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Phytotoxic effects of several essential oils on two weed species and Tomato

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ABSTRACT

Essential oils (EOs) composition of *Rosmarinus officinalis* L., *Satureja hortensis* L. and *Laurus nobilis* were analyzed by GC and GC–MS instrument. Then phytotoxic activities of mentioned EOs and a combination of *R. officinalis* and L. *nobilis* EOs (R + L) were evaluated against germination and growth of two weeds species, *A. retroflexus* (dicot), *B. tectorum* (monocot) and tomato. Applied EOs strongly inhibited the germination and seedling growth of the tested species, in a dose dependent manner with *A. retroflexus* being significantly more sensitive than others. Indeed, at 400 $\mu L.L^{-1}$ EO of *R. officinalis*, germination of *A. retroflexus* decreased 91.3%, while for the same dose, germination and seedling growth of *B. tectorum* and *tomato* were reduced by 56.7% and 26.7%, respectively, compared with control. *R. officinalis* EO caused the most germination inhibitory for *A. retroflexus* and tomato while *B. tectorum* germination was well inhibited by *S. hortensis* EO. Seedling growth were also affected by EO application in a dose response manner. *A. retroflexus* shoot length was inhibited by the R + L EO more than other EOs while most root growth inhibition caused by *S. hortensis* EO. For *B. tectorum*, and tomato, *S. hortensis* had the strongest inhibitory effect on root and shoot elongation.

1. Introduction

Weeds are uninvited guests in farms that contribute to numerous direct and indirect losses in the crop fields. They compete with the crops and suppress their growth and development. Yield reduction by weeds in the farms is usually greater than the losses caused by disease, pests and insects. In addition, weeds might reduce biodiversity and interfere with the accessibility of water by blocking water channels (Gnanavel, 2015; Jabran et al., 2015). Even though using chemical herbicides is a solution to control weeds but many weeds are evolving resistance to them. Beside this, negative effects of synthetic herbicides on nature and organisms are undeniable. Thus environmental friendly herbicides with new modes of action are in highly demand (Abrahim et al., 2000; Dayan and Duke, 2014).

Today's agriculture is shifting toward organic agricultural and allelopathy brings new hopes to control weeds without applying synthetic herbicides. Allelochemical have been found in different forms such as phenolic compounds, alkaloids, flavonoids, terpenoids, momilactones, hydroxamic acids, brassinosteroids, jasmonates, salicylates, glucosinolates, carbohydrates, and amino acids which are usually identified as secondary metabolites (Weston, 1996; Ma et al., 2012; Farooq et al., 2013). Terpenes which are the most abundant compound in EOs are the largest group of secondary metabolites with great diversity (Gershenzon and Dudareva, 2007). EOs have been extensively tested in order to evaluate their herbicidal effects. Although synthetic herbicides are cost effective but organic herbicides based on EOs can be potential alternative because of their safety for human and environment (Amri et al., 2013). Natural products including EOs have quick biodegradation which means they will degrade in a short time and do not remain on the products after the harvest. Beside this, they may possess different modes of action from synthetic herbicides. More interestingly they can improve pollination by acting as attractant agents for pollinators while deter other harmful insects (Romagni et al., 2000; Singh et al., 2002; Blazquez, 2014).

Rosmarinus officinalis L., *Satureja hortensis* L. and *Laurus nobilis* are native to Mediterranean area and belong to Lamiaceae family. Plants in this family are significantly important because of their uses as ornamental, medicinal and aromatic plants. Also EOs extracted from species of this family are utilizing in traditional medicine, pharmaceutical, food and cosmetic industries (Mamadalieva et al., 2017). Additionally, EOs of Lamiaceae family species have promising herbicidal activity which is contributed to presence of individual components in their EOs that are highly active such as α-Pinene, Limonene, 1,8-Cineole, Carvacrol, Camphor and Thymol (Batish et al., 2007; De Martino et al., 2010;

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Mutlu et al., 2010; Sadgrove and Jones, 2015; Hazrati et al., 2017).

R. officinalis known as rosemary is a medicinal and aromatic shrub with dark green leaves and white to blue flowers. It's EO has light yellow color and its major compounds are including 1, 8 Cineole, α-Pinene and Camphor (Ventura-Martlneza et al., 2011; Cui et al., 2012; Alipour and Saharkhiz, 2016).

S. hortensis (Summer savory) is an annual horticultural plant which is broadly planted around the world (Bezic et al., 2009). The plant is economically important in culinary and cosmetic industry. Also it is widely used in medicine because of its carminative, stomachic, anti-diarrheal and diuretic influences. Also antibacterial, antioxidant and antifungal activities of EO and methanol extracts of the *S. hortensis* have been reported (Gulluce et al., 2003; Saharkhiza et al., 2011).

