University of Birjand

تنشهای محیطی در علوم زراعی
Environmental Stresses In Crop Sciences
Env. Stresses Crop Sci.
Vol. 15, No. 1, pp. 185-197 (Spring 2022)

http://dx.doi.org/10.22077/escs.2020.3603.1881

*Original article*

# Physiological response of two oregano species medicinal plant to foliar spraying of chitosan under water deficit stress conditions

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Received 11 August 2020; Accepted 21 September 2020

## *Extended abstract*

### Introduction

Origanum is a plant of the Lamiaceae family that is considered one of the most important and best-selling medicinal plants in the world. The aerial parts, especially the leaves of different species of Origanum, have always been used as one of the most popular spices and flavorings in the food, perfume and cosmetics industries. Drought is one of the most important abiotic stresses may cause deleterious damage to plants. Lack of access to adequate amount of water or increase in transpiration can induce drought stress in plants and alter the production of metabolites. Drought stress reduces the water and turgor potentials, thereby negatively affecting various physiological processes. Chitosan is a natural biodegradable substance derived from crustacean shells such as crabs and shrimp. Having unique biological and physiological properties, it has found several applications in various industries such as pharmaceuticals, medicine and agriculture. As a bio-elicitor, chitosan prevents severe damage to plants under stress conditions by increasing the plant's defense activity.

### Materials and methods

This experiment was performed in 2019 in the research greenhouse of Azarbaijan Shahid Madani University, Iran as a factorial experiment in the complete randomized block design with three replications. The experiment treatments included three different concentrations of chitosan as foliar application (0, 250, 500 mg/l), and drought stress treatment at two levels (no stress and water deficit stress) in two species of Origanum (*Origanum vulgare*, *Origanum majorana*). The Origanum seeds were obtained from the Forest and Rangeland Research Organization and planted inside the seedling trays. Then, as the plants reached the desired growth of 4- and 5-leaves stages, each of the plant seedlings was transferred to plastic pots with the opening diameter of 15 cm, height of 25 cm, and capacity of 4 kg. The soil transferred to the pots had a loamy texture and a mixture of 33.32% sand, 40% silt, and 26.48% clay. Also, the soil inside the pots had a pH equal to 7.96, electrical conductivity of 2.8, organic matter of 2.415%, organic carbon of 1.401%, and calcium carbonate of 12.5%. The pots were kept in the greenhouse for 16 hours in light at 24 °C and 8 hours in dark at 18 °C. The application of water deficit stress treatment and foliar application of chitosan was performed in three stages three weeks before the complete flowering. In this experiment, a TDR moisture meter was used to measure the soil moisture of each pot for applying the water deficit treatment.

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## Results and discussion

The results of the present study show that the water deficit stress reduces total shoot dry weight. This reduces the yield when regarding the yield of aerial part of the plant. It should be noted in this study that the foliar application of chitosan under the water deficit conditions improved this trait. In this study, the foliar application of chitosan at the concentration of 500mg/l under the water deficit stress conditions increased the shoot dry weight. The results showed that the water deficit stress reduced relative water content, chlorophyll a, chlorophyll b, total chlorophyll and carotenoids, while increasing the hydrogen peroxide ($H_2O_2$), malondialdehyde (MDA) and proline contents. The foliar application of chitosan under water deficit stress conditions increased RWC, chlorophyll a, carotenoids, total chlorophyll, and proline contents.

## Conclusions

According to the results of this study, by conducting extensive research, the use of chitosan as a bio-elicitor to reduce the water deficit stress in the medicinal plant of *Origanium* can be suggested and the positive aspects of chitosan to be used for improving the physiological parameters in Origanium and other medicinal plants. In the end, it is suggested to perform this research in the areas with different climates, especially arid and semi-arid climates, with different concentrations of chitosan on different plants.

