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Exogenous application of chitosan on biochemical and physiological characteristics, phenolic content and antioxidant activity of two species of basil (*Ocimum ciliatum* and *Ocimum basilicum)* under reduced irrigation

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ABSTRACT

Chitosan is a biopolymer with various industrial, medicinal, pharmaceutical, and agricultural applications. Effects of exogenous application of chitosan, a marine polysaccharide with unique bioactive properties, under normal irrigation and stressed conditions on morphology, physiology and biochemical characteristics of two species of sweet basil, including *Ocimum ciliatum* and *O. basilicum* in a pot experimental at semiarid and cold climate, southwestern Iran were investigated. Treatments comprised control, 0.0, 0.2, and 0.4 g/L chitosan applied to plants under normal irrigation, slight and mild drought stress conditions. Results indicated that drought stress had significant effects on some morphology, physiology and biochemical characteristics. In both species of basil, drought stress decreased the content of photosynthetic pigments and growth parameters. Exogenousapplication of chitosan (in particular 0.4 g/L) increased plant growth parameters in both species of basil under stressed or non-stressed conditions as compared to untreated plants. In addition, results indicated that the different levels of chitosan had significant effects on total phenol content and antioxidant activity of the extracts of two species. In conclusion, it is suggested that chitosan could be a promising material used to reduce the harmful effect of water stress on the growth parameters of basil plants and as a whole, treatment with chitosan partly could alleviate the effect of drought stress.

1. Introduction

Ocimum L. (basil) is considered as one of the largest genera of the family Lamiaceae and comprises annual, perennial herbs and shrubs native to the tropical and subtropical regions (Moghaddam et al., 2014). Most culinary and ornamental basils are cultivars of sweet basil (*O. basilicum*), which it widely cultivated in many countries. *O. ciliatum*as a main species of Iranian herbs (Makari and Kintzios, 2008; Moghaddam et al., 2015) is grown in home gardens and leaves and herbaceous parts of plants used as medicinal, vegetable and culinary herb (Moghaddam et al., 2011).

In arid and semiarid regions, the growth of medicinal and aromatic plants is influenced by various environmental factors especially water deficit stress (Bettaieb et al., 2009; Ghasemi Pirbalouti et al., 2014). Drought is a major environmental stress affecting on plant morphology, physiology and biochemistry characteristics (Shao et al., 2008). Water deficit stress inhibits the photosynthesis of plants, causes changes in chlorophyll contents and components and damage to the photosynthetic apparatus (Nayyar and Gupta, 2006). Overall, under drought stress conditions, fresh and dry weights of the herbs, proline, total carbohydrate and protein contents were significantly influenced (Khalid, 2006).

Plants under water stress can avoid the harmful of drought throw several ways among them stomata closure, leaf rolling, osmotic adjustments, reductions and consequently decreases in cellular expansion, and alterations of various essential physiological and biochemical processes that can affect growth, productivity and yield quality (Farouk and Amany, 2012; Hefny, 2011). In this

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respect, Bittelli et al. (2001) reported that occasional or episodic drought events can be counteracted through the use of anti-transpirants, compounds applied to foliage to limit the water loss. These compounds are able to increase leaf resistance to water vapor loss, thus improving plant water use and increasing biomass or yield (Tambussi and Bort, 2007).

Chitosan (CHT, 2-amino-2-deoxy-b-D-glucosamine), deacetylated chitin, is currently obtainedfrom the outer shell of crustaceans such as crabs and shrimps (Sandford, 1989). Chitin and chitosan are polysaccharides, chemically similar to cellulose differing only by the presence or absence of nitrogen. Chitosan/chitin is the second most important natural polymer in the world. The agricultural and horticultural uses for chitosan, primarily for plant defense and yield increase, are based on how this glucosamine polymer influences the biochemistry and molecular biology of the plant cell. Chitosan is an anti-transpirant compound that has proved to be effective in many crops (Khan et al., 2002; Karimi et al., 2012); it was used to protect plants against oxidative stress (Guan et al., 2009) and to stimulate plant growth (Farouk et al., 2008, 2011). Chitosan was first characterized as an elicitor in plants (Limpanavech et al., 2008). Chitosan is a natural low toxic and inexpensive compound that is biodegradable and environmentally friendly with various applications in agriculture. Chitin and chitosan have been improved soil fertility, and enhanced the mineral nutrient uptake of plant (Dzung, 2005, 2007), increased the content of chlorophylls, photosynthesis, and chloroplast enlargement, escalating nitrogen fixing nodes of species of leguminous plants (Dzung and Thang, 2004), and educed the effects of abiotic stress on plants (like drought stress), by increase the key enzymes related to the closure of the plant's stomata resulting in reduction of water loss (Song et al., 2006). Bittelli et al. (2001) reported that foliar application of chitosan decreased transpiration in pepper plants, and reduced water use while maintaining biomass production and yield. In addition, Sheikha and AL-Malki (2011) indicated that chitosan enhanced bean shoot and root length, fresh and dry weights of shoots, root, and leaf area as well as the level of chlorophylls. Results of previous investigations (Farouk et al., 2008; Ghoname et al., 2010) indicated that foliar application of chitosan resulted in higher vegetative growth and improvement in fruit quality of pepper, radish, and cucumber. However, the beneficial effect of chitosan is generally depending on its concentration, application methods, environmental conditions and growth status.

