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RESEARCH ARTICLE

Chemical composition and herbicidal effects of *Pistacia lentiscus* L. essential oil against weeds

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Abstract: The aims of this study were designed to examine the chemical composition of the hydrodistillated essential oil obtained from the leaves of *Pistacia lentiscus* L. and to evaluate their herbicidal effects against germination and seedling growth of four weeds (*Sinapis arvensis*, *Trifolium campestre*, *Phalaris canariensis* and *Lolium rigidum*). GC and GC–MS analysis of the essential oil were resulted in determination of 27 different components representing 98.9% of the total oil. α-pinene (20.6%), limonene (15.3%), β-pinene (9.6%) followed by germacrene D (8.4%) and terpen-4-ol (8.2%) were determined as the major compounds of the oil. On the other hand, essential oils of *Pistacia lentiscus* have shown very important herbicidal activities with a special attention for the inhibition of all weeds germination and seedling growth. In fact, at the dose of 3µl/ml, germination of *T. campestre*. *P. canariensis* and *L. rigidum* was totally inhibited and only reduced to 18.33% for *S. arvensis* and the phytotoxic effects of the oil exceeds the activity of the synthetic herbicide (2,4-D, isooctyl ester). In the light of these findings, we suggested that *P. lentiscus* essential oil may be considered as an interesting source of bio-herbicide components used as potent agents in weeds control.

Keywords: Allelopathy; essential oil; herbicidal activity; *Pistacia*; weeds.

Introduction

Weeds are defined as plants with no real use; these plants can grow in different habitats, especially cultivated fields. The presence of weeds in crop fields is generally unwanted by farmers for a number of reasons; Firstly, it reduces crop production by competing with the desired plants for the resources that a plant typically needs that soil nutrients, water and space for growth and most important, they are considered in most cases as host plants for pests (Buriro et al. 2003; Kolahi et al. 2009). According to an estimate conducted in the United States by Pimentel et al. (2001), weeds causes' crop loss which reaches 12% and costing to nearly US$ 33 billion to control them. Furthermore, the increasing herbicidal resistance of weeds has resulted in a dramatic increase in the use of herbicides. Now days, scientists have focused on the increase of pesticide residues in food. This has encouraged researchers to look for news alternative pesticides. Recently they have been considerable interest in biologically active compounds from plants as source of biopesticides. Essential oils from aromatic plants are examples of compounds with potential to control pests; they are becoming more popular because many synthetic drugs are connected with unpleasant side effects. Volatile oils also represent an interesting alternative due to

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emerging resistance pests against synthetic agents. (Ghasemi et al. 2007; Koudou et al. 2008; Shonouda et al. 2008; Nour et al. 2009). In this context, the use of secondary metabolites implicated in allelopathic interactions as sources for news agrochemical models could satisfy the requirements for crop protection and weeds management (Razavi 2011; Singh et al. 2003). Many plants have been demonstrated to control weeds when used as mulch under field conditions (Hong et al. 2003; Salam and Kato-Noguchi 2010). The phytotoxic potential of essential oils and their pure components have been also studied, in fact, earlier studies have documented that volatile oils are potent seed germination inhibitors (Amri et al. 2011ab; Amri et al. 2012abc; Abrahim et al. 2003; Scrivanti et al. 2003; Singh et al. 2002; Singh et al. 2006; Batish et al. 2006; Salamci et al. 2007), which explain their possible use as alternative herbicide. *Pistacia lentiscus* L. is a shrub belonging to the Anacardiaceae family which grows in many Mediterranean countries (Zrira et al. 2003). Traditional preparations from the leaves and fruit of *Pistacia lentiscus* are known by their use in treatment of many diseases such as hyper-tension and ulcer, also their effects were considered diuretic. Many researchers reported the chemical composition of the essential oil from leaves of *P. lentiscus* L. of diverse origins (Boelens and Jimenez 1991; Castola et al. 2000; Zrira et al. 2003; Ben Douissa et al. 2005; Chryssavgi et al. 2008; Mecherara-Idjeri et al. 2008) and most of them indicate that α and β-pinene, limonene and β-myrcene are the major components of *Pistacia lentiscus* essential oil.