## Acknowledgements

This study was financially supported by Deputy of Research and Technology of Azarbaijan Shahid Madani University (98/D/897), Tabriz, Iran

***Keywords*:** Chitosan, Origanum species, Physiological parameters, Water deficit stress

**Table 1. Physical and chemical characteristics of the studied soil.**

| Texture | Sand | Silt | Clay | OC | OM | $CaCO_3$ | EC | pH |
|---|---|---|---|---|---|---|---|---|
| | ----------%---------- | | | | | | $dSm^{-1}$ | |
| Loam | 33.32 | 40 | 26.48 | 1.401 | 2.415 | 12.5 | 2.8 | 7.96 |

**Table1. Analysis of variance physiological traits of two *Oregano* species under water deficit stress**

| S.O.V | df | dry matter | RWC | Chlorophyll a | Chlorophyll b | Total chlorophyll |
|---|---|---|---|---|---|---|
| **Block** | **2** | $0.02^{ns}$ | $29.06^{ns}$ | $0.00032^{ns}$ | $0.0013^{ns}$ | $0.0012^{ns}$ |
| **Chitosan application (C)** | **2** | $2.87^{**}$ | $179.67^{**}$ | $0.00594^{**}$ | $0.0031^{**}$ | $0.0142^{**}$ |
| **Water regimes (W)** | **1** | $14.24^{**}$ | $761.79^{**}$ | $0.00516^{**}$ | $0.0076^{**}$ | $0.0251^{**}$ |
| **Species (S)** | **1** | $0.78^{**}$ | $6.25^{ns}$ | $0.00027^{ns}$ | $0.0207^{**}$ | $0.0163^{**}$ |
| **C × W** | **2** | $0.96^{**}$ | $66.64^{*}$ | $0.00022^{ns}$ | $0.0015^{ns}$ | $0.0009^{ns}$ |
| **C × S** | **2** | $0.11^{ns}$ | $1.23^{ns}$ | $0.00001^{ns}$ | $0.0121^{**}$ | $0.0122^{**}$ |
| **W × S** | **1** | $0.57^{**}$ | $0.81^{ns}$ | $0.00033^{ns}$ | $0.0019^{*}$ | $0.0006^{ns}$ |
| **C × W× S** | **2** | $0.67^{**}$ | $20.04^{ns}$ | $0.00007^{ns}$ | $0.0168^{**}$ | $0.0187^{**}$ |
| **Error** | **22** | 0.07 | 14.09 | 0.00020 | 0.0004 | 0.0004 |
| **CV%** | | *11.62* | *4.71* | *11.57* | *17.46* | *9.50* |

**Table 1. Continued**

| S.O.V | df | Carotenoid | $H_2O_2$ | MDA | Proline | Essential oil |
|---|---|---|---|---|---|---|
| **Block** | **2** | $0.0004^{ns}$ | $0.0015^{ns}$ | $0.085^{ns}$ | $5.36^{ns}$ | $0.0008^{ns}$ |
| **Chitosan application (C)** | **2** | $0.0032^{**}$ | $0.0018^{ns}$ | $4.745^{**}$ | $89.32^{**}$ | $0.106^{**}$ |
| **Water regimes (W)** | **1** | $0.0001^{ns}$ | $0.3522^{**}$ | $24.169^{**}$ | $633.48^{**}$ | $0.726^{**}$ |
| **Species (S)** | **1** | $0.0045^{**}$ | $0.2393^{**}$ | $0.112^{ns}$ | $36.93^{**}$ | $0.859^{**}$ |
| **C × W** | **2** | $0.0004^{ns}$ | $0.0161^{**}$ | $4.591^{**}$ | $16.89^{**}$ | $0.064^{**}$ |
| **C × S** | **2** | $0.0023^{*}$ | $0.0001^{ns}$ | $0.257^{ns}$ | $17.30^{**}$ | $0.084^{**}$ |
| **W × S** | **1** | $0.0007^{ns}$ | $0.0800^{**}$ | $0.609^{*}$ | $55.03^{**}$ | $0.234^{**}$ |
| **C × W× S** | **2** | $0.0013^{ns}$ | $0.0037^{ns}$ | $0.051^{ns}$ | $6.03^{ns}$ | $0.126^{**}$ |
| **Error** | **22** | 0.0004 | 0.0013 | 0.091 | 1.92 | $0.005^{**}$ |
| **CV%** | | *14.26* | *18.97* | *16.44* | *13.63* | *15.81* |

*, **: significantly different at the 5 and 1% probability level, respectively, ns: non-significant

