Journal of Plant Nutrition

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/lpla20

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To cite this article: Abdollah Beyk-Khormizi, Siavash Hosseini Sarghein, Mohammad Reza Sarafraz-Ardakani, Seyed Mohammad Moshtaghioun, Seyed Mousa Mousavi-Kouhi & Ali Ganjeali (2022): Ameliorating effect of vermicompost on *Foeniculum vulgare* under saline condition, Journal of Plant Nutrition, DOI: 10.1080/01904167.2022.2092513

To link to this article: https://doi.org/10.1080/01904167.2022.2092513

Published online: 25 Jun 2022.

JOURNAL OF PLANT NUTRITION
https://doi.org/10.1080/01904167.2022.2092513

Ameliorating effect of vermicompost on *Foeniculum vulgare* under saline condition

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ABSTRACT

Fennel (*Foeniculum vulgare* Mill) is a medicinal plant that all parts of it can be used in various industries such as food, chemical and pharmaceutical industries. To investigate the effect of vermicompost (VC: 0 and 5%), as an organic fertilizer, on the morphophysiological and biochemical characteristics of two fennel landraces (Shiraz and Urmia) under saline conditions (0, 40, 80, and 120 mM NaCl), a factorial experiment was conducted in greenhouse condition. After 3 weeks of culturing, plants were irrigated with experimental treatments until 5 weeks more. The results showed that salinity stress induced a significant decrease in leaf area, biomass, photosynthetic pigments content, relative water content, membrane stability index, minerals (Fe, K, Ca, and Mg) concentration, total protein content, and gibberellin concentration of the shoot. A significant increase in malondialdehyde, total phenol, and the activity of catalase and peroxidase enzymes in the same organ was also observed. In contrast, for most of these variables, the opposite results were obtained under VC alone or its interaction with salinity stress. In comparison between two fennel landraces, Shiraz was less affected by salinity stress, while the effect of VC on Urmia was more evident. It can be concluded that VC can limit the adverse effects of salinity stress on fennel by affecting photosynthetic pigments, membrane integrity, minerals concentration, water status, protein content, gibberellins biosynthesis, and antioxidants activity.

Abbreviations: VC: vermicompost; EC: electrical conductivity; OC: organic carbon; DW: dry weight; FW: fresh weight; Chl: chlorophyll; Carot: carotenoids; Conc: concentration; RWC: relative water content; TPC: total protein content; CAT: catalase; POX: peroxidase; MDA: malondialdehyde; MSI: membrane stability index; TP: total phenol; GA: gibberellins; ROS: reactive oxygen species; PGR: plant growth regulators

ARTICLE HISTORY
Received 21 March 2022
Accepted 16 June 2022

KEYWORDS
antioxidant enzymes; fennel; gibberellin; malondialdehyde; organic products; photosynthetic pigments

Introduction

Fennel (*Foeniculum vulgare* Mill) from Apiaceae family is a well-known traditional and medicinal plant (Shamkant, Vainav, and Atmaram 2014) with many uses (medicinal, industrial, and food) for humans. All parts of this plant, including seeds, shoot, and roots are used by humans in different ways (Barros, Carvalho, and Ferreira 2010): leaves as a vegetable, seeds as a spice and to

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Table 1. Some properties of VC and soil used in the test.

Sample	EC (dS m^{-1})	OC (ppm)	Zn (ppm)	Na (ppm)	Fe (ppm)	Mg (ppm)	Ca (ppm)	K (ppm)	P (ppm)	N (ppm)
VC	7.68	158700	127	4200	15100	18900	52000	15900	22200	15400
Soil	3.81	150	0.14	0.0248	0.08	0.0075	0.0108	136.4	4.3	10
mixture of VC & Soil	4.87	990	0.22	0.0241	0.36	0.0108	0.0184	390	15.62	90

EC: electrical conductivity; OC: organic carbon.

extract essential oils, and flowers as a source of yellow and brown colors (Malhotra 2012). This medicinal plant is cultivated in different parts of the world such as Europe, China, India, Iran, and Pakistan (Ahmad et al. 2004).

