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Research Article

Zeolite alleviates physiological and defense responses in drought stressed carrot (*Daucus carota* L.)

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Abstract

Drought stress is one of the main restrictions in plant production in arid and semi-arid regions. Addition of superabsorbent agents that maintain water in the soil, is among strategies to cope with drought stress. Therefore, in order to investigate the effect of zeolite superabsorbent on the physiological properties of carrot plants, the experiment was carried out as a factorial in a completely randomized blocks design. The factors were zeolite (0, 2.5 and 5% of soil) and irrigation regime (25, 50, 75 and 100% of the field capacity). The results indicated that the highest total phenol was related to 2.5% zeolite treatment with 75% of field capacity irrigation. The highest amounts of carotenoids, *chlorophyll a*, total chlorophyll and carbohydrate belonged to 5% zeolite treatment with 25% of field capacity irrigation. The highest levels of *chlorophyll b* and anthocyanin were related to 5% zeolite treatment with 75 and 50% of field capacity, respectively. In conclusion, the application of zeolite in combination with soil, maintained the plant moisture in drought stress conditions and, under 25 and 50% of field capacity the application of 5 % zeolite, improved the physiological capacity of carrots.

Keywords: Antioxidants, Drought, Photosynthetic pigments, Zeolite

Introduction

Carrot (*Daucus carota* L.), a biennial herbaceous plant, belongs to Apiaceae family. Carrot is one of the most important root vegetables, rich in biologically active compounds such as phenols and dietary fibers (Al-Snafi, 2017). Carrot root is a source of β-carotene (Augspole *et al*., 2014), kaempferol, quercetin and luteolin (Ching and Mohamed, 2001).

The fast growth of population rate and scarcity of water and food are global challenges. Water deficiency reduces the plant crops production in most arid areas i.e. Iran (Abedi and Pakniyat, 2010). Addition of soil modifier agents which improve the physical properties of the soil and increase water usage efficiency is an effective strategy to cope with water insufficiency problem. These modifier materials include superabsorbent polymers, perlite and zeolite (Xiubin and Zhanbin, 2001).

Zeolite, hydrated alumina silicate crystals with high cationic exchange capacity, maintains moisture for a long period and improves soil physical conditions (Caspersen and Ganrot, 2018). Zeolite, due to its high porosity and crystalline structure, can absorb water up to 60% of its weight (Pulit *et al*., 2004). The positive impact of zeolite on morphological, physiological, biochemical and yield parameters has already been reported in drought-exposed rice (Zheng *et al*., 2018), *Aloe vera* (Hazrati *et al*., 2017) and tomato (Bassam Al-Qarallah *et al*., 2013).

The edible part of the root and tuberous vegetables is in direct contact with the soil and highly sensitive to water deficit. Water stress leads to cracking of their edible parts, resulting in the loss of marketing quality. Carrots are highly sensitive to water deficit or irregular watering (Wicks, 2004). Irregular irrigation causes cracking, deformation and bitter taste in carrots; therefore, the supportive treatments which lead to proper usage of water, can greatly improve the quality and quantity of the product. Given the importance of vegetables in daily meals, the efficiency of zeolite polymer in drought-exposed carrots is the main concern of this research.

Material and methods

This experiment was conducted in the research farm and physiology lab of the Department of Horticulture, Faculty of Agriculture, Hormozgan University in 2019. The experiment was carried out as a factorial in a randomized complete block design. The factors included zeolite (0, 2.5 and 5% of soil around the roots) and the irrigation regimes (25, 50, 75 and 100% of field capacity) in six replications (four seeds in each replication). The viability of seed was 99.99% (direct germination test). Plant spacing was 5cm in row and 8 cm between rows. The sowing depth was 2 cm.

The combination of zeolite with soil was performed prior cultivation. The field capacity (FC) was calculated based on weight method. In addition the volume of water for each treatment was calculated as the amount

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of water per time (based on calculated FC). Irrigation was done daily in the first week and then continued as once every two days. The water stress was treated from the first week until the end of experiment. Thinning and weeding were done in the 5^{th} week. Fertilizing with NPK (10 g per 10 L of water) was carried out in the 6^{th} week. Plants were harvested in the 15^{th} week.

