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Phytotoxic activity and variation in essential oil content and composition of Rosemary (*Rosmarinus officinalis* L.) during different phenological growth stages

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ABSTRACT

Phytotoxic activity and variation in the quantity and quality of the essential oil (EO) of *Rosmarinus officinalis* L. (Lamiaceae) were examined at different phenological growth stages of full flowering (FF), fruit set (FS) and full ripened fruit (FRF). The EO yields (w/w %) at different stages were in the order of: FF (0.94%), FS (0.98%) and FRF (1.15%). The EO samples were analyzed by gas chromatography (GC) and GC–mass spectrometry (GC–MS). The main oil components at different phenological stages were found to be α-pinene (25.8–27.7%), camphor (8.6–9%), camphene (6.5–7.7%) and 1, 8-cineole (9.4–9.6%). The EO phytotoxicity activity of the mentioned stages was determined at 0, 300, 600, 900, 1200, 1500 and 1800 $\mu L L^{-1}$ concentrations on a weed plant, prickly lettuce (*Lactuca serriola* L.), and a crop, radish (*Rhaphanus sativus* L.). The inhibitory effect of rosemary was found to be depended on the plant's phenological growth stages and EO concentrations.

1. Introduction

Rosmarinus officinalis (Lamiaceae), more popularly known as "rosemary", is an aromatic evergreen shrub, attaining a height of about one meter with upright stems, whitish-blue flowers and dark green leaves. It is native to the Mediterranean areas, and is widely distributed in many parts of the world (Cui et al., 2012; Albuquerque et al., 2007; Ventura-Martlneza et al., 2011; Al-Sereitia et al., 1999). This plant is used fresh, dried or for obtaining EO, which is largely used in traditional and modern medicine (Pintore et al., 2002). The yield of EO from dried leaves is 1–2% (v/w). The oil is a colorless or pale yellow liquid having the characteristic odor of the plant. Its main components are 1,8-cineol, α-pinene, camphor, bornyl acetate, camphene, linalool, D- limonene, borneol, myrcene, terpineol, rosmaron and caryophyllene (Zargari, 1990; Al-Sereitia et al., 1999; Rao et al., 1998; Pintore et al., 2002). An earlier report (Moghtaderand Afzali, 2009) has also shown that α-pinene (15.5%), camphor (11.6%), verbenone (11.1%) and 1,8-cineole (10.3%) were the major oil components of *R. officinalis* from Kerman Province (I.R. Iran). Moreover, rosemary contains high levels of biologically active compounds such as carnosic acid, carnosol, ursolic acid, betulinic acid, rosmarinic acid, rosmanol, oleanolic acid, and micrometric acid (Razborsek et al., 2008; Zegura et al., 2011; Hernandez-Hernandez et al., 2009).

Nowadays, the importance of allelopathy in the natural control of weeds and crop productivity is highly recognized (Nourimand et al., 2011). Allelopathy phenomenon is defined as the ability of plants to protect themselves using natural allelochemicals (Gniazdowska and Bogatek, 2005); another definition is the chemical communication between microbe–microbe, plant–microbe, plant–insect or plant–herbivore (Weir et al., 2004). While phytotoxicity is a toxic effect by a compound on plant growth, such damage may be caused by a wide variety of compounds including trace metals, salinity, pesticides, phytotoxins or allelochemicals. Allelochemicals are usually considered as secondary products or waste products of the main metabolic pathways in plants, containing complex compounds (Jinhu et al., 2012). They are phenolics, alkaloids, flavonoids, terpenoids, momilactones, hydroxamic acids, brassinosteroids, jasmonates, salicylates, glucosinolates, carbohydrates and amino acids (Farooq et al., 2013). Aromatic plants are rich in EOs and phenolic components. They can play an important role in plant interactions, and are considered as a major source of allelochemicals (Saharkhiz et al., 2010). It has been demonstrated that the EO of catnip (*Nepeta cataria* L.) from Lamiaceae family has inhibitory effects on the seed germination and seedling growth of *Hordeum spontaneum* Koch, *Taraxacum officinale* and *Avena fatua* L., as well as the three crop seeds of *Lipidium sativum*, *Nepeta cataria* and *Ocimum basilicum* (Saharkhiz et al., 2016). The allelopathic potentials of aqueous extracts and

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leaf powders of three *Satureja* species, namely: *S. khuzestanica* Jamzad, *S. bachtiarica* Bunge and *S. rechingeri* Jamzad (Lamiaceae) have been previously reported (Taban and Saharkhiz, 2015).

Variation in the chemical compositions of the EOs and extracts of medicinal and aromatic plants depends on the factors such as origin, environmental conditions, ontogeny, genetic modifications, analytical methods, different plant parts, and developmental stages of collected plant materials (Nejad Ebrahimi et al., 2008; Eyres et al., 2005; Saharkhiz and Mohammadi, 2011; Msaada et al., 2007). In recent years, numerous publications regarding the chemical compositions of the EOs of medicinal and aromatic plants have reported that growth stage and harvesting time have major impacts on the plants' EOs content and chemical compositions (Saharkhiz et al., 2009; Msaada et al., 2007).

There are substantial data on the chemical composition of rosemary EO. However, in the present work, the EO content and chemical compositions of the plant during different phenological stages along with the phytotoxic activities of the EO from each growth stage were determined and comparatively discussed.