Decreased water quality can induce salinization of arable soils. Salinity has become one of the most important factors in reducing plant growth and production in many parts of the world (Kaya et al. 2009). Munns and Tester (2008) reported that more than 800 million hectares of land worldwide are affected by salt, which is equivalent to more than 6% of the world's land area. Besides, the increasing use of low-quality water and traditional agriculture has led to the development of this problem (Lakhdar et al. 2008). Unfortunately, salinization of agricultural lands will reduce arable land by up to 30% in the next 25 years and up to 50% in 2050 (Bahmani et al. 2015). Under salt stress in plants, all important plant processes such as photosynthesis, protein synthesis, lipid metabolism, and energy conversion are affected (Parida and Das 2005). The presence of salt in the root environment can induce reduced photosynthesis, increased respiration, disrupted protein synthesis and nucleic acid metabolism, reduced osmotic potential, and consequently reduced availability of water to root cells (Munns and Tester 2008).

The use of chemical fertilizers has led to a decline in the quality of agricultural products and reduced soil fertility. In recent decades, the use of organic fertilizers has been an important method for soil reclamation and fertility in areas under salinity stress (Lakhdar et al. 2009; Melero et al. 2007). Vermicompost (VC) is the product of the vermicomposting process by the cumulative activity of earthworms and microbes (Bhat et al. 2018). This fertilizer with a wide active microbial biodiversity is used for soil reclamation and remediation (Arancon et al. 2004a). VC has many pores, good drainage, high ventilation capacity, and water retention. It also contains Humat, which is a humic substance having an effect similar to plant growth regulators (PGR) and hormones. Another advantage of VC is the high content of macro and micronutrients in a form that is easily absorbed and available to the plant (Atiyeh et al. 2002).

Since fennel is relatively sensitive to salinity stress, this study aims to investigate the effect of VC on morphophysiological and biochemical properties of two fennel landraces under saline conditions to find an organic factor for ameliorating adverse effects of salt stress, a major problem in most areas where it is cultivated.

Material and methods

Experimental layout, plant material, sampling and measurement of morphological traits

VC was prepared in a mixture of cow manure and egg carton waste in a ratio of 90 to 10% bed using *Eisenia foetida* worm within six months. Some characteristics of VC, the soil used in the experiment, and a mixture of 5% VC with soil (v/v) are shown in Table 1.

In this study, the effect of 0 and 5% (v/v) VC with loamy-clay soil at salinity levels of 0 (control), 40, 80 and 120 mM NaCl, which are equivalent to 0, 3.652, 7.305 and 10.957 deciSiemens per meter (dS/m), respectively, were studied in two fennel landraces: Urmia and Shiraz. A factorial experiment was conducted in a randomized complete block design (RCBD) with three replications in greenhouse condition with a temperature of 25–30° C, and16/8 h day/light.

The seeds of fennel landraces were soaked in water for 24 hours and then planted in the plastic pots and irrigated according to the field capacity. After 3 weeks of irrigating pots with distilled

water (without salinity levels) and ensuring germination, the seedlings were thinned and to remaine 3 seedlings in each pot. The pots were then irrigated according to the experimental treatments (different salinity levels). Plants were sampled 8 weeks after culturing. Leaf area was determined by Leaf Area Meter and dry weight of roots and shoots was also measured. Finally, some physiological and biochemical variables were determined.

Physiological and biochemical analysis

Photosynthetic pigments

Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were determined using Lichtenthaler (1987) method. Plant leaves (0.2 g) were homogenized with 15 ml of 80% acetone and the homogenate was centrifuged at 3000 rpm for 5 min. The absorbance of the supernatant was measured by spectrophotometer (Model Analytic Jena 210) at 647, 663, and 470 nm, and the pigment concentrations were calculated using the following equations.

$$\text{Chla} = 12.21\text{A663} - 2.79\text{A647} \times (\text{V}/1000\text{W})$$

$$\text{Chlb} = 21.21\text{A647} - 5.1\text{A663} \times (\text{V}/1000\text{W})$$

$$\text{Chlt} = 7.15\text{A663} + 18.71\text{A647} \times (\text{V}/1000\text{W})$$

$$\text{Car} = (1000\text{A470} - 1.8\text{Chla} - 85.02\text{Chlb})/198 \times (\text{V}/1000\text{W})$$

Where Chla = chlorophyll a, Chlb = chlorophyll b, Chlt = total chlorophyll, Car = carotenoids, V = final volume of the extract in acetone and W = fresh weight of the tissue for extraction in grams.

