy is considered as an efficient approach which can be alternatively used to combat this weed in cropping systems and the understanding of allelopathic interactions between plants are one of the most interesting strategies for herbicide discovery.
Among natural substances, essential oils possess allelopathic activity and are regarded as potent inhibitors of seed germination and the development of different plant species, which accredits these compounds to be used as bioherbicides **(Dayan et al., 1999)**. This work has as objective to characterize chemically the essential oil of *Artemisia campestris* originating from Tunisia and to assess its allelopathic effect over the germination.

MATERIAL AND METHODS

Plant material

Leaves of *Artemisia campestris*L. (Asteraceae) used in the present study were collected in the region of Tajerouine, Le Kef (North-West of Tunisia) Latitude: 35°53'30" N; Longitude: 8°33'10" E; Altitude (elevation), in meters: 660; Lat, 10°45'10.76″ E Long, 12 m, Elevation, 300 Km south of Tunisia). Samples were collected on November 2014 and identified by Dr. Imtinen BEN HAJ JILANI from the National Institute for Agricultural Research of Tunis and deposited in the Herbarium of the same institution. The collected material was air-dried at room temperature for two weeks.

Essential oil extraction

The dried leaves of *A. campestris* were hydrodistilled over 180 min using a Clevenger type apparatus. The resulting distillate was extracted using diethyl-ether as solvent (1/1, v/v) and dried over anhydrous sodium sulphate. The organic layer was then concentrated at 35°C using a Vigreux column and the essential oil was stored at -20°C prior to analysis.

Analysis of the essential oil

Leaves of *Artemisia campestris*L. essential oil 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×0.25 mm ID, film

thickness of 0.25 mm). The oven temperature was held at 50°C for 1 min then programmed at rate of 5°C/min to 240°C and held isothermal for 4 min. The carrier gas was nitrogen at a flow rate of 1.2 mL/min; 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 Hewlette Packard 5972 MSD System. An HP-5 MS capillary column (30 m × 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/min. Oven temperature was programmed (50°C for 1 min, then 50e240°C at 5°C/min) and subsequently held isothermal for 4 min. 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: 40e300 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 C9eC28 on the HP-5 and HP-20M columns).

Germination test

The seeds of *Daucuscarota*, *Cicer arietinum*, *Phaseolus vulgaris* and *Triticumsativum* were cleaned and sorted to eliminate broken or damaged ones. Thereafter, they were dipped in water for 12 hours. To avoid contamination, the seeds were washed for 5 min in 95° alcohol and then rinsed with sterilized distilled water. The seeds were placed in gearbox-type germination boxes containing two sheets of sterilized blotting paper, which were soaked with solutions containing different concentrations of the essential oil, whose volumes were equivalent to 2.5 times the mass of the dry paper. The germination boxes were maintained in a BOD at 20˚C using a 12 h photoperiod. Seed germination was monitored for seven days, with daily counts of let- tuce seedlings until the seventh day of sowing and assessment of normal seedlings. The verification of the allelopathic effect of *A. campestris* essential oil was performed using three concentrations C1 = 100 ppm, C2 = 1000 ppm and C3 = 2000 ppm.

RESULTS AND DISCUSSION

Essential oil composition

The yield (v/w) of the essential oil from the air-dried leaves of *A. campestris*was of 0.24% and it was similar to the four areas. Twenty-seven compounds, representing more than 95% of the whole oil, were identified (Table 1). Monoterpene hydrocarbons accounting for 72.6% of the whole oil constituted its main fraction. They were followed by oyygenated monoterpenes, oxygenated sesquiterpenes and hydrocarbon terpenes presented at the respective rates of 20.5%, 2%, 1% and 0.3% of the whole oil. The major component of our oil was β-pinene with a percentage of 35%. It was followed by 1,8-cineole, p-cymene and myrcene whose amounts were of 14.4, 11.2 and 10.9%. We detected α-pinene at an amount of 8.1%.

Thirteen to fifteen components were identified in each sample of the essential oils of the aerial parts of several populations of *A. campestris*, collected from four areas of south-eastern of Tunisia (Bengardane, Benikhdache, Jerba and Tataouine). These compounds accounted for more than 95% of the total oil. All samples were dominated by the presence of β-pinene (24.2–27.9%), p-cymene (17.4–22.3%) and α-pinene (4.1–11.0%), representing more than 45% of the whole oil. The distribution of volatiles in these oils was qualitatively and quantitatively irregular. This variability of the composition can be attributed to the climatic and geographical conditions among areas **(Akrout et *al.*, 2001)**.