Total phenol content: First, 0.1 g of leaf sample was mixed with 10 ml of 80% methanol. Then the extract was centrifuged (10000 rpm, 10 mins.) and the supernatant (10 µL) was mixed with distilled water (490 µL) and folin ciocalteu reagent (500 µL) and then placed in the dark for 3 mins. Afterwards, 500 µL of 1% sodium carbonate was added. Finally, the optical absorbance of the extract was recorded at 765 nm. Total phenol content was calculated from gallic acid standard curve and was expressed in GAE/g FW (Spanos and Wrolstad, 1990).

Chlorophyll and carotenoids: Briefly, 0.5 g of leaf tissue was homogenized with 10 ml of 80% acetone. Then the mixture was centrifuged (10000 rpm, 10 mins.) and the absorbance was recorded at 470, 645 and 663 nm (Arnon, 1949). The *chlorophyll a* (Chl_a), *chlorophyll b* (Chl_b), total chlorophyll (Chl_{total}) and carotenoids (C_{x+c}) contents were calculated using the following formulas and were expressed in mg/g FW.

$Chl_a = (12.7 \times A_{663}) - (2.69 \times A_{645})$

$Chl_b = (22.9 \times A_{645}) - (4.68 \times A_{663})$

$Chl_{total} = (20.2 \times A_{645}) + (8.02 \times A_{663})$

$C_{x+c} = ((1000 \times A_{470}) - (1.82 \times Chl_a) - (85.02 \times Chl_b)) /198$

Carbohydrate content: For this purpose, 0.5 g of leaf tissue was homogenized with 5 ml of 80% ethanol and then incubated in a water bath at 70ºC for 10 mins. After centrifuging (6000 rpm, 15 mins.), the supernatant was condensed using indirect heat to reach one-fifth of the original volume. Then it was mixed with chloroform in the ratio of 1 to 5 (extract-chloroform) and the mixture was vortexed and then centrifuged (6000 rpm, 10 mins.). The supernatant (25 µL) was mixed with distilled water (175 µL). Then it was mixed with 3 ml of Antron agent (containing 76 ml of 98% sulfuric acid, 30 ml of distilled water and 150 mg of antron) and incubated in a water bath (70°C, 21 mins.). Finally, the optical absorbance was recorded at 620 nm. Total carbohydrate was calculated from glucose standard curve and was expressed in mg/g FW (Lee *et al*., 2011).

Anthocyanin content: Leaf sample (0.5 g) was homogenized with 1 ml of acidic methanol (Methanol: Hydrochloric acid, 99:1). Then, the extract was kept in the refrigerator under dark conditions for 24 hours. The mixture was then centrifuged (10000 rpm, 4ºC, 15 mins.). Finally, the absorbance was recorded at 520 and 637 nm. Total anthocyanin was calculated from following formula and was expressed in mg/g FW (Chen *et al*., 2015).

Anthocyanins = [OD520 - 0.25 OD657] × TV/ [Fw × 1000]

Where OD, TV and FW are optical density; total volume of the extract in ml and fresh weight of tissue in g, respectively.

The experiment was performed as a factorial in a randomized complete block design. The analysis of variance was carried out using SAS.9. The mean comparisons were calculated using the Tukey test ($P<0.01$). The charts were drawn in Sigma Plot 10.0.

Results and discussion

Based on variance analysis results, irrigation regime had a significant effect on anthocyanin, *Chlorophyll a* and *Chlorophyll b* contents. All the parameters except total phenol were influenced significantly by zeolite levels. In addition, the interaction of irrigation regime and zeolite were significantly affected all the parameters (Table 1).

Total phenol content (TPC): Data analysis indicated that the interaction of superabsorbent and irrigation regimes was significant on the total phenol content. Increased irrigation water led to an increase in the leaf phenol content in 2.5% of zeolite. The highest phenol content (10.77 mg GAE/g FW) was related to 2.5% zeolite with 75% of FC and the lowest amount (7.26 mg GAE/g FW) was in 5% zeolite with 25 and 75% of FC (Figure 1). Increasing the levels of phenolic compounds is one of the antioxidant mechanisms of plants under drought stress (Bettaieb *et al*., 2011). Drought by stimulating the expression of phenylpropanoid biosynthesis genes, causes an increase in the biosynthesis of antioxidants, including polyphenolic compounds. Drought stress has led to an increase in phenolic compounds in grapes (Peterlunger *et al*., 2000), which were in line with the results of this experiment. The study of the effect of superabsorbent application under drought stress conditions in apples had significant effects on the phenol content. In fact, superabsorbent retains water and nutrients at the root zone and thus improves the antioxidant properties of plant by accumulation of phenolic components, which ultimately reduces the intensity of oxidative stress (Keivanfar *et al*., 2019; Sultana *et al*., 2016; Valizadeh Ghale Beig *et al*., 2014).

