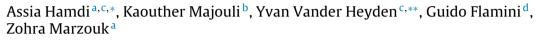
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Phytotoxic activities of essential oils and hydrosols of Haplophyllum tuberculatum



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ABSTRACT

Phytotoxic properties of plants and their compounds against other plants or seeds are being increasingly reported. Moreover, essential oils may provide a new source of phytotoxic agents with possibly novel mechanisms of action. The aim of this study is to determine the chemical composition of the leaves, stems and leaves + stems essential oils by GC/MS and to assess their phytotoxic activities extracted from *Haplophyllum tuberculatum* A. Juss. (Forssk) as well as of the aromatic water obtained from these parts and of the roots. The phytotoxic activities of the essential oils and hydrosols were evaluated against *Triticum aestivum* L. and *Raphanus sativus* L. seeds. The GC–MS analysis revealed the presence of limonene, *cis-p*-menth-2-en-1-ol, *trans-p*-menth-2-en-1-ol, *cis*-piperitol, *trans*-piperitol, 1-octyl acetate, piperitone and isobornyl acetate as major compounds. Phytotoxic results showed that the assayed stems essential oils had a significant activity against the tested seeds with IC₅₀ anged from 0.70 to 1.46 mg/mL for radical and epicotyls/coleoptiles percentage inhibition against *T. aestivum* L and *R. sativus* L. While the hydrosols of leaves, stems and leaves + stems inhibited seeds germination at a higher concentration. The essential oils of this plants cannot be used as biologic herbicide with these cultivated plants.

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1. Introduction

Plant communities are structured by species interactions, which can occur between the same species, called intraspecific, or between species, named interspecific. Allelopathy is a specific interaction between plants, direct or indirect, harmful or beneficial for one plant. Depending on the benefit for a given plant, symbiosis, commensalism, parasitism, neutralism, amensalism and competition are the six principal interactions found. Different categories of secondary metabolites from various plant parts are released into the environment by leaching, root exudates, volatilization, residue decomposition, and are implicated in the allelopathy (Schulze et al., 2002; Rice, 1984).

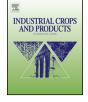
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http://dx.doi.org/10.1016/j.indcrop.2016.12.053 0926-6690/© 2016 Elsevier B.V. All rights reserved. Recently, several studies concentrated on different methods to increase the efficiency of agricultural plants (Li et al., 2013; Burn et al., 2015). One of these methods is the use of synthetic pesticides. However, in developing countries, the use of pesticides has dangerous effects on people and their environment. These effects are the results of insufficient regulation, lack of surveillance, limited enforcement, lack of training, inadequate access to information systems and poorly maintained or nonexistent personal protective equipment. Other studies showed that in these countries the annual incidence rate of acute pesticide poisoning in agricultural workers is as high as 18.2 per 100 000 full time workers and 7.4 per million among school children. The World Health Organization (WHO) defines acute pesticide poisoning as any illness or health effect resulting within 48 h from suspected or confirmed exposure to a pesticide (Thundiyil et al., 2008).

Therefore scientists have focused on searching for plant compounds to develop bio-pesticides as alternative. The allelopathic potential of plants may develop a healthy and sustainable agriculture by reducing the use of synthetic pesticides. Allelopathic







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compounds as phenols, flavonoids, tannins, coumarins, terpenoids, alkaloids and polyacetylenes can provide excellent inhibition of weed seed germination, insect, bacterial and fungal influences (Duke et al., 2000). The volatile compounds of essential oils, especially terpenes, showed important allelopathic action (Verdeguer et al., 2009). Because of the complex and variable mixtures of compounds occurring in essential oils, the relevant quantitative relationships between chemical structures and effects have not yet been established. Chemical compounds have the potential to either inhibit or stimulate the growth of plants and this can be by different mechanisms and acting on different sites of action. Various factors in this effect as season, changing concentrations, population, interactions of allelochemical components (antagonistic and synergistic) can be involved (Vokou et al., 2003; Asplund, 1968). Previous studies were interested in the effect of isolated or synthetic compounds on some seeds, but few researches were concentrated on the antagonistic or synergic compounds of the volatile oils (Vokou et al., 2003; Einhellig and Rasmussen, 1978).

Rutaceae is a large family comprising about 161 genera and 1815 species. The genus *Haplophyllum* (Rutaceae) contains about 70 species colonized from subtropical zones of Eurasia and the northern tropical zone of eastern Africa (Townsend, 1986). In Tunisia, Rutaceae is represented by 3 genera: *Ruta*, *Citrus* and *Haplophyllum*. The genus *Haplophyllum* is composed by 3 species *Haplophyllum linifolium* (L.) A. Juss (*Haplophyllum hispanicum* Spach), *Haplophyllum tuberculatum* A. Juss (Forssk.) and *Haplophyllum buxbaumii* Poiret (Pottier-Alapetite, 1979).

Haplophyllum tuberculatum (Forsskal) A. Juss is a perennial herb, which grows in sandy depressions and spills (Pottier-Alapetite, 1979). *H. tuberculatum* exhibits a biochemical diverse composition. The chemical composition of the essential oils from this species collected from different regions has been studied and varied considerably across the regions of collection (Al-Rehaily et al., 2014; Al-Yousuf et al., 2005; Al-Burtamani et al., 2005). In addition, several classes of compounds such as alkaloids, lignans, coumarins and flavonoids have been isolated from the aerial parts of *H. tuberculatum* (Al-Shamma et al., 1979; Ulubelen and Öztürk, 2008; Sheriha et al., 1987).