Relative water content (RWC)

To determine RWC of the leaves, plant leaves were immersed in distilled water for 48 h. After removing the leaves from the water, their surface was dried with tissue paper and their weight was measured again. The leaves were then dried in an oven at 70 °C for 48 hours and their weight was measured. Finally, RWC was calculated using the following equation (Bian and Jiang 2009).

$$\text{RWC} = (\text{FW} - \text{DW}/\text{TW} - \text{DW}) \times 100$$

Where FW = leaf fresh weight, DW = leaf dry weight, and TW = leaf weight in complete turgid mode.

Membrane stability index (MSI)

To determine MSI, 0.1 g of the leaves were placed in two groups of test tubes containing 10 ml of distilled water. One group of tubes was placed at 40 °C for 30 min and the other group was placed at 100 °C for 10 min. After reducing the temperature of the tubes to ambient temperature, the electrical conductivity (EC) of the samples was measured by EC meter (Jenway model) and then MSI was calculated based on the following equation (Sairam and Saxena 2000).

$$\text{MSI} = [1 - (\text{C1}/\text{C2})] \times 100$$

Where C1 = EC of the sample at 40 °C for 30 min, and C2= EC of the sample at 100 °C for 10 min.

Malondialdehyde (MDA)

For determination of MDA (Heath and Packer 1968), 0.2 g of plant fresh tissue was homogenized with 5 ml of 0.1% Trichloroacetic acid (TCA). The resulting homogenate was centrifuged at 10,000 rpm for 5 min. Then, 4 ml of 20% TCA solution containing 0.5% Thiobarbituric acid (TBA) was added to 1 ml of supernatant solution. The resulting mixture was placed in a hot bath at 95 °C for half an hour and then cooled rapidly. The mixture was centrifuged at 10,000 rpm for 10 min and the absorbance of the supernatant was read at 532 and 600 nm. The concentration of MDA was calculated using the extinction coefficient of 156 $mM^{-1} cm^{-1}$ according to the following equation:

$$MDA = (A532–A600/156) \times 100$$

Total phenol (TP)

To measure TP, 0.1 g of plant tissue was homogenized in 5 ml of 95% methanol and left in the dark for 24 hours. Then, 450 μl of distilled water and 250 μl of Folin–Ciocâlteu reagent were added to 50 μl of homogenate, followed by adding 1.25 ml of 20% sodium carbonate solution. The mixture was placed at laboratory temperature (25 °C) for 20 min. After that, the mixture was centrifuged at 2000 rpm for 10 min and then the absorbance of the supernatant was read at 735 nm. Finally, the amount of TP was calculated using the standard curve (Singleton and Rossi 1965).

Total protein content (TPC) and activity of antioxidant enzymes

To extract total protein, 0.5 g fresh plant tissue was homogenized by adding 50 mg of Poly Vinyl Pyrrolidone (PVP) and 3 ml of extraction buffer containing 50 mM Potassium Phosphate (pH = 7) and 1 mM Sodium Metabisulfite. Homogenate was transferred to the test tubes and centrifuged at 4 °C and 14,000 rpm for 30 min. The supernatant (Protein extract) was used to determine the TPC and activity of antioxidant enzymes.

The concentration of protein was measured according to the Bradford method (Bradford 1976), using the standard curve. Catalase (CAT) activity was measured by Aebi (1984) method. The reaction mixture consisted of 2.5 mL of 50 mM potassium phosphate buffer (pH = 7), 0.4 mL of 15 mM hydrogen peroxide (H_2O_2), and 0.2 ml of protein extract. The absorbance change curve was recorded at 240 nm for 3 min. Each enzyme unit was accounted for the amount of enzyme that degrades one mM H_2O_2 in one min.