Chlorophyll and carotenoids: According to our results, *chlorophyll a* was affected by zeolite levels, irrigation regimes and the interaction of both factors. Decrease in irrigation level had a descending effect on the content of this pigment. Zeolite treatment induced an increase in *chlorophyll a* content. The most impact was observed in 5% zeolites-treated plants. The highest amount of *chlorophyll a* (17.29 mg/g fresh weight) was obtained in 5% zeolite treatment with 25% of FC and the lowest amount (14.19 mg/g fresh weight) was observed in 2.5% zeolite treatment with 75% of FC (Figure 2). The results of this study indicated that *chlorophyll b* was affected by the superabsorbent treatment, drought conditions and their interaction. Reduction in FC made a downward trend in the content of chlorophyll *b*. A significant increase in *chlorophyll b*

Table 1. The analysis of variance of irrigation regime and zeolite on physiological parameters of carrot

S.V	D.F	TPC	Chl_a	Chl_b	Chl_{total}	Carotenoid	Carbohydrate	Anthocyanin
Irrigation regime	3	6.36	40.82*	17.32*	137.25	3.71	†47.14	0.05*
Zeolite	2	2.52	1.92*	1.62*	8.92*	0.28**	299.54*	0.04*
Irrigation regime × Zeolite	6	1.28*	42.83*	6.38**	72.84*	2.54**	894.54*	0.02**
Block	5	1.72	7.32	0.08	5.72	2.91	104.26	0.02
Error	55	0.72	8.61	1.26	20.71	1.07	352.73	0.02

†The mean square values are given. * and **: state significant at 5 and 1% respectively

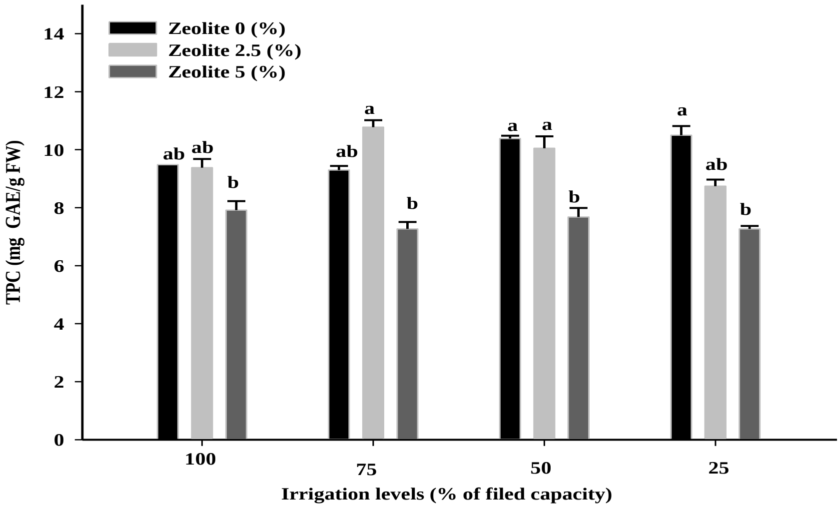

Figure 1. The influence of zeolite on total phenol content of carrot under different irrigation levels. The same letter denotes no differ significantly Tukey (P<0.01).