2. Materials and methods

2.1. Plant material

The aerial parts of *R. officinalis* were collected during three periods: namely FF (100% of plants at flowering stage) on the 14th of April, FS (green fruits) on the 6th of May, and FRF (brown fruits) on the 20th of May 2012 from Sadra Medicinal and Aromatic Plants Botanical Garden (Shiraz, Iran) at an altitude of 1846 m above the mean sea level, latitude 29.8° north and 52.4° east, the mean value for yearly temperature 14.5 °C, and the mean value for yearly rainfall 437 mm. The plant species was identified and authenticated by A.R. Khosravi, a plant taxonomist at Shiraz University Herbarium, Shiraz, Iran.

2.2. Essential oil isolation

The aerial parts of the plant were air-dried at room temperature (less than 25 °C) in a shady location for 10 days. Samples (50 g, three replicates for each stage) were hydrodistilled for 3 h using an all glass Clevenger-type apparatus to extract the EOs according to the method recommended by the European Pharmacopoeia (Anonymous, 1997). The extracted EOs were dried over anhydrous sodium sulphate and stored in sealed vials at low temperature (4 °C) before gas chromatography (GC) and gas chromatography-mass spectrometric (GC–MS) analyses.

2.3. EO analysis procedure

The components of volatile oil from the aerial parts of the plants were identified using GC and GC–MS analyses. The GC analysis was performed using an Agilent gas chromatograph series 7890-A equipped with a flame ionization detector (FID). The analysis was carried out on fused silica capillary HP-5 column (30 m × 0.32 mm i.d.; film thickness 0.25 μm). The injector and detector temperatures were kept at 250 °C and 280 °C, respectively. Nitrogen was used as carrier gas at a flow rate of 1 ml.min; oven temperature program was 60–210 °C at the rate of 4 °C min, which was then programmed to 240 °C at the rate of 20 °C min, and finally, held isothermally for 8.5 min. The split ratio was 1:50. The GC–MS analyses was carried out by the use of Agilent gas chromatograph equipped with fused silica capillary HP-5MS column (30 m × 0.25 mm i.d.; film thickness 0.25 μm) coupled with 5975-C mass spectrometer. Helium was used as carrier gas with the ionization voltage of 70 eV. Ion source and interface temperatures were 230 °C and 280 °C, respectively. Mass range was from 45 to 550 amu. The oven temperature program was the same as for the GC. The retention indices for all components were determined according to the method using n-alkanes as standard.

2.4. Identification of EO components

The compounds were identified by comparing their retention indices (RI, HP-5) with those reported in the literature and also by comparing their mass spectra with the Wiley GC–MS Library, Adams Library, Mass Finder 2.1 Library data, and published mass spectra data (Adams, 2007).

2.5. Bioassay

Radish as a horticultural crop and prickly lettuce as a cosmopolitan weed with worldwide distribution (Bown, 2010) were selected for phytotoxic experiments. Uniform and healthy seeds of the examined plants were collected from the Research Field Station of Faculty of Agriculture, Shiraz University (Fars Province, Iran). The seeds were stored at 4 °C for future examinations. Determination of the phytotoxic effects of the EOs on the crop plant compared to the weed species was the main reason for selecting a crop for germination tests. In order to detect the phytotoxic effects of rosemary, different concentrations of 300, 600, 900, 1200, 1500, and 1800 $\mu l L^{-1}$ of the EOs obtained at the FF, FS and FRF stages were used. The EO concentrations were selected based on the preliminary tests and the literature review (Verdeguer et al., 2009; Saharkhiz et al., 2010; Taban et al., 2013; Mahdavikia and Saharkhiz, 2015). Distilled water was served as the control. Whatman No. 1 filter paper was placed in 9 cm diameter petri dishes moistened with 4 ml of EO solution. Three replications of 50 seeds of radish and prickly lettuce were used for each treatment. To prevent evaporation, the petri dishes were sealed with parafilm and placed in a growth chamber (1300 STC Mod, Noor-Sanat-Ferdows Company, Karaj, Iran) at 25 ± 2 °C, 4000lx and 16 h photoperiod. They were monitored daily and moistened with distilled water as needed. After 8 days, all of the germinated and non-germinated seeds were counted (No germination was happened after this period). Seeds showing radicle emergence (2 mm) were recorded as germinated. The germination percentages were determined (Germination Percentage (GP) is an estimate of the viability of a population of seeds). The equation to calculate germination percentage is: (GP = seeds germinated.total seeds × 100). In addition, the length of the primary roots and shoots (using a scientific measuring ruler) was measured and recorded (Saharkhiz et al., 2010).

2.6. Statistical analysis

The experiment was arranged based on a completely randomized design (CRD) with three replications for each treatment. The normality test was done by using Minitab statistical software (version 15) to assess data normality, and transformation of the data was performed as needed. One-way analysis of variance (ANOVA) was used to determine the significant differences among the treatments. All data were analyzed using SAS software (version 9.0), and the means were compared using LSD test at 5% level.

3. Results

The EOs were obtained by hydrodistillation of 50 g of the air-dried samples. The content of EOs (w/w %) at different phenological growth stages was in order of: FF (0.94%) < FS (0.98%) < FRF (1.15%). Therefore, the highest amount of EO was obtained in the