Bakchiche et *al.*, (2014) mentioned that essential oil yield was of 0.33% for aerial parts of *A. campestris* growing in Algeria. In their essential oil, the major constituents in aerial parts were α-pinene (75.8%) and sabinene (16%). Moreover, hydrodistillation of fresh aerial parts of *A. campestris*originating from Algeria resulted in 1.0 % as yield of a yellowish essential oil. The authors identified 48 compounds **(Ghorab*et al.*, 2013)**. Their essential oil was characterized by the main presence of β-myrcene (16.2 %), α-pinene (14.18 %), *trans*-β-ocimene (12.61%), p-cymene (8.15%) and camphor (5.85%). Essential oils of Tunisian and Italian *A. campestris* were mainly composed by β-pinene (24.0-49.8%) **(Bellomaria *et al.*, 2001; Akrout *et al.*, 2003)**. The same authors found that the species growing in Tunisia and Algeria have been reported to contain important amounts of α-pinene (5.9-12.5%) and (18%) of the whole oil, respectively.

Belhattab et al., (2011) collected leaves *A. campestris* essential oil with a yield of 0.66%. They reported as main components α-terpenyl acetate and α-pinene (19% and 18% respectively) followed by camphor (9%), camphene (8%), limonene and borneol (5% both).

Caryophyllene oxide (8.5-38.8%) has also been reported from the essential oil of *A. campestris*, growing in Lithuania **(Judzentiene *et al.*, 2010)**. According to **Belhattab *et al.*, (2011)**, the essential oil of *A. campestris* growing in Southern Algerian is characterized by relatively important amounts of camphor (9%).

Table 1 Chemical composition (%) of essential oil from Tunisian *Artemisia campestris.*

Volatile compound	Amount (% of whole EO)	Ki
α-pinene	8.1	974
camphene	0.1	983
sabinene	3.8	1000
β-pinene	35	1003
myrcene	10.9	1011
p-cymene	11.2	1037
1,8-cineole	14.4	1041
artemisiaketone	3.8	1055
g-terpinene	3.2	1064
artemisia alcool	0.1	1079
terpinolene	0.3	1089
linalool	0.1	1104
α-thujone	0.1	1119
camphor	0.2	1141
pinocarvone	0.1	1160
terpinene-4-ol	0.8	1170
α-terpineol	0.2	1189
myrtenol	0.1	1200
Z-chrysanthemylacetate	0.2	1261
bornylacetate	0.1	1287
sabinylacetate	0.2	1294
carvacrol	0.1	1309
β-caryophyllene	0.1	1432
g-selinene	0.2	1451
spathulenol	1.8	1540
caryophylleneoxide	0.1	1593
1-epi-cubenol	0.2	1630
Hydrocarbonmonoterpenes (%)	**72.6**	
Oxygenatedmonoterpenes (%)	**20.5**	
Hydrocarbonsesquiterpenes (%)	**0.3**	
Oxygentedsesquiterpenes (%)	**2.1**	
Total (%)	**95.5**	

Allelopathic activity of *Artemisia campestris* essential oil

According to figure 1, it is obvious that the essential oil of *A. campestris* affected the seed germination rate of all the tested species by causing a decrease at the concentrations 1000 ppm and 2000 ppm. Contrary to the concentrations 1000 ppm and 2000 ppm, the concentration 100 ppm resulted in an increase of the seed germination rate of all the seeds. Particularly, the rate of germination of the weed *D. carota* undergoes a decrease with 1000 ppm and 2000 ppm whereas we noticed an increased germination rate (11.65%) compared to the control (10%) at the 100 ppm concentration. According to these findings, *A. campestris* EO could have a stimulating effect at low concentrations.

It seems clear that at high dilutions, the essential oil of *A. campestris* could have a stimulating effect instead of an inhibitory impact on seed germination. Our findings were in agreement with those of **Abdel-Fattah *et al.*, (2011)** who found that allelopathic effects can cause both stimulatory and suppressive effects at lower and higher concentrations respectively.