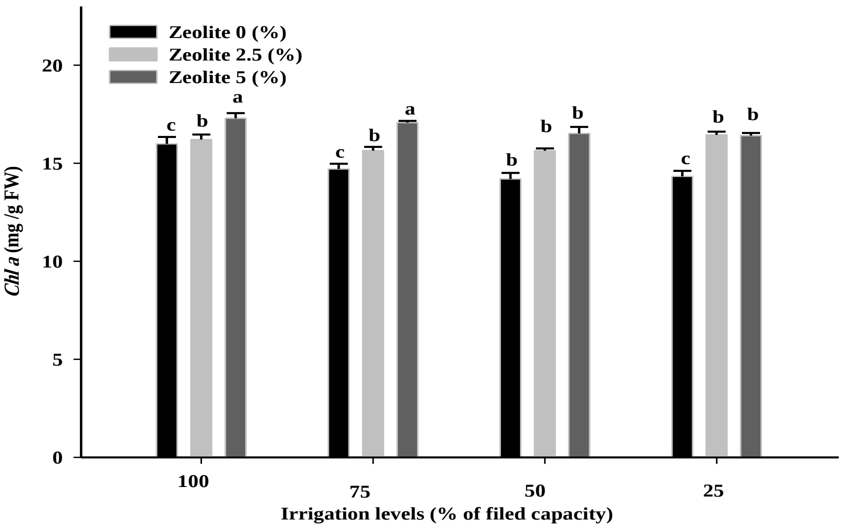

Figure 2. The influence of zeolite on *chlorophyll a* content of carrot under different irrigation levels. The same letter denotes no differ significantly Tukey (P<0.01).

content was observed in 5% zeolite treated plants. The highest amount of *chlorophyll b* (8.79 mg/g fresh weight) obtained by the application of 5% zeolite with 75% of FC and the lowest value (6.29 mg/g fresh weight) was belonged to 2.5% zeolite treatment with 100% of FC (Figure 3). In addition, the total chlorophyll content was also affected by zeolite treatment and its interaction with irrigation treatments. Low water conditions decreased the pigment, while zeolite application increased the values. However, observed differences at all irrigation levels were not statistically significant. The highest value was observed in the highest level of zeolite. But there was less difference between irrigation regimes. The highest content of total

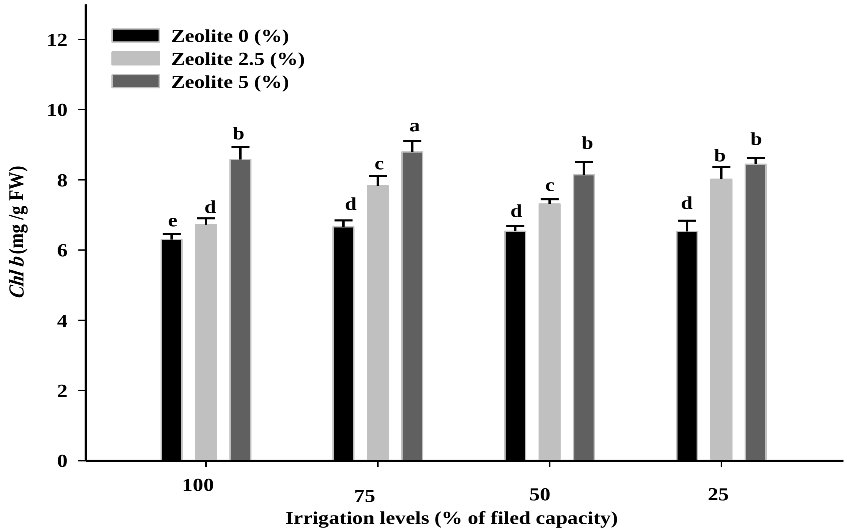

Figure 3. The influence of zeolite on *chlorophyll b* content of carrot under different irrigation levels. The same letter denotes no differ significantly Tukey (P<0.01).

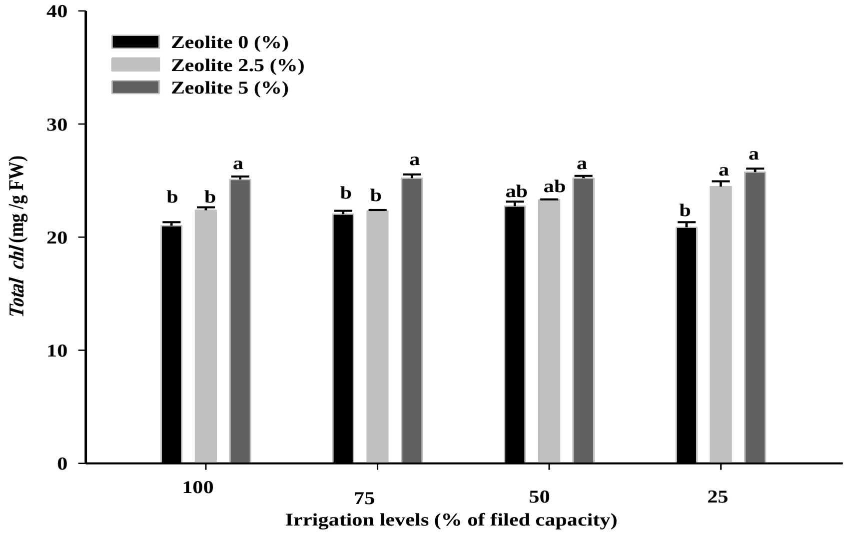

Figure 4. The influence of zeolite on total chlorophyll content of carrot under different irrigation levels. The same letter denotes no differ significantly Tukey (P<0.01).