**Table 2. Mean comparison of water regimes, species and chitosan spray interaction**

| Water regimes | گونه‌ها Species | Chitosan concentration | Total dry matter | Chlorophyll b | Total chlorophyll | Essential oil yield |
|---|---|---|---|---|---|---|
| | | mg/lit | g | mg $gr^{-1}$ FW | | w/w% |
| Well-watered | *O.vulgare* | 0 | $2.576^{b} \pm 0.194$ | $0.009^{e} \pm 0.005$ | $0.121^{de} \pm 0.006$ | $0.309^{cd} \pm 0.025$ |
| | | 250 | $3.297^{a} \pm 0.237$ | $0.084^{d} \pm 0.012$ | $0.221^{bc} \pm 0.012$ | $0.319^{cd} \pm 0.015$ |
| | | 500 | $3.651^{a} \pm 0.155$ | $0.087^{cd} \pm 0.007$ | $0.254^{ab} \pm 0.006$ | $0.911^{b} \pm 0.090$ |
| | *O.majorana* | 0 | $1.612^{c} \pm 0.024$ | $0.125^{ab} \pm 0.018$ | $0.230^{b} \pm 0.015$ | $0.349^{c} \pm 0.015$ |
| | | 250 | $2.770^{b} \pm 0.046$ | $0.156^{a} \pm 0.004$ | $0.285^{a} \pm 0.002$ | $0.427^{c} \pm 0.037$ |
| | | 500 | $3.501^{a} \pm 0.206$ | $0.086^{cd} \pm 0.009$ | $0.233^{b} \pm 0.014$ | $0.209^{de} \pm 0.008$ |
| Water deficit stress | *O.vulgare* | 0 | $1.376^{c} \pm 0.165$ | $0.006^{e} \pm 0.002$ | $0.092^{e} \pm 0.004$ | $1.111^{a} \pm 0.065$ |
| | | 250 | $1.298^{c} \pm 0.029$ | $0.122^{abc} \pm 0.023$ | $0.240^{b} \pm 0.012$ | $0.348^{c} \pm 0.028$ |
| | | 500 | $2.320^{b} \pm 0.181$ | $0.008^{e} \pm 0.002$ | $0.130^{d} \pm 0.009$ | $0.396^{c} \pm 0.024$ |
| | *O.majorana* | 0 | $1.600^{c} \pm 0.089$ | $0.104^{bcd} \pm 0.010$ | $0.189^{c} \pm 0.007$ | $0.161^{e} \pm 0.011$ |
| | | 250 | $1.664^{c} \pm 0.168$ | $0.010^{e} \pm 0.004$ | $0.127^{de} \pm 0.013$ | $0.446^{c} \pm 0.044$ |
| | | 500 | $1.601^{c} \pm 0.030$ | $0.122^{abc} \pm 0.028$ | $0.249^{b} \pm 0.023$ | $0.360^{c} \pm 0.015$ |