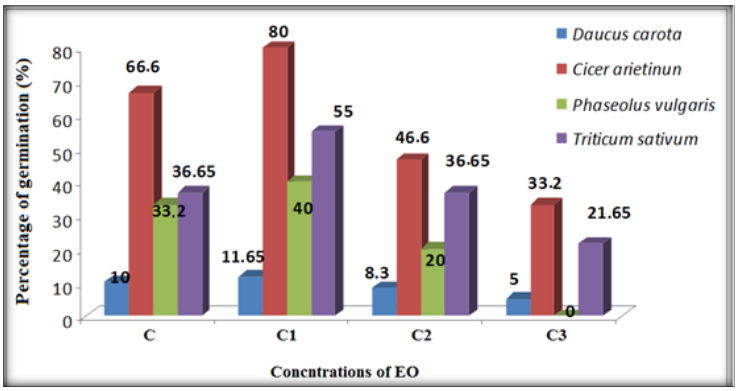

Figure 1 Variation of seeds germination rate of the tested species according to the concentration of *Artemisia campestris* essential oil. C: Control; C1 = 100 ppm; C2 = 1000 ppm; C3 = 2000 ppm.

According to figure 2, the speed of germination or the time required by *D. carota* seeds to germinate was also affected. Compared to the control, it increased from 6 to 8 days and from 6 to 11 respectively for the concentrations 1000 ppm and 2000. Such a delay caused at the sowing time may not be favourable for the

weeds to benefit from rains which can lead to preventing their growth especially during the first crop growth cycle.

According to **Ferreira and Áquila (2000)**, the allelopathic effect does not affect germination, but the germination rate or other process parameters, so the monitoring of germination must be daily. The authors mentioned that these changes in the pattern of germination may affect membrane permeability, DNA transcription, RNA translation, the operation of secondary messengers, breathing for the abduction of oxygen (phenol), the conformation of enzymes and receivers, or alternatively, the combination of these factors.

It has been reported that Eos and their constituents inhibited cell division in growing root tips and interfered with DNA synthesis in growing meristems **(Nishida *et al.*, 2005).** They also may induce oxidative stress, inhibit root growth, enhance lipid peroxidation and hydrogen peroxide accumulation, and increased electrolyte leakage in root tissue **(Scrivanti *et al.*, 2003; Singh *et al.*, 2006)**.

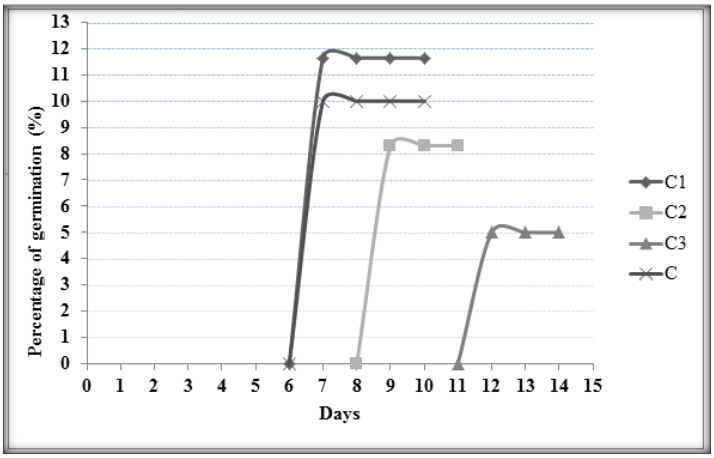

Figure 2 Variation of seeds Germination rate of *Daucuscarota* to the concentration in relation to time. C: Control; C1 = 100 ppm; C2 = 1000 ppm; C3 = 2000 ppm.

Yun and Maun (1997) conducted in greenhouse tests to verify the allelopathic effects of *A. campestris* subsp. *caudata* on seed germination, seedling elongation or increase of dry weight in plants at low concentrations (10 and 50%), but a 100% concentration of the extracts causes varying degrees of inhibition dependent on the species tested. A mixture of dry leaves of seedlings *A. campestris* in sand causes severe inhibition of seedlings of *Elymuscanadensis*. Furthermore, at low concentration, the aqueous extracts of *A. monosperma*aerial parts stimulated the germination percentage of common bean seeds. At high concentrations, *Artemisia campestris* had great inhibitory effects on the germination percentage and early seedling growth of common bean seeds. This indicates that the aqueous extract contained growth inhibiting allelochemicals and their effects were dependent on the concentration of *Artemisia* extract. **Adrian *et al.*, (2000); Lixf*et al.*, (2010)** and **Yang *et al.*, (2012)** revealed that the inhibitory effect of *Artemisia* water extract was directly related to the allelochemical concentration.

The allelopathic effect of *A. herba-alba* shoots on annual plants by means of volatile phytochemicals was previously reported in the Negev desert **(Friedman *et al*,. 1977)**. The allelopathic effect not only leaded to lower germination, but also a delay in germination, which in turn suggests a problem with the effective water for germination and seedling survival.