chlorophyll (25.73 mg/g fresh weight) was obtained by the application of 5% zeolite and 25% of FC. The lowest content (20.85 mg/g fresh weight) was observed in zeolite-free treatment and 25% of FC. Also, the total chlorophyll content showed an upward trend by increasing of zeolite in 25% of FC (Figure 4). Furthermore, carotenoid was affected by the levels of applied zeolite and the interaction of zeolite levels and irrigation regimes. The most value (7.59 mg/g fresh weight) was obtained by the application of 5% zeolite with 25% of FC. The lowest value (4.38 mg/g fresh weight) was observed in zeolite-free conditions with 75% of FC. The carotenoid content significantly increased by increasing zeolite in all irrigation regimes (in 25, 50, 75 and 100% of FC) (Figure 5).

Leaf chlorophyll has been considered as a useful criterion for assessing the physiological status of plants (Wang and Huang, 2004). Drought stress caused chloroplasts degradation and chlorophyll reduction in radish (Misra and Srivastatva, 2000) and sunflower (Mohsenzadeh *et al*., 2006). This decrease is related to increase in chlorophyllase activity (Reddy and Vora, 1986). In our research the *chlorophyll a* decreased more than *chlorophyll b*, which could be related to high sensitivity of *chlorophyll a* to the drought treatment, because of its conversion to *chlorophyll b* (Fang *et al.*, 1998; Saeidi and Abdoli, 2015). Carotenoids are essential in photosynthesis. They have a protective role to induce the antioxidant properties of drought- exposed plants. Increased carotenoids content under stress conditions, is due to their role in the antioxidant defense mechanism to protect photosynthetic pigments. Drought stress resulted in increasing the content of carotenoids (Abdalla and El-Khoshiban, 2007; Hazrati *et al*., 2016).

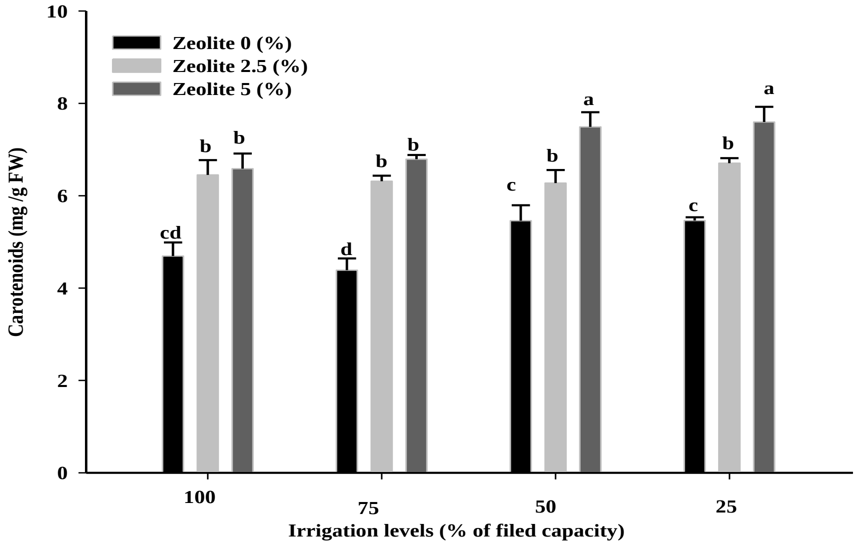

Figure 5. The influence of zeolite on carotenoids content of carrot under different irrigation levels. The same letter denotes no differ significantly Tukey (P<0.01).