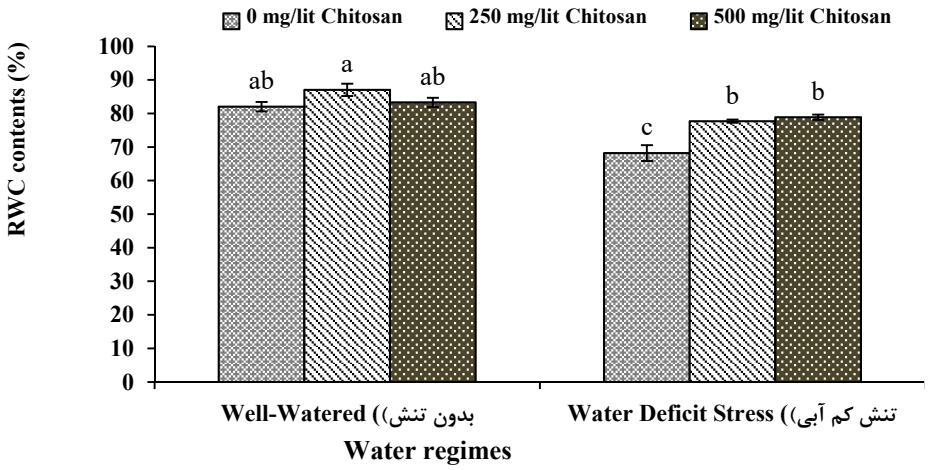


**Fig.1. Effect of water regimes and chitosan application on relative water content**

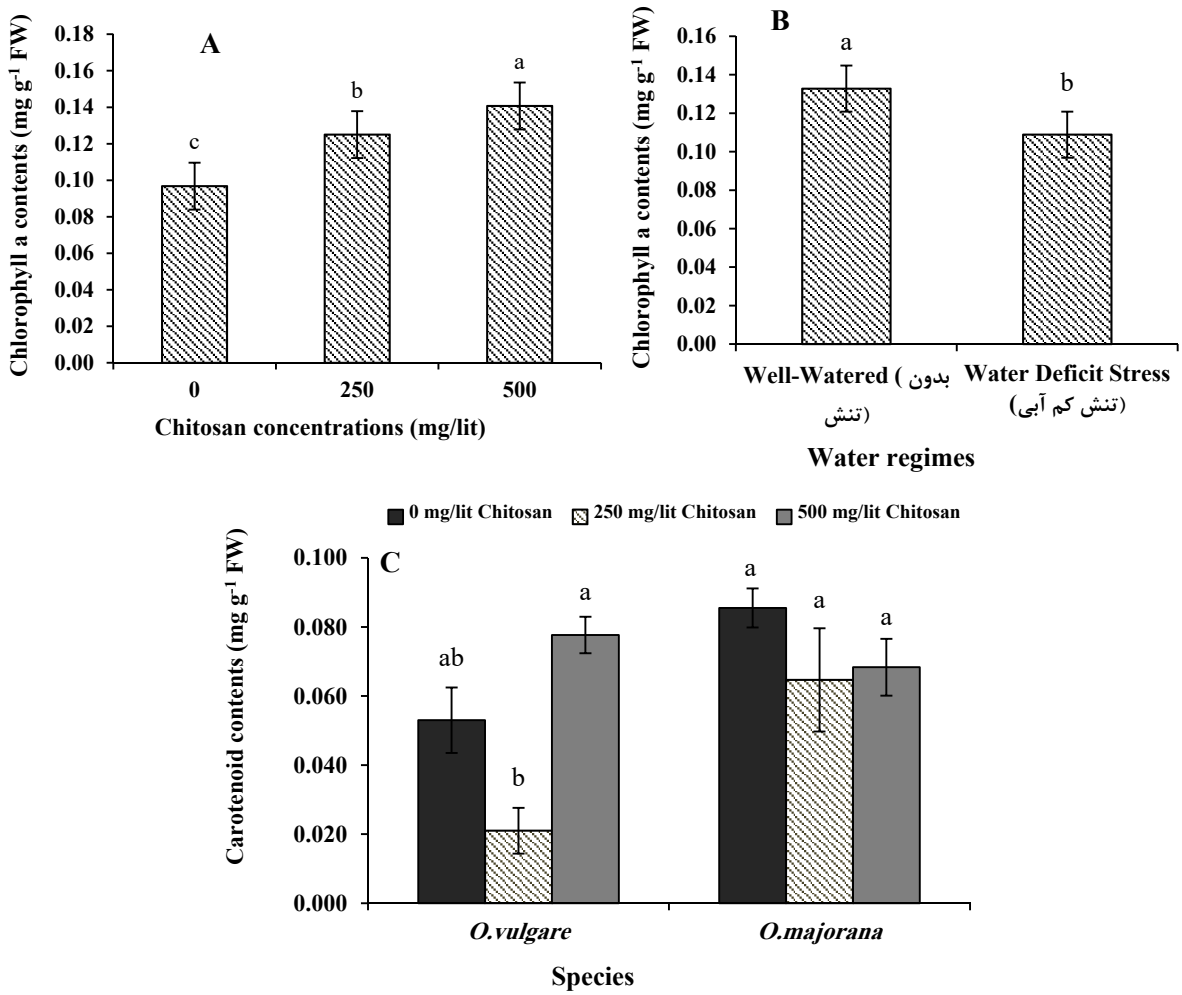


**Fig. 2. Effect of chitosan application (A) and water regimes (B) on chlorophyll a contents, and Effect of chitosan application and Oregano species on carotenoid contents (C)**