Finally, *Sinapis arvensis, Trifolium campestre, Phalaris canariensis* and *Lolium rigidum* are four serious weeds in summer crops in the Mediterranean area and are considered very aggressive in cereal field (Amri et al. 2012a, b, c). In order to continue our studies in the exploitation of allelopathic interactions between plants in the search for natural substances with phytotoxic effects (Amri et al. 2011a, b; Amri et al., 2012a, b, c), the aims of the present study were to investigate the essential oil composition of *Pistacia lentiscus* grown in Tunisia and to the best of our knowledge; this is the first report on the phytotoxic effects of *Pistacia lentiscus* essential oil against germination and seedling growth of weeds.

Materials and methods

Plant material

The leaves of *Pistacia lentiscus* were collected from the I.N.R.G.R.E.F. arboretums (National Institute of Researches on Rural Engineering, Water and Forests) in October 2010 from the region of Korbous. Five samples collected from more than five different trees were harvested, mixed for homogenization, and used in three replicates for essential oil extractions. The specimen of the plant was submitted to the herbarium division of the institute and identification was confirmed in the Laboratory of Forest Ecology

Extraction of the essential oils

The essential oils were extracted by hydrodistillation of fresh plant material (100 g of each sample in 500 ml of distilled water) using a Clevenger-type apparatus for 3 h according to the standard procedure described in the *European Pharmacopoeia* (2004).

The oils were dried over using anhydrous sodium sulfate (a pinch/10 ml^{-1}) and stored in sealed glass vials at 4 °C before analysis. Yield was calculated based on dried weight of the sample (mean of three replications).

Analysis of the essential oils

The composition of the oils was investigated by GC and GC/MS. The analytical GC was carried out on an HP5890-series II gas chromatograph (Agilent Technologies, California, USA) equipped with Flame Ionization Detectors (FID) under the following conditions: the fused silica capillary column, apolar HP-5 and polar HP Innowax (30 m x 0.25 mm ID, film thickness of 0.25 mm). The oven temperature was held at 50 °C for 1 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% solu-

tion (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 x 0.25 mm ID, film thickness of 0.25 mm) was directly coupled to the mass spectrometry. The carrier gas was helium, with a flow rate of 1.2 ml/min. Oven temperature was programmed (50 °C for 1 min, then 50-240 °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: 40-300 amu. Software adopted to handle mass spectra and chromatograms was ChemStation. The identification of the compounds was based on mass spectra (compared with Wiley 275.L, 6th edition mass spectral library) Further confirmation was done from Retention Index data generated from a series of alkanes retention indices (relatives to C9-C28 on the HP-5 column) (Davies, 1990; Adams, 2001).

Seed germination and seedling growth studies

The seeds of *Sinapis arvensis, Trifolium campestre, Phalaris canariensis* and *Lolium rigidum* were collected from parent plants growing in Tunisia, in July 2009. Seeds were sterilized with 15% sodium hypochlorite for 20 min, and then rinsed with abundant distilled water. Empty and undeveloped seeds were discarded by floating in tap water and the remaining seeds were air-dried. Two filter papers were placed on the bottom of each Petri dish (9 cm diameter) and 20 seeds of the respective plant species were placed on the filter papers (Tworkoski, 2002). The oil was dissolved in tween– water solution (1%; v/v). The final concentrations of the treatments were 1, 2 and 3µl/ml. The emulsions (6 ml) were transferred to Petri dish placed on the bottom two layers of filter paper. A similar set-up but without essential oil served as control and in addition, 2,4-D, isooctyl ester was used as reference. Afterward Petri dishes were lined with a piece of Whatman no. 1 filter paper held in place with a transparent tape. The Petri dishes were closed and sealed with adhesive tape to prevent the volatile oils from escaping and kept at 25°C on a laboratory bench supplied with 12 h of fluorescent light (Tworkoski, 2002). The number of germinated seeds was daily counted and seedling lengths were measured. The assays were arranged in a completely randomized design with three replications including controls.