The zeolite causes an increase in water availability which causes the improvement of plant growth rate via increase in efficiency of mesophilic cells, water consumption and photosynthesis rate. Zeolite also stimulates the synthesis of photosynthetic pigments, chlorophyll and carotenoids, indirectly (Bahador and Tadayon, 2018). Zeolite, duo to its particular structure for storage of water, could be effective in reducing water stress. Also, it has reported that zeolite is useful soil amendment with a significant role in keeping macro and micro nutrients of soil (Ippolito *et al*., 2011; Hazrati *et al*., 2017). Zeolite, with a negative charge, provides best trap for positive cations (such as potassium and calcium), and positively charged groups (such as water and ammonia) (Polat *et al*., 2004). The characteristics of zeolite especially for NH_4^+ improves soil nitrogen retention and nitrogen availability to plants (Sepaskhah and Barzegar, 2010). In addition, zeolite improves plant growth, leaf area index and florescence chlorophyll index, which finally support photosynthetic machinery to biosynthesis of related pigments (Liang *et al*., 1997; Pulite *et al*., 2004). Our results indicated that zeolite treatment had an ascending impact on chlorophyll and carotenoid stabilities under low water stress which were in line with the results of Gholam Hosseini *et al*. (2009), Karami *et al*. (2018) and Khadem *et al*. (2010).

Carbohydrate content: The results of this experiment indicated that the soluble carbohydrate was affected by zeolite levels and the interaction of zeolite levels and irrigation regimes. Increasing the irrigation regime in 2.5 and 5% zeolite caused an increasing trend in carbohydrate content, compared with related zeolite-free treatments. However, a descending trend was observed with increasing the irrigation regime in zeolite-free conditions. The highest amount of carbohydrate (50.91 and 51.17 mg/g FW) was in 5% zeolite treatment with 25 and 50% of FC and the lowest amount (10.53 mg/g FW) was observed in zeolite-free conditions with 25% of FC (Figure 6). Drought stress has increased the soluble solids content in plants (Faci Gonzalez *et al*., 2014). Environmental stresses are perceiving by signal molecules, which relay a signaling cascade and evolve adaptive responses to reduce oxidative stress. Carbohydrates have dual role in plants. They involve in metabolic processes and act as molecule signals regulating various genes, particularly those involve in photosynthesis, sucrose metabolism and osmolyte synthesis (Rosa *et al*., 2009). Carbohydrates accumulation under low water conditions regulates the osmotic balance (Sperdouli and Moustakas, 2012). Non-photosynthetic pathways and degradation of insoluble carbohydrates, also causes increased soluble carbohydrates (Damayanthi *et al*., 2010; Masoudi-Sadaghiani *et al*., 2011). Superabsorbent materials under such conditions assist maintain water storage of roots and collect carbohydrate (Kosterna *et al*., 2012). The combination of superabsorbent and drought treatments was related with improved photosynthesis and higher carbohydrate production (Ranjbar *et al*., 2004).

Anthocyanin content: Based on table 1, Zeolite levels and the interaction of zeolite levels and irrigation regimes significantly influenced the anthocyanin content. According to our data, in 5% of zeolite treatment anthocyanin content non-significantly increased when irrigation decreased. The highest amount of anthocyanin (1.47 mg/g fresh weight) was obtained by the application of 5% zeolite treatment with 25% of FC and the lowest amount (1.02 mg/g fresh weight) was observed in no-zeolite treatment with 100% of FC (Figure 7). Plants protect themselves against reactive oxygen radicals through both enzymatic and non-enzymatic antioxidant mechanisms, such as accumulation of anthocyanins (Ashraf and Harris, 2013; Abdalla and El-Khoshiban, 2007). Anthocyanin is highly water soluble, especially as glycosides, and is