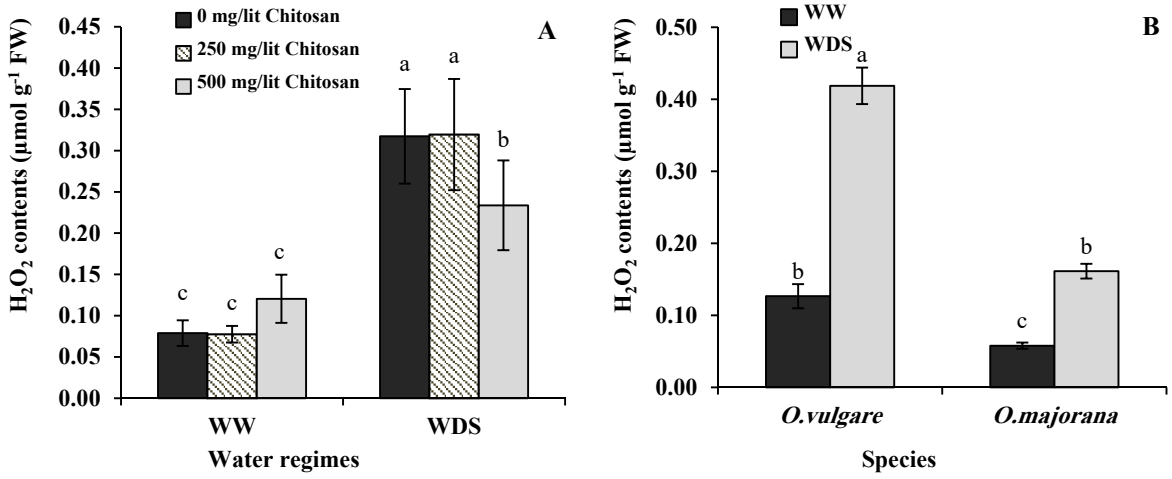


**Fig. 3. Effect of water regimes (water deficit stress-WDS and well watered-WW) and chitosan on $H_2O_2$ content (A), Effect of water regimes and species on $H_2O_2$ content (B).**

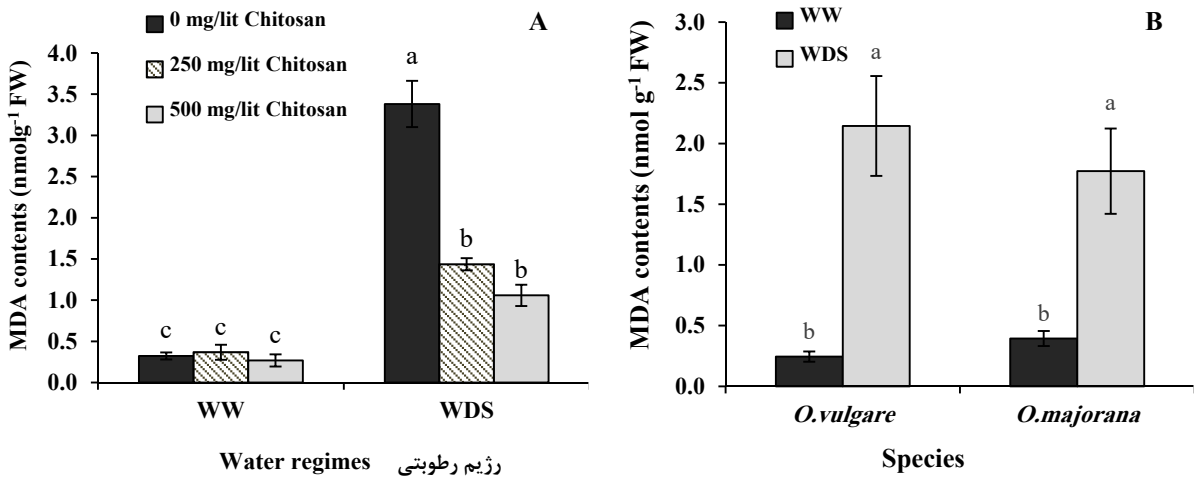


**Fig. 4. Effect of water regimes (water deficit stress-WDS and well watered-WW) & chitosan on MDA content (A), and Effect of water regimes and species on MDA content (B)**