Statistical analysis

Data of seed germination and seedling growth assays were subjected to one-way analysis of variance (ANOVA), using the SPSS 13.0 software package. Differences between means were tested through Student-Newman-Keuls (SNK) and values of $p \leq 0.05$ were considered significantly different.

Results and discussion

Chemical composition of the essential oil

The essential oil yield of our study was 0.14% (v/w). GC–MS analyses of hydrodistillated essential oil of *P. lentiscus* allowed the identification of 27 different components representing 98.9% of the total oil. The global chromatographic analysis of *P. lentiscus* oil showed a complex mixture consisting mainly of mono- and sesquiterpenes. It was dominated by monoterpene hydrocarbons (63.9 %) and sesquiterpenes hydrocarbons (19.6%), while oxygenated mono and sesquiterpenes were only present in small percentage (respectively 11.7 and 3.7 %). The major components detected in the oil were α-pinene (20.6%), limonene (15.3%), β-pinene (9.6%) followed by germacrene D (8.4%) and terpen-4-ol (8.2%) (Table 1).

Essential oil composition of *P. lentiscus* has previously been published elsewhere (Zrira et al. 2003; Ben Douissa et al. 2005; Chryssavgi et al. 2008). According to these authors, monoterpene hydrocarbons and sesquiterpenes constituted the major components of *P. lentiscus* oil. On the other hand, essential oil composition of Tunisian *P. lentiscus* was determined by Ben Douissa (2005), data revealed from this study showed the abundance of α-pinene 17%, δ-terpinene (9%) and terpen-4-ol (12%), while

limonene was present at low amount. However, other reports show that β-myrcene was the major compound of essential oils from Algeria (Mecherara-Idjeri et al. 2008), Spain (Boelens and Jimenez 1991) and France (Castola et al. 2000). These differences found between the main constituents of oils obtained from *P. lentiscus* growing in Tunisia and these from other countries could be related particularly to climate, soils and the genetic background of tree.

Table 1: Essential oil composition of *Pistacia lentiscus* L.

Peak	Compounds	RI[a]	RI[b]	Area%	Identification
1	Tricyclene	926	1015	0.2	RI, MS
2	α-thujene	931	1020	0.65	RI, MS
3	α-pinene	939	1026	20.6	RI, MS, Co-GLG
4	camphene	953	1052	0.9	RI, MS
5	sabinene	976	1125	1.9	RI, MS, Co-GLG
6	β-pinene	980	1115	9.6	RI, MS, Co-GLG
7	myrcene	991	1152	3.4	RI, MS
8	α-phellandrene	1005	1160	3.85	RI, MS
9	α-terpinene	1018	1183	4.1	RI, MS
10	*p*-cymene	1026	1258	1.2	RI, MS
11	limonene	1031	1218	15.3	RI, MS, Co-GLG
12	α-terpinolene	1088	1287	2.2	RI, MS
13	terpen-4-ol	1177	1571	8.2	RI, MS
14	α-terpineol	1189	1673	3.5	RI, MS
15	α-cububene	1386	1537	0.6	RI, MS
16	α-copaene	1376	1515	1.1	RI, MS
17	β-bourbonene	1384	1520	1.2	RI, MS
18	β-elemene	1388	1588	1.3	RI, MS
19	*Z*-caryophyllene	1418	1608	2.6	RI, MS
20	α-humulene	1454	1670	0.9	RI, MS, Co-GLG
21	α-muurolene	1474	1688	1.1	RI, MS
22	germacrene D	1480	1721	8.4	RI, MS
23	β-bisabolene	1498	-	1.6	RI, MS
24	δ-cadinene	1524	1772	0.8	RI, MS
25	caryophyllene oxide	1581	2008	1.1	RI, MS
26	humulene epoxide	1606	2002	0.7	RI, MS
27	α-cadinol	1653	2225	1.9	RI, MS
Yield (w/w) % :				0.14±0.026	
Total identified % :				98.9	
Hydrocarbonated monoterpenes % :				63.9	
Oxygenated monoterpenes % :				11.7	
Hydrocarbonated sesquiterpenes % :				19.6	
Oxygenated sesquiterpenes % :				3.7	

RI: Retention Index; MS: mass spectrometry; Co-GLC: co-injection.
[a] Apolar HP-5 MS column.
[b] Polar HP Innowax column.