L. *nobilis* is an evergreen tree widely cultivated in warm area as an ornamental plant. Fresh or dry leaves of tree are used as food flavor and its EO can be used in cosmetic and pharmaceutical industries. In addition, the EO has antibacterial, antifungal and antioxidant activities (Bakkali et al., 2008). Major components in the extracted EO of leaves are 1, 8 Cineole and Linalool (Pino et al., 1993; Flamini et al., 2007). Finally, *Amaranthus retroflexus* L. and *Bromus tectorum* L. are two serious weeds in summer crops in the most part of the world and are considered to be very aggressive in farms (Holm et al., 1991).

The aim of the present work was to evaluate the phytotoxic effects of three EOs individually and in combination on two important weed species and Tomato as a horticultural crop. Such information might be used as basic requirements for achieving the formulation of natural herbicides in organic agricultural systems.

2. Materials and methods

2.1. Plant material

The aerial parts of *R. officinalis* were collected during full flowering stage from Sadra Medicinal and Aromatic Plants Botanical Garden (Shiraz, Iran). Leaves of L. *nobilis* were collected during full flowering stage from Eram Botanical Garden (Shiraz, Iran). At the beginning of the fruit set stage, the aerial parts of *S. hortensis* were harvested from Experimental field of Agriculture College, Shiraz University, Shiraz, Iran. All the harvested materials were air-dried at room temperature (less than 25 °C) in a shady location for 14 days.

The plant species was identified and authenticated by A. Khosravi, a plant taxonomist at Shiraz University, Shiraz, Iran. The voucher specimens were deposited in the herbarium.

2.2. Essential oil isolation

The samples (30 g, three replicates) were hydrodistilled for 3 h using an all glass Clevenger-type apparatus to extract the EO (W/W%) according to the method recommended by the European Pharmacopoeia (Anonymous, 1997). The EO of each species was separated from the aqueous layer and dried over anhydrous sodium sulfate. The EO samples were stored in sealed vials at low temperature (4 °C) until analyzed by GC and GC–MS and future experiments.

2.3. Essential oil 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 sample volume injected into the GC was 0.2 μL pure EO. The temperature of injector and detector was set at 250 °C and 280 °C, respectively. Nitrogen was used as carrier gas at a flow rate of 1 mL min; the 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 analysis 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. The sample volume injected into the capillary column was 0.1 μL pure EO in the split mode (1:50). Helium was used as carrier gas with the ionization voltage of 70 eV. The temperature of ion source and interface was 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 essential oil 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 and Mass Finder 2.1 Library data, as well as the published mass spectra data (McLafferty and Stauffer, 1989; Adams, 2007).

2.5. Bioassay

Uniform and healthy seeds of the examined plants (*A. retroflexus* and *B. tectorum*) were collected from the Research Field Station of Faculty of Agriculture, Shiraz University. Tomato seeds of cultivar Cal-J N3 were used. The seeds were stored at 4 °C for future examinations. In this stage, we determined the Phytotoxicity effect and selectiveness of *R. officinalis*, *S. hortensis*, L. *nobilis* and combination of *R. officinalis* (50%) and L. *nobilis* (50%) EOs (R + L) in several concentrations (100, 200, 400, 800, 1000 and 1200 $\mu L.L^{-1}$) on the studied weeds and tomato. A solution of 2.0% v/v Tween 80 in distilled water was served as the control. Four replications were used for each treatment. Whatman No. 1 filter paper was placed in 9 cm diameter Petri dishes moistened with 4 mL of EO solutions. Each petri dishes contained 20 seeds from each species. 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, 4000 lx and 16 h photoperiod. They were monitored daily. After 7d, all germinated and non-germinated seeds were counted. No seed germinations were observed after this time. Seeds showing radicle emergence (1 mm) were recorded as germinated. Final germination percentage (GP) which is an estimate of the viability of a population of seeds and germination rate (GR) were calculated for each trial by method of Hartman et al. (1990) (the below equations). Moreover, the length of the seedling's primary root and shoot was measured by scientific 20-in. ruler.

$$GP = \frac{n}{N} \times 100$$

n = Number of newly germinating seeds
N = Total number of seeds

$$GR = R_s = \sum_{i=1}^{n} \frac{Si}{Di}$$

Rs = Maquer germination rate (number of seeds per day)
Si = Number of germinated seeds.
Di = Number of days

2.6. Statistical analysis

All experiments were conducted using a randomized complete block design with four replications. Data were subjected to an analysis of variance (ANOVA) by using general linear model, and the means were compared by least significant difference (LSD) method at 5% level using SAS (version 9.1).