Guaiacol peroxidase (GPX) activity was determined based on the absorbance of Tetraguaiacol change curve at 470 nm for 3 min (Putter 1974). The reaction mixture consisted of 2.65 ml of 50 mM phosphate buffer (pH = 7.4), 300 μl of 3% H_2O_2, 30 μl of 99% guaiacol, and 0.2 ml of protein extract.

The concentration of mineral elements

Oven-dried (at 70 °C) and ground shoot samples (1 g) were digested with 10 ml of 2 M hydrochloric acid (HCl) under 550 °C for 4 h. The extract was filtered through filter paper (Whatman No. 1). The filtered solution was brought to a final volume of 100 ml with distilled water (Walsh 1971). Finally, the concentration of Mg and Fe in the solution was measured using an atomic absorption spectrometer (NOVAA 300).

The concentration of K and Ca in the shoot was determined by Flame Photometry (model PFP7). For this purpose, 0.05 g of dried and ground shoot samples were mixed with 3 ml of

Table 2. Interaction of VC and salinity stress on morphological traits, chlorophyll content, and carotenoids of two fennel landraces.

Fennel landraces	VC Conc.	Salinity Conc. (mM NaCl)	Shoot DW (mg)	Leaf area (mm^2)	Root DW (mg)	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)	Total Chl (mg g^{-1} FW)	Carot (mg g^{-1} FW)
Urmia	0	0	0.0448h	106.30h	0.0181f	0.2082h	0.0977h	0.3059ij	0.1426i
		40	0.0203l	91.20i	0.0123h	0.1720hi	0.0822h	0.2543jk	0.0962j
		80	0.0157m	77.22j	0.0116i	0.1526ij	0.0645i	0.2172kl	0.0884jk
		120	0.0059p	47.75k	0.0061n	0.1071j	0.0408j	0.1479m	0.0752k
	5%	0	0.1832b	292.71b	0.0431b	0.8919a	0.2680c	1.1590b	0.3821b
		40	0.0955d	216.50c	0.0307d	0.8111b	0.2386d	1.0490c	0.3131d
		80	0.0633e	160.72e	0.0174g	0.4125f	0.1516f	0.5641g	0.1991g
		120	0.0249j	94.16i	0.0112jk	0.2102h	0.1362fg	0.3464i	0.1471i
Shiraz	0	0	0.0329i	133.30f	0.0173g	0.4743e	0.1875e	0.6619e	0.3585c
		40	0.0228k	117.11g	0.0114ij	0.4532ef	0.1774e	0.6306ef	0.2891e
		80	0.0133n	109.90gh	0.0100l	0.3019g	0.1529f	0.4548h	0.1642h
		120	0.0173o	80.45j	0.0077m	0.1195ij	0.0616i	0.1809 lm	0.0751k
	5%	0	0.2303a	310.7a	0.0415c	0.9117a	0.4102a	1.3211a	0.4823a
		40	0.1818c	215.11c	0.0435a	0.7194c	0.3201b	1.0410c	0.3813b
		80	0.0542f	181.8d	0.0300e	0.6367d	0.1803e	0.8170d	0.2970de
		120	0.0478g	117.10g	0.0110k	0.4746e	0.1291g	0.6037fg	0.2649f

In each column, the means that have at least one common letter are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).
DW: dry weight; FW: fresh weight; Chl: chlorophyll; Carot: carotenoids; Conc: concentration.

concentrated nitric acid in a closed container. After 48 h, the mixture was gently heated until it was discolored. The volume of the remaining solution was reached to a final volume of 50 ml with distilled water (Chapman and Pratt 1982). The solution was used for determining the concentration of K and Ca.

Gibberellin (GA) content

GA content was measured by Asghari and Yasmeen (2010) method. Shoot sample (0.25 g) was thoroughly homogenized with 2.5 ml of 85% cold methanol and filtered with filter paper (Whatman No. 1). Then, acidified ethyl acetate (pH = 2.5) with HCl was added to the filtered solution (1:1). The resulting solution was centrifuged for 5 min at 16,000 rpm under 4 °C. Purified supernatant by a syringe filter was used to measure GA using HPLC device (Agilent 1260) equipped with UV detector and c18 column (4.6 × 100 mm, 3.5 µm).