Escudero*et al.*, (2000) reported that phytochemicals involved in allelopathy may be thermo-labile. Results of the cool extraction treatments on scarified seeds suggest that allelochemicals, which might be easily leached by rainfall, rapidly reach a critical amount able to act on seeds.

Several studies have reported the role of volatile compounds in suppression of neighbouring vegetation **(Kohli and Singh, 1991)**. For example, they mentioned the chaparral species *Salvia* sp. and *Eucalyptus* sp. reputed for creating "monocultural islands" with no surrounding vegetation. Furthermore, the influence of foliar terpenes on the germination of competing species has been mentioned in many studies relative to Artemisia species. In fact, foliar tissues generate continuously volatiles. The emission and the accumulation of these secondary metabolites depend not only on the season, climatic and environmental conditions but also on genotype and plant age **(Fajer*et al.*, 1992)**.

When terpene storage structures are ruptured, these potential allelochemicals are immediately released in atmosphere and associated with soil organic matter as observed in many forest and shrubby ecosystems **(Hayward *et al.*, 2001)**. This association of phytotoxic terpenes with soil particles constitutes an eventual mechanism for allelopathic interaction in natural settings. It has been supposed that the synergistic combination of phytotoxic terpenes results in an alleloathic interaction with competiting species.

Abrahim et *al.*, (2000) demonstrated that β-pinene at 1.0 and 5.0 mM inhibited respiration in *Zea mays* roots. This may be due to the fact that monoterpenes are lipophilic molecules. Consequently, they readily penetrate into mitochondrial membranes, alter dehydrogenases activity and impair respiratory metabolism **(Penuelas *et al.*, 1996; Abrahim*et al.*, 2000)**.

It is known that water stress, high sun radiation, and high temperatures favour the production of volatile toxins in *A. herba-alba*, mainly α-pinene, camphor and 1,8-cineole **(Black, 1995)**. In fact, when there was more than 350 mm of annual rainfall, no reduction in the germination of annual plants was detected **(Black, 1995)**. In *A. californica*a greater amount of essential oil is extractable in autumn, after summer drought stress, than in winter, and some terpenoids become more abundant in late summer and autumn **(Halligan, 1975)**. *A. herba-alba* produces sesquiterpene hydrocarbons only when growing in thermophytic plant communities **(Cossini Lokar *et al.*, 1987)**.

This environment-driven depletion of volatile phytotoxin production could explain the highly diversified and productive annual plant community which appears interspersed among *A. herba-alba* shrubs and even below the canopy of the mature plants **(Somolinos, 1997; Escudero*et al.*, 1999)**.

VonPoser *et al.*, (1996), found that monoterpene vapors can cause anatomical and physiological changes in lettuce seedlings. Results obtained by Menezes et al., (2000) show that in the presence of light, lettuce germination and early development occur in a wide range of temperatures with 20°C being the best. The temperature of 35°C induces secondary dormancy. Corroborating with the first author, **Nascimento and Cantliffe (2002)**, working with lettuce seeds, stipulate that conditioning at suboptimal temperatures, lettuce seeds can reduce germination at low values.

Contradictory results according to the allelopathic potentie of *S. terebinthifolius* essential oil were reported. **Barbosa *et al.*, (2007)** reported that *S. terebinthifolius* essential oil at a concentration of 10,000 µg/mL did not cause significant inhibition of seed germination of *Lactuca sativa* and *Cucumis sativus* L. However, **Pawlowski *et al.*, (2012)** reported a reduction of lettuce germination percentage by 65.2%. This fact could be attributed to the variability of the chemical composition of this oil. In fact, **Barbosa *et al.*, (2007)** mentioned that *S. terebinthifolius* essential oil was mainly composed of sesquiterpenes, while **Pawloski *et al.*, (2012)** reported the predominance of monoterpenes.

Oils with high percentage of oxygenated monoterpenes were reported to have a great allelopathic potential **(De Almeida *et al.*, 2010, Vokou *et al.*, 2003)**. According to these authors, the potent phytotoxic activity was linked to the high percentage of oxygenated monoterpenes. Moreover, **Srivastava *et al.*, (2003) and Lopez *et al.*, (2009)** found that the Eos with high percentages of oxygenated compounds were more active than those with high percentages of hydrocarbon compounds

CONCLUSION

Artemisia campestris essential exhibited allelopathic properties against the tested species and particularly against the weed *Daucus carota*. This study should be continued by the investigation of seedling growth of Daucus carota thus holding a potential for use in weed management. However, in order to incorporate it as bioherbicide, there is a need to study the long term crop/weed allelopathic interactions under field conditions and to explore the physiological and biochemical mechanism of action.

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