Herbicidal activity of P. lentiscus essential oil

Herbicidal effects of *P. lentiscus* oil were tested on seed germination and seedling growth of *S. arvensis*, *L. rigidum*, *P. canariensis* and *T. campestre*, which are considered very aggressive weeds in cereal field. The results show different degrees of inhibition on germination and seedling growth of these plant species relative to the control. The suppressing effects of the oil on germination were also higher than commercial herbicide, germination of all tested weeds were significantly reduced at lower doses (1 and 2 µl/ml), at the dose of (3 µl/ml), the germination was totally inhibited for all tested weeds and reduced to (18.33%) for *S. arvensis* while, at the same dose 2,4-D, isooctyl ester partially reduced the rate germination of *S. arvensis*, *L. rigidium*, *P. canariensis* and *T. campestre* respectively to 80, 88.33, 75 and 61.66%. As seen Table 2, as there was no germination of tested weeds there was no growth of these seedlings, while at low doses in the range of (1 and 2µl/ml), the oil partially inhibit germination, and inhibiting the aerial parts and roots of seedling while, inhibitory effects of the oil were lower compared to 2,4-D, isooctyl ester and their effects on root growth were most effective than aerial parts (Table 2).

The obtained results on the herbicidal activity of *P. lentiscus* oil confirm the allelopathic properties of Anacardiaceae family reported by others reports (Bulut et al. 2006; Scrivanti et al. 2003; Morgan et al. 2005; Donnelly et al. 2008; Zahed et al. 2010; Amri et al. 2012a). The herbicidal effects of essential oils against weeds have been previously reported and their phytotoxicity was generally attributed to the allelopathic properties of some terpenes (Abrahim et al. 2003; Scrivanti et al. 2003; Singh et al. 2002, Singh et al. 2006; Salamci et al. 2007). In our study, the oil was rich in monoterpenes, especially α-pinene, β-pinene, limonene, terpen-4-ol and sesquiterpenes that germacrene D and (*Z*)-caryophyllene which are known by their phytotoxic effects. Our results are in agreement with several studies that have demonstrated the herbicidal activity of monoterpenes in particular that of oxygenated components (De Feo et al. 2002; Salamci et al. 2007; Kordali et al. 2008). Essential oils of *P. lentiscus* were characterized by the relatively high content of limonene, α and β-pinene, and exhibited potent inhibitory effects on seed germination and seedling growth of all tested weeds. These findings suggest that the strong herbicidal effects of the oils are probably due to these components.

Table 2: Inhibitory effects of *P. lentiscus* essential oil on the germination and seedling growth of weeds.