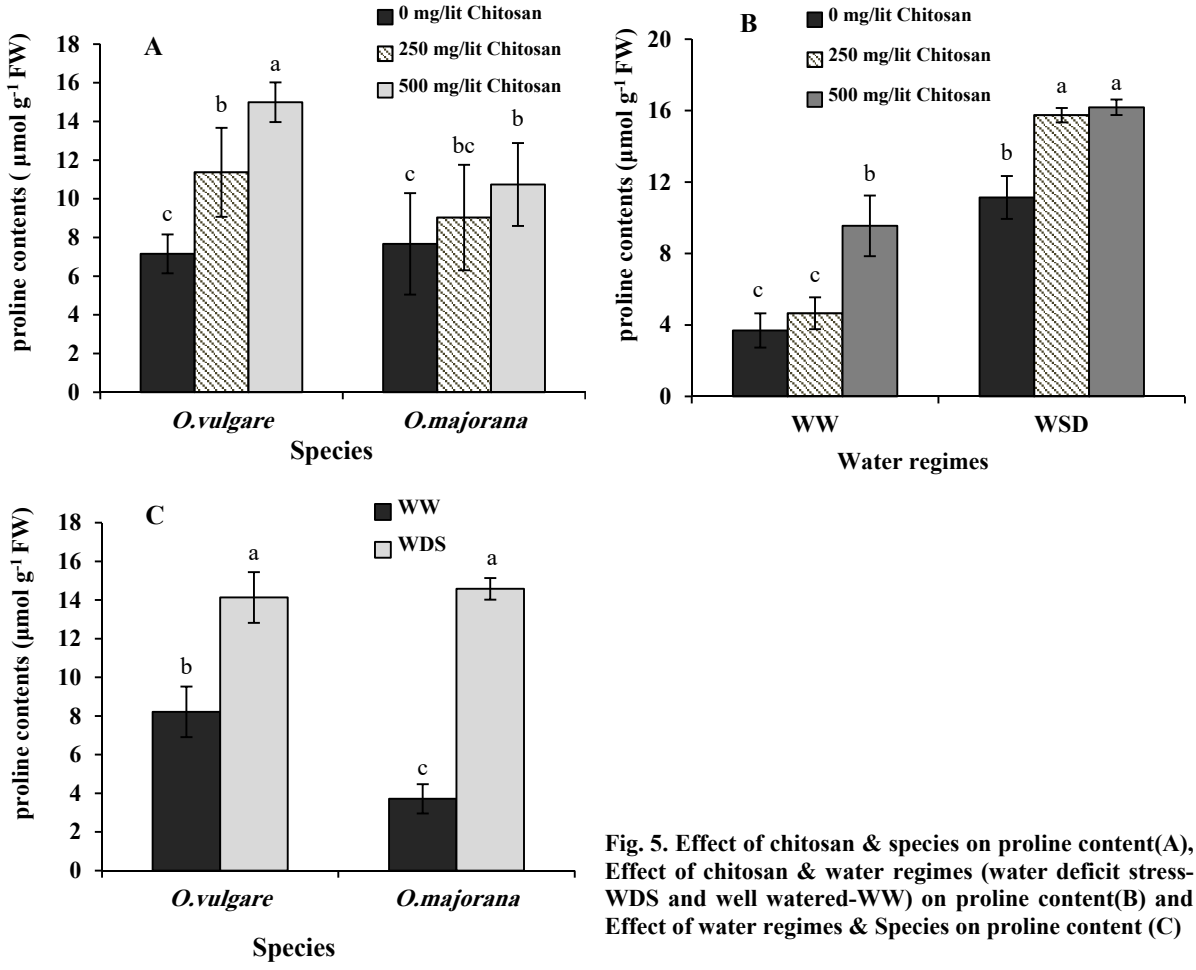


**Fig. 5. Effect of chitosan & species on proline content(A), Effect of chitosan & water regimes (water deficit stress-WDS and well watered-WW) on proline content(B) and Effect of water regimes & Species on proline content (C)**