Hydrosols, also named hydrolates, distillate water and aromatic water are the by-products or co-products produced during water or steam distillation of plant material. They are rich in water-soluble components and traces of essential oils. The hydrosols of various plants showed many biological activities: antibacterial (Tornuk et al., 2011; Sagdiç, 2003), anti-tyrosinase (Lante and Tinello, 2015), antifungal (Boyraz and Ozcan, 2006) and antioxidant (Aazza et al., 2011).

The objective of our work is to investigate the volatile extracts of *H. tuberculatum* obtained from leaves, stems and the combination leaves + stems, as well as their hydrosols for having a phytotoxic effect on *Triticum aestivum* L. and *Raphanus sativus* L. seeds. This is the first report on the phytotoxicity of *H. tuberculatum* A. Juss essential oils on *T. aestivum* L. and *R. sativus* L. seeds germination and growth.

2. Materials and methods

2.1. Plant material

Plant material from the *H. tuberculatum* A. Juss (Forssk.) species was collected from Beni Ghzayel, Medenine in South-East Tunisia (Jeffara, Medenine). This region has the geographical coordination 33°21'17" North 10°30'19" East with an arid climate. The herbarium specimens were authenticated by examining the morphological and anatomical features in the Botany Department, Faculty of Pharmacy Monastir Tunisia and Botany Department

of Faculty of Sciences Sfax Tunisia, according to the flora of Tunisia (Pottier-Alapetite, 1979) and a voucher specimen (H.t-01.03) deposited in the Biological Laboratory of the Faculty of Pharmacy of Monastir. The leaves, stems, leaves + stems and roots were cut into small pieces and weighed before extraction of the volatile compounds.

2.2. Extraction of essential oils and hydrosols

Essential oils and hydrosols of different parts of *H. tuberculatum* were extracted using a Clevenger-type apparatus (Marzouk et al., 2008). A volume of 500 mL distilled water was added to 100 g leaves, stems or roots of the plant. The aerial part essential oil was obtained from 100 g of the mixture of leaves + stems (70% stems + 30% leaves) from *H. tuberculatum*. The hydrosols and the volatile oils are kept at 3 °C. The roots of the plant did not contain essential oils, but its hydrosols were kept.

2.3. Analytical GC-MS

Gas chromatography (GC) analyses were carried out with an HP-5890 Series II instrument equipped with HP-WAX and HP-5 capillary columns ($30 \text{ m} \times 0.25 \text{ mm}$, $0.25 \mu \text{m}$ film thickness), working with the following temperature program: 60°C for 10 min, ramp of 5 °C/min up to 220 °C; injector and detector temperatures 250°C; carrier gas was helium (2 mL/min); detector dual FID; split ratio 1:30; injection of 0.5 µL (10% hexane solution). Components identification was carried out, for both columns, by comparing their retention times with those of pure authentic samples and by means of their linear retention index (LRI), relative to the series of *n*-hydrocarbons. Gas chromatography-electron impact mass spectroscopy analyses were performed with a Varian CP-3800 gas chromatograph, equipped with a HP-5 capillary column ($30 \text{ m} \times 0.25 \text{ mm}$; coating thickness $0.25 \mu \text{m}$) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions are as follows: injector and transfer line temperatures 220 and 240 °C, respectively; oven temperature programmed from 60 to 240 °C at $3 \,^{\circ}$ C/min; carrier gas helium at 1 mL/min; injection of 0.2 μ L (10% hexane solution); split ratio 1:30. Constituents identification was based on comparison of retention times with those of authentic samples; this implied comparing their LRIs with the series of *n*-hydrocarbons and using computer matching against commercial (NIST 98 and ADAMS) and home-made library mass spectra (built up from pure substances and components of known oils and mass spectra literature data) (Stenhagen et al., 1974; Massada, 1976; Jennings and Shibamoto, 1980; Swigar and Silverstein, 1981; Davies, 1990; Adams, 1995). Moreover, molecular weights of all identified substances were confirmed by gas chromatographychemical ionisation mass spectrometry, using methanol as the chemical ionizing gas.

2.4. Phytotoxic activity

Leaves (LEO), stems (SEO) and leaves+stems (LSEO) essential oils were dissolved in diethyl ether and placed in Petri dishes lined with filter paper. The solvent was evaporated and 2 mL of distilled water was added. The concentration of the essential oils used in the experiments was chosen based on our experience. Four final concentrations were evaluated 0.25, 0.50; 1.00 and 2.00 mg EO/mL distilled water. The hydrosol extracts: leaves (LHd), stems (SHd), leaves+stems (LSHd) and roots hydrosols (RHd) were forwarded to an allelopathic assay on filter paper at the same concentrations as the essential oils. The phytotoxic properties of these hydrosols (Hd) were revealed by a concentration of 100% of the hydrosols (2 mL of Hd). Seedlings watered with distilled water were used as control. Download English Version:

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