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Essential oil composition and total phenolic, flavonoid contents, and antioxidant activity of sage (*Salvia officinalis* L.) extract under chitosan application and irrigation frequencies

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ABSTRACT

Sage (*Salvia officinalis* L.), which is one of the most important pharmaceutical herbs, has been exploited for many uses. It is an important industrial crop that natural products in the form of extracts and essential oil from the leaves of this herb are used in pharmaceutical, perfumery, and food industries. Response of sage to foliar application of chitosan (control, 0.0, 0.25 and 0.50 g/L), a marine polysaccharide with unique bioactive properties, under three irrigation frequencies (4, 6, and 8 every days) was evaluated in an experimental field at semiarid and cold climate, Southwestern of Iran. Chitosan and irrigation frequencies treatments had significant effects on studied parameters, including quantity and quality of essential oil, antioxidant activities, and the amounts of total phenolic and flavonoids of the extract of sage. The results clearly indicated that application of chitosan was beneficial to plants under deficit irrigation treatments. The foliar application of chitosan reduced the adverse effect of reduced irrigation on essential oil yield and improved content of the essential oil. Elicitation of sage with chitosan had positive influences on the amounts of secondary metabolites, such as α-pinene, β-pinene, limonene, α-thujone, β-thujone, camphor, and 1,8-cineole in the essential oil from sage under reduced irrigation. In reduced irrigation conditions, the antioxidant activity, and the amounts of total phenolic and flavonoid of the extracts increased when the plants were sprayed with chitosan. In conclusion, results of this study indicated that the spray of chitosan elicitor can be have useful impacts on the essential oil quality and quantity, antioxidant activity, and the amounts of total phenolic and flavonoid of sage under reduced irrigation conditions or drought stress.

1. Introduction

Sage (*Salvia officinalis* L.), belonging to the family Lamiaceae is an ornamental, culinary, medicinal, and aromatic plant (Porte et al., 2013). It has shown various biological activities, including antioxidant, antibacterial, antiviral, and antifungal properties (Martins et al., 2015). The leaves of this important industrial crop are used in pharmaceutical, perfumery, and food industries (Martins et al., 2015). The essential oil from *Salvia* species is widely used in treatment of various diseases such as the nervous system, heart and blood circulation, respiratory system, digestive system, metabolic, and endocrine diseases (Loizzo et al., 2007; Radulescu et al., 2004). According to the results of phytochemical analysis of *Salvia* species oils, the main constitutes of the oils are monoterpenes (α–and *β*-thujone, 1,8–cineole, camphor, and linalool), sesquiterpenes (α–humulene), and phenolics (Bernotiene et al., 2007; Rahimmalek et al., 2012; Roby et al., 2013; Martins et al., 2015).

In medicinal and aromatic plants, growth and biosynthesis of secondary metabolites are strongly influenced by genetic, environmental factors, and genetic × environmental effects (Ghasemi Pirbalouti and Craker, 2015; Ghasemi Pirbalouti et al., 2015; Ghasemi Pirbalouti and Imaniyan-Fard, 2016; Moghaddam and Ghasemi Pirbalouti, 2017; Bajalan et al., 2017; Lei et al., 2011; Ghasemi Pirbalouti et al., 2017b). Plant physiologists have been looking for new alternatives to conventional methods for improvements in production of secondary metabolites. One of these methods is elicitation, which it can be an important strategy towards obtaining improved production of bioactive

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compounds (Hussain et al., 2012). Some chemical compounds that could be used as elicitors to modify secondary metabolites and subsequently the bioactivity of medicinal and aromatic plants (Malekpoor et al., 2016a,b; Ghasemi Pirbalouti et al., 2014a,b,c). Recent years, the applications of signal components as elicitors have evolved an effective strategy for the production of target secondary metabolites in plant (Ghasemi Pirbalouti et al., 2014b; EmamiBistgani et al., 2017a).

Chitosan is a marine polysaccharide and considered a useful natural polymer. Chitosan is produced by alkaline *N*-deacetylation of chitin. Chitin and chitosan have been introduced as a material to improve on quantity and quality productions of agronomic and horticultural crops. In addition, they improve secondary metabolites of medicinal and aromatic plants (Chakraborty et al., 2009; Wiktorowska et al., 2010). Beneficial effects of chitin and chitosan in enhancing tolerance of plants to biotic and abiotic stresses crop plants (Farouk and Amany, 2012). Chitosan is considered as a potent elicitor of secondary metabolite accumulation in plants. Several reports indicated that chitosan positively affected growth and development, ion uptake and transport, and transpiration rate of various plant species (Karimi et al., 2012; Bitelli et al., 2001). For example, EmamiBistgani et al. (2017b) reported that the foliar spray of 400 µL/L chitosan improved biosynthesis of phenols in Iranian thyme (*Thymus daenensis* Celak.). They stated the application of chitosan elicitor can to some degree compensate the negative impact of deficit irrigation on its biomass, essential oil yield, some secondary metabolites, and antioxidant activity.

Water deficit, as an important abiotic stress especially in arid and semiarid regions, influences on physiological, biochemical, and phytochemical characteristics of plant species. Secondary metabolites of plants can be altered by ecological conditions, specially biotic and abiotic stress on many aspects of plant metabolism (EmamiBistgani et al., 2017a,b). Many investigators (Turtola et al., 2003; Ghasemi Pirbalouti et al., 2014b; Malekpoor et al., 2016a,b; EmamiBistgani et al., 2017a,b; Ghasemi Pirbalouti et al., 2017a) have shown the influences of drought stress on secondary metabolites of the herbs such as essential oils.