Weeds	Samples	Dose (µl /ml)	Germination (%)	Seedling Growth (mm)	
				Aerial parts	Roots
S. arvensis	Control	0	98.33±1.66 a	84.33±6.43a	74±4.58a
	Essential oïl	1	71.66±4.4b	90±1.73a	45±2.88b
		2	51.66±6.0c	61.66±4.4b	31.66±1.66c
		3	18.33±1.66d	32.66±4.33c	26.66±1.66c
	2,4,d	3	80±2.88b	10.33±0.33d	11.66±0.33d
T. campestre	Control	0	71.6±4.4a	75±2.88a	70.33±3.17a
	Essential oïl	1	45±2.88c	71±4.58a	58.66±4.37b
		2	21.66±4.4d	51.66±1.66b	35.33±5.48c
		3	0.0±0.0e	0.0±0.0c	0.0±0.0d
	2,4,d	3	61.66±1.66b	7.66±1.45d	6.33±1.33d
P. canariensis	Control	0	100±0.0a	69.33±2.33a	86±2.64a
	Essential oïl	1	51.66±4.4c	51±4.93b	60.66±2.33b
		2	33.33±4.4d	34±2.3c	34.66±3.28c
		3	0.0±0.0e	0.0±0.0d	0.0±0.0d
	2,4,d	3	75±2.88b	9.66±1.85e	6.66±0.88d
L .rigidum	Control	0	96.66±3.33a	71±5.85a	91.33±2.9a
	Essential oïl	1	61.66±6b	57.66±3.38a	65.66±2.96b
		2	28.33±7.26c	41±2.08b	38.33±9.76c
		3	1.66±1.66d	7.33±7.33c	11±11d
	2,4,d	3	88.33±6a	10±0.57c	9.331.45d

Means in the same column by the same letter are not significantly different of the test Student-Newman-Keuls ($p \leq 0.05$).

In line with our findings, Scrivanti (2003) showed that limonene and α-pinene inhibited the root growth of the *Zea mays*; De Feo (2002) has demonstrated that both of α-pinene and limonene inhibited the germination of *Raphanus sativus* by (68 and 28% respectively). Our results were consistent with previous studies on the allelopathic potential of these compounds that the essential oils isolated from *Schinus areira* (Anacardiaceae family) and it majeur compound α-pinene (85.3%) possess potent herbicidal effect on *Zea mays* root growth (Scrivanti et al. 2003). On the other hand, other reports have demonstrated the herbicidal activity of sesquiterpenes that (*Z*)-caryophyllene and undecanone (kong et al. 1999; Kil et al. 2000; De Feo et al. 2002; Wang et al. 2009). For these reasons, the herbicidal activity of our oil was attributed to the presence of both sesquiterpenes and monoterpenes and the synergism between components do play an important role. Although the exact mechanisms of action of essential oil on germination and seedling growth inhibition remain unclear however, a number of effects and hypothesis have been reported by many authors. In general, the majority of reports agree that essential oils have phytotoxic effects that may cause anatomical and physiological changes in plant seedlings leading to accumulation of lipid globules in the cytoplasm, reduction in some organelles such as mitochondria, possibly due to inhibition of DNA synthesis or disruption of membranes surrounding mitochondria and nuclei (Koitabashi et al. 1997; Zunino and Zygadlo 2004; Nishida et al. 2005). It has documented that α-pinene enhanced solute leakage, and increased levels of malondialdehyde, proline and hydrogen peroxide, indicating lipid peroxidation and induction of oxidative stress, which explain the increase of antioxidant enzymes activities that superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, catalase and glutathione reductase (Singh et al. 2006). Abrahim (2003) have demonstrated that α-pinene acts on the seedling growth of *Zea mays* by two mechanisms: uncoupling of oxidative phosphorylation and inhibition of electron transfer, which result the uncoupling of mitochondrial energy metabolism and inhibition of the mitochondrial ATP production. In the same report it demonstrates that the actions of α-pinene on isolated mitochondria are consequences of unspecific disturbances in the inner mitochondrial membrane.

Conclusion

New trends in crop protection lead to a reduction in the levels of pesticides or/and to the use of "naturally-derived" pesticide from plants, animal or microbial origin. Among natural substances, essential oils and extracts from several types of plants used as flavouring agents are known to possess many biological activities and seem to be suitable for different types of products as bio-herbicide. In this sense, the development of natural herbicides would help to decrease the negative impact of synthetic agents, such as residues, resistance and environmental pollution. In this respect, natural herbicides may be effective, selective, biodegradable, and less toxic to environment. In our study *P. lentiscus* oil showed phytotoxic effects against weeds. Based on the present results, the oil could be suggested as alternative herbicides. However, further studies are required to determine the cost, applicability, safety and phytotoxicity against the cultured plants of these agents as potential herbicide.

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