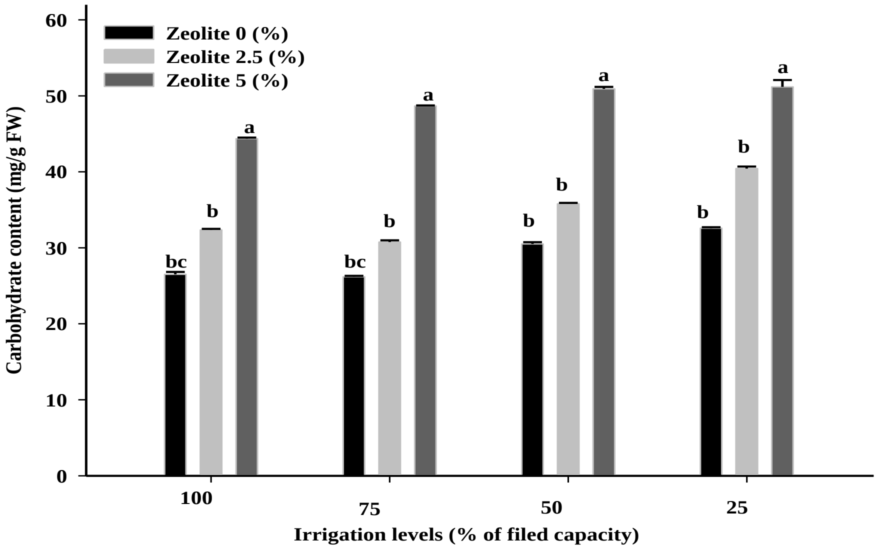

Figure 6. The influence of zeolite on carbohydrate content of carrot under different irrigation levels. The same letter denotes no differ significantly Tukey (P<0.01).

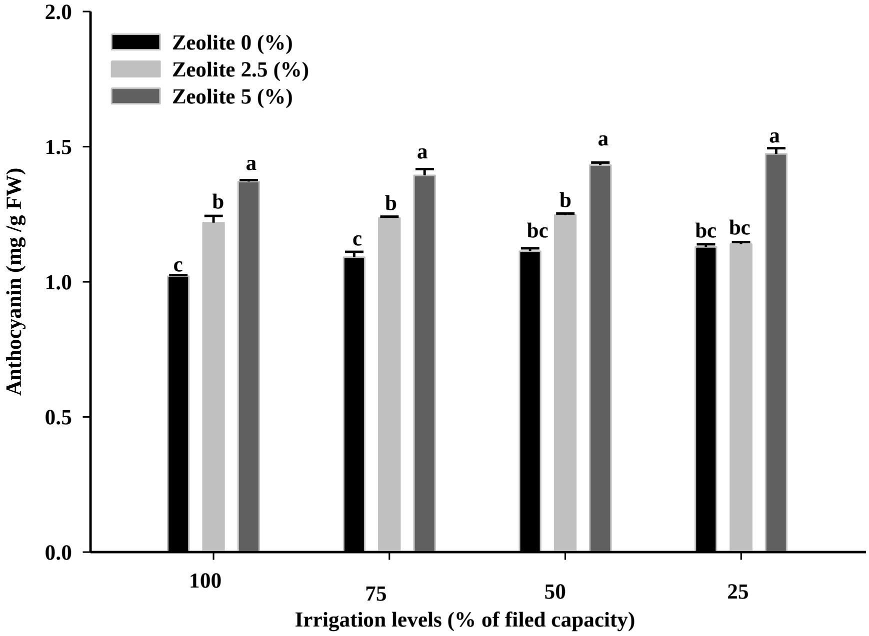

Figure 7. The influence of zeolite on anthocyanin content of carrot under different irrigation levels. The same letter denotes no differ significantly Tukey (P<0.01).

regularly found in vacuoles (Chalker-Scottbitats, 2002). Drought increases synthesis of anthocyanin in plants which acts as osmotic adaptation and antioxidant agent to protect plants against free radicals (Tahkorpi, 2010; Chalker-Scott, 2002; Keivanfar *et al*., 2019). The increased accumulation of anthocyanin in plant allows it to maintain deficit water conditions (Chalker-Scottbitats, 2002). Drought stress has significantly increased the anthocyanin content in the leaves of bean (Bahador *et al*., 2015), which was in line with the results of this study. Zeolite influences water efficiency of leaves and improves cations balance. Also, it protects photosynthetic pigments (Ippolito *et al*., 2011; Hazrati *et al*., 2017; Pulite *et al*., 2004), which ultimately can induce defensive responses to stress. Under such conditions the plant can normally carry out primary and secondary metabolism, and even enhance their metabolic capacity to improve adaptability to stress (Martin *et al*., 2011; Wang *et al*., 2016). In our case, accumulation of anthocyanin, as a secondary metabolite, may increase plant defense system under low water conditions.

Conclusion

Low water availability causes degradation of photosynthetic pigments and decreases photosynthesis rate by generation of free radicals, which finally reduces crops production. Addition superabsorbent to soil increases water usage efficiency and recovers the injury induced by oxidative stress. The results of the present research indicated that the application of zeolite in different irrigation regimes reduced oxidative stress. Besides it improved the photosynthetic pigments content of carrot plants. In conclusion, under 25 and 50% of FC the application of 5% zeolite, increased *chlorophyll a*, *chlorophyll b*, total chlorophyll, carotenoids, anthocyanin and carbohydrate content of carrots.

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