Limited investigations have been documented to identify the effects of foliar application of chitosan on growth, phytochemical traits, and antioxidant activity of sage (*S. officinalis*) under field and various irrigation frequencies conditions. We hypothesized that application of chitosan can be used as an applicable method to enhance essential oil, total plenolic and flavenoid contents, and antioxidant activity in *S. officinalis* under reduced irrigation.

2. Material and methods

2.1. Plant material and experimental site description

Sage (*S. officinalis*) seeds were obtained from the Research Center of Natural Products, Shahid-Beheshti University, Iran. In the spring 2016, the seedlings of *S. officinalis* were transplanted to research farm at Islamic Azad University of Shahrekord, Iran (32° 20′ N; 50° 51′ E; altitude. 2070 m asl). The climate of the area of study is classified as cold, semiarid, and semi humid (see meteorological data in Fig. 1). The soil of plots was filled with C.L. at pH of 7.96; containing 0.117% O.C comprised of 0.011% total N, 6.5 ppm P (available), 214 ppm K (available), and salinity level E.C.: 1.143 dS/m.

2.2. Experimental design and treatments

The experiment was done in a split plot arrangement based on randomized complete block design (RCBD) with three replications.

Various irrigation frequencies, including IF_1: every four days or normal irrigation (irrigation in field capacity); IF_2: every six days (irrigation in 75% field capacity when 25% of maximum total available soil water was depleted in the upper 30 cm of the soil profile); IF_3: every eight days (irrigation in 60% field capacity when 40% of maximum total available soil water was depleted in the upper 30 cm of the soil profile) were assigned to the main plots.

Foliar spray levels of chitosan were C1: no foliar application or negative control ($Chit_0$); C2: foliar application by water and acetic acid (Merck Co., Darmstadt, Germany) as a solvent or positive control ($Chit_{0A}$); C3: foliar application by 0.25 g/L chitosan ($Chit_1$); C4: foliar application by 0.50 g/L chitosan ($Chit_2$). The treatments of chitosan were assigned to the sub plots. Chitosan was sprayed five times in the vegetative period. Chitosan (Sigma–Aldrich Co., Steineheim, Germany) was dissolved in acetic acid 5%, diluted in distilled water with various concentrations. These solutions were sprayed at dew point (approximately 100 mL per plant) with a hand sprayer.

Each experimental plot was 2.5 × 5 m, and plants were grown in 5 rows, with a spacing of 30 cm in rows 50 cm apart per replication. Adjacent subplots, main plots and replications (blocks) were 1.0, 2.0, and 2.5 m apart, respectively. Finally, the aerial parts of sage at full flowering stage were harvested after seven days after the last applied of chitosan in September 2016.

2.3. Essential oil isolation

For about 100 g of small pieces of dried aerial part of sage for three hours in an all-glass Clevenger-type apparatus were subjected with 500 mL distilled water to hydro-distillation; then for drying obtained oils, anhydrous sodium sulphate applied and before analysis stored in sealed vials at 4 °C. The obtained oils essential oils were clear and yellow liquid.

2.4. Extract preparation

The aerial parts, especially the leaves of *S. officinalis* were macerated in 100 mL of ethanol 97% (Merck Co., Darmstadt, Germany) and filtered then were dried at 35 °C under rotary vacuum (Zirbus, Italy).

2.5. Identification of the oil constituents

Chemical constituents of the essential oils of sage was determined by GC (Agilent Technologies 7890 GC, Agilent Technologies, Santa Clara, CA) equipped with a single injector and a flame ionization detector (FID). A nonpolar HP-5 capillary column (30 m × 0.25 mm, 0.25 µm film thicknesses) was used. Initial column temperature was 60 °C and programmed to increase at 4 °C/min to 280 °C. The injector temperature was set at 280 and 300 °C. Split injection was conducted with a ratio split of 1:100. GC–MS analyses were performed on an Agilent Technologies 7890 gas chromatograph coupled to Agilent 5975C mass selective detector (MSD) and quadruple EI mass analyzer (Agilent Technologies, Palo Alto, CA, USA). A HP–5MS 5% column (coated with methyl silicone) (30 m × 0.25 mm, 0.25 µm film thicknesses) was used as the stationary phase. Helium was used as the carrier gas at 0.8 mL/min flow rate. The temperature was programmed from 60 to 280 °C at 4 °C/min ramp rate. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 50–550.

The essential oils components were identified based on their retention indices (RI) determined with reference to homologous series of C_5-C_{24} (*n*-alkanes), by comparison of their mass spectra with those reported in the literature (Adams, 2007) and stored in libraries of NIST 08 and Willey.

2.6. Determination of total phenolic content

The total phenolic content in the extracts was determined by the Folin–Ciocalteu (Sigma–Aldrich Co., Steineheim, Germany) assay (Singleton and Rossi, 1965). The absorbance of the samples was measured at 765 nm against a reagent blank using a UV–vis spectrophotometer (Perkin–Elmer Lambda, US). Gallic acid (Merck Co., Darmstadt, Germany) equivalent (GAL) was used as the reference