Although there is some research about influences of chitosan application on different plants, little is known about how chitosan foliar would affect the growth and yield of basil. On the other hand, the effects of deficit irrigation on plant growth and productivity are frequently investigated, but these studies cannot accurately predicate plant responses to deficit irrigation and foliar application of elicitor chitosan. To the best of our knowledge there has been no previous report regarding the combined effects of foliar application of chitosan and drought stress on basil plant growth and yield. Therefore, the purpose of this study was to determine the ability of chitosan to alleviate the deleterious effects of water stress on two species of basil plants.

2. Materials and methods

2.1. Experimental design and treatments

Pot experiment was conducted at the Research Field, Islamic Azad University of Shahrekord (latitude. 32° 20′ N; longitude. 50° 51′ E; altitude. 2070 m asl.), southwestern Iran, from May to August 2014. The climate of the area study is cold, semiarid and semi humid with temperate summer by Emberger's climatology method. In addition, the climate of area study is very cold winter by Karimi's climatology method (IRIMO, 2012).Seeds of two Iranian species of basil (*O. ciliatum* and *O. basilicum*) were sown in plastic pots with a diameter of 20 cm and a depth of 25 cm. The pots were filled with clay loam with a pH of 7.23, containing 0.8% organic matter comprised of 0.01% total N, 11.20 mg/kg available phosphorus, 694 mg/kg available potassium, and a saline value measured at E.C.: 1.35 dS/m. The soil moisture for all pots was kept at 100% field capacity until 35 days after sowing.

Experimental treatments were arranged as $3 \times 4 \times 2$ factorial. Each treatment included three replicates, producing a total of 72 experimental units (pots). Factor A included three water conditions, viz., I_1 (unstressed or control), I_2 (irrigation in 70% field capacity when 30% of maximum total available soil water was depleted in the upper 30 cm of the soil profile), and I_3 (irrigation in 40% field capacity when 60% of maximum total available soil water was depleted in the upper 30 cm of the soil profile). Factor B comprised three chitosan treatments (control, 0.0, 0.2, and 0.4 g/L) sprayed thrice at 10–12 leaves, before flowering, and two weeks later. Chitosan 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 (untreated plants were sprayed with an equivalent volume of distilled water). Factor C comprised two species of basil, including *O. ciliatum* and *O. basilicum*. Field water capacity or field capacity (FC) is defined "the amount of water held in soil after the excess gravitational water has drained away and after the rate of downward movement of water has materially decreased". FC is the upper limit of the available soil water (AW) reservoir, from which water can be released but not necessarily absorbed by plants, until the permanent wilting point (PWP) is reached (Vanderlinden and Giraldez, 2014).

2.2. Morphological characteristics

Three uniform plants were selected from each pot at the full blooming stage (95 days from sowing) to measure morphological and physiological characteristics. In this experiment, morphological traits were measured plant height, inflorescence, number of branches, leaf area, and fresh herbal and dry weights. Plant height was measured from the soil surface to the tip of the tallest flowering stem. Plants were cut from 1 cm area just above the lignified parts of the stem, immediately weighed (fresh weight) and then dried in room temperature until it reached a constant weight (dry weight). The fresh and dry weigh of roots were determined also.

2.3. Physiological characteristics

Samples of fresh leaves were taken for chlorophyll and carotenoids determination. Extraction in acetone 80% was repeated until all pigments were extracted. Chlorophyll concentrations (a, b, and total) as well as carotenoids content were determined in the samples of fresh leaves according to method described by Arnon (1967). The absorbance of acetone extract of the leaves was measured at three wavelengths (646.8, 663.2 and 470 nm) using a UV–vis spectrophotometer (Perkin–Elmer Lambda, US). The concentration of the pigment fraction was calculated as mg g^{-1} fresh weight using the following equations:

$$\text{Chlorophyll a} = (19.3 * A_{663} - 0.86 * A_{645})V/100W$$

$$\text{Chlorophyll b} = (19.3 * A_{645} - 3.6 * A_{663})V/100W$$

$$\text{Carotenoids} = 100(A_{470}) - 3.27(\text{mg chl. a}) - 104(\text{mg chl. b})/227$$

V = The volume of filtrate (centrifuged solution)

W = Fresh weight of sample (gram)