Statistical analysis

Statistical analysis was performed using Mstat-C software. Duncan's multiple range test was used to compare the means at $P \leq 0.05$.

Results and discussion

Morphological characteristics

The results showed that salinity stress induced a significant decrease in shoot and root biomass, and leaf area of both fennel landraces, where the decrease was more in Urmia than Shiraz landrace at different levels of salinity. In the presence of VC alone or its combination with salinity stress, a significant increase in later variables was observed in both landraces. The effect of VC under saline and non-saline conditions on leaf area of Urmia landrace was more evident than Shiraz one (Table 2).

Consistent with the results of the present study, Semiz et al. (2012) reported a decrease in fennel biomass under salinity and considered fennel as a relatively sensitive plant to salinity. Cucci et al. (2014) reported that the sodium-induced salinity could affect the growth of fennel plant, possibly due to the side effects of sodium on the plant (i.e., toxicity and competition in nutrient uptake) as well as on the soil (i.e., reduced physical and chemical fertility). Lacerda et al. (2003) showed that salinity could disturb the absorption of nutrients and the ionic balance in plants. Therefore, reduced root growth, and leaf and stem development can be attributed to nutrient deficiencies and nutritional disorders induced by salinity.

The positive effect of VC on increasing leaf area of basil (Cabanillas, Stobbia, and Ledesma 2013), and improving the dry weight of bean (Beyk-Khormizi et al. 2016) have been reported in other studies. Arancon et al. (2008) reported that humic acid, folic acid, and other organic acids of VC or those produced by its microorganisms can stimulate growth and improve plant yield. Atiyeh et al. (2000) also reported an increase in the weight of VC-treated tomato plants due to the changes in physical, chemical, microbial, and biological characteristics of the culture medium. Arancon et al. (2004b) attributed a VC-induced increase in strawberry leaf area to the increase in the microbial population in this fertilizer.

Abou El-Magd, Zaki, and Habou Hussein (2008) stated that the effect of organic fertilizer on fennel plant growth might be due to the improvement of soil structure by increasing its water holding capacity and proper ventilation and drainage, which improves root growth and nutrients absorption. Consistent with the results of the present study, some reports indicate a positive effect of VC application on plant growth in saline conditions (Chinsamy, Kulkarni, and Van Staden 2013; Patel and Saraf 2013). Oliva et al. (2008) reported that the salinity tolerance of VC-treated tamarind seedlings might be due to the organic matter in the fertilizer, which is capable of trapping materials such as heavy metals. Biological factors such as mycorrhizal fungi may also be involved in the salinity tolerance mechanism of plants. Li et al. (2016) concluded that the positive effect of VC on plant growth in saline conditions is probably due to having nutrients, PGR, or microorganisms. It also changes the structure of the soil and has a positive effect on plant growth.

Photosynthetic pigments

Chlorophyll a, b, and total chlorophyll of both fennel landraces under 80 and 120 mM NaCl, and carotenoids under all salinity levels were significantly reduced. The reduction of these pigments under salinity was more in Shiraz landrace than Urmia one. In Urmia landrace under VC, chlorophyll a, b, total, and carotenoids were increased more than 4, 2.5, 3.5, and 2.5 times, respectively. In Shiraz landrace under the same treatment, chlorophyll a, b, total, and also carotenoids were increased by about 100 and 34%, respectively. In the interaction of salinity and VC, these pigments were significantly increased in both fennel landrace (Table 2). Similar to the results of the present study, a decrease in photosynthetic pigments in fennel (Shafeiee and Ehsanzadeh 2019) and in fenugreek (Banakar et al. 2022) under salinity has been reported. Plants that grow in saline environments are at the risk of producing reactive oxygen species (ROS) (Tayyab et al. 2016). ROS can induce peroxidation and degradation of chlorophyll pigments (Ramírez et al. 2014). Weakening of chlorophyll binding to thylakoid proteins, increasing chlorophyllase activity, and increasing PGR such as abscisic acid and ethylene are the reasons for decreasing chlorophyll content in saline conditions (Orabi, Salman, and Shalaby 2010). A competition between glutamine kinase (proline catalytic enzyme) and glutamate ligase (the first enzyme in the chlorophyll biosynthesis pathway) during salinity stress which causes glutamate (precursor of chlorophyll and proline synthesis pathway) to be used more in the proline production pathway can also lead to limiting chlorophyll biosynthesis (Bybordi, Tabatabaei, and Ahmadev 2010).

Table 3. Interaction of VC and salinity stress on relative water content (RWC), total protein content (TPC), catalase (CAT), and peroxidase (POX) activity, and concentration of some minerals in the shoot of two fennel landraces.

Fennel landraces	VC Conc.	Salinity conc. (mM NaCl)	RWC (%)	TPC (mg g^{-1} FW)	CAT activity (U $Protein^{-1}$)	POX activity (U $Protein^{-1}$)	Mg (mg g^{-1} DW)	Fe (mg g^{-1} DW)	K (%)	Ca (%)
Urmia	0	0	63.55c	230.8e	0.3536g	5.753l	290.3bc	171.9h	3.391gh	0.2480d
		40	54.04de	142.1h	0.7040e	13.240f	251.5d	135.5j	2.925hi	0.0897e
		80	50.2 e	114.5i	1.2160c	19.481d	161.4f	82.0l	2.615i	0.0590e
		120	44.77f	81.7j	2.0970a	37.731a	108.6g	70.8m	1.994j	0.0167e
	5%	0	75.78a	382.5b	0.1339h-j	2.747o	305.4ab	437.8b	7.953b	0.5840a
		40	75.37a	336.3c	0.1677hi	4.247m	271.6 cd	208.0e	6.712c	0.3310c
		80	70.16b	226.4e	0.3261g	8.297i	183.5ef	104.3k	5.719d	0.1953d
		120	53.13de	145.9gh	0.6538e	16.041e	122.1g	77. 7 lm	3.639g	0.0693e
Shiraz	0	0	70.75b	327.3c	0.3026g	5.967k	296.9a-c	198.1f	5.532d	0.3767c
		40	66.18c	265.1d	0.4458f	9.070h	278.8c	188.3g	4.663f	0.3533c
		80	62.91c	166.4g	0.9536d	19.570c	189.3e	145.0i	3.267gh	0.1000e
		120	55.47d	135.0hi	1.7330b	37.630b	118.2g	72.5m	3.112hi	0.0583e
	5%	0	78.54a	542.6a	0.0477j	2.217p	319.5a	451.9a	10.530a	0.6260a
		40	75.92a	392.7b	0.0914ij	3.573n	307.1ab	329.3c	6.277c	0.4580b
		80	70.72b	238.3e	0.2073h	6.510j	197.9e	306.3d	5.408de	0.2060d
		120	65.53c	199.4f	0.5128f	12.731g	124.7g	76.3 lm	4.943ef	0.1003e

In each column, the means that have at least one common letter are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).
DW: dry weight; FW: fresh weight; Conc: concentration.

Similar to the results of the present study, an increase in chlorophyll and carotenoids in lettuce (Kiran 2018) under VC treatment has been reported. Amiri, Ismaili, and Hosseinzadeh (2017) attributed the increase in chlorophyll in the presence of VC to the nutrients of this organic fertilizer, including K and N, which play a role in regulating osmotic pressure. The researchers reported that under VC, less glutamine was involved in the proline biosynthesis so that chlorophyll content was increased. On the other hand, Hosseinzadeh, Amiri, and Ismaili (2016) attributed this issue to the presence of microelements such as Fe in VC, because Fe acts as a prosthetic group of antioxidant enzymes such as CAT, peroxidase (POX), and superoxide dismutase (SOD) (Atik 2013) and therefore the ability of scavenging ROS in VC-treated plants can be increased. It is believed that the chlorophyll content is proportional to the amount of its protectors, i.e., carotenoids (Ebrahimi et al. 2014). Therefore, an increase in carotenoid content under VC treatment can be associated with an increased chlorophyll synthesis (Amiri, Ismaili, and Hosseinzadeh 2017).

Relative water content (RWC)

The results showed a significant decrease in RWC under different levels of salinity stress in both fennel landraces, where the decrease was more in Urmia. In VC treatment, a significant increase in RWC of Urmia (19%) and Shiraz (11%) landraces was observed. Also, the use of VC under salinity stress conditions induced a significant increase in RWC in both fennel landraces, where it was more evident in the Urmia (Table 3).

Consistent with the present study, Banakar et al. (2022) and Sadat Hosseini et al. (2022) reported that RWC was significantly decreased in Plants studied under salt stress. Decreased RWC under salinity stress is due to impaired water uptake by roots and reduced leaf water potential (Colom and Vazzana 2003). Jabeen and Ahmad (2009) reported that osmotic potential and water potential were decreased in sunflower leaves with increasing salt concentration, whereas they were increased with the use of organic fertilizer. The researchers stated that the change in internal water potential requires an increase in osmotic pressure by absorbing solutes from the soil or by synthesizing metabolic substances in the cell. The application of organic fertilizers leads to the accumulation of K^+ and some organic ions in the cell and consequently increases the osmotic activity and reduces the water potential and water movement into the cell. Beyk-Khormizi et al. (2016) also reported a decrease in RWC under salinity stress and its improvement under VC. They showed that VC can improve leaf water potential due to its phytohormones, porous structure, and high water holding capacity.

Total protein content (TPC)

TPC of fennel landraces was significantly decreased with increasing salinity stress. Under salinity levels of 40, 80, and 120 mm NaCl, TPC was decreased 1.6, 2, and 2.8 times in the Urmia landrace, and 1.2, 1.9, and 2.4 times in the Shiraz landrace, respectively, compared to the control.

Under VC treatment, TPC of Urmia and Shiraz landraces was increased by about 66%. Also, under different levels of salinity stress, the presence of VC induced a significant increase in the TPC of both fennel landraces (Table 3). Following salinity stress, secondary stresses such as oxidative stress also occur, in which the production and accumulation of active radicals lead to oxidation of proteins, lipids, and ultimately cell death (Molassiotis et al. 2006; Nasir Khan et al. 2007).

Amiri, Ismaili, and Hosseinzadeh (2017) attributed the increase in protein under VC to the availability and higher uptake of nitrogen in plants. An important feature of VC is the increase in some microbial populations of soil, such as nitrogen-fixing bacteria and symbiotic mycorrhizal fungi (Kale et al. 1992). The deficiency of some other elements also affects the amount of protein.

Salama et al. (2006) reported that in corn and chickpea, TPC was decreased under Zn deficiency conditions. Therefore, VC with a high concentration of elements and the presence of microorganisms can increase protein production and on the other hand, due to reduced production of ROS, prevents damage to proteins.

Activity of antioxidant enzymes

Salinity stress, VC, and their interaction had a significant effect on the activity of CAT and GPX. With increasing NaCl, the activity of these enzymes was significantly increased in both fennel landraces, where the increase was more in Urmia compared to Shiraz. Under VC treatment, a significant decrease in the activity of both enzymes was observed in both landraces, especially Shiraz. The application of VC in saline conditions significantly reduced the activity of both antioxidant enzymes in fennel landraces (Table 3).

Salinity stress can induce osmotic stress due to disrupting water potential homeostasis and ion distribution both at the cell and whole plant level. This water deficiency leads to the formation of ROS such as hydrogen superoxide and hydroxyl radicals. These cytosolic ROS disrupt metabolism through oxidative damage to lipids, proteins, and nucleic acids, and increase the activity of antioxidant enzymes such as CAT and GPX (Sorkheh et al. 2012). Plant cells use a set of antioxidant systems including a system of low molecular weight antioxidants such as glutathione, ascorbate, and carotenoids, and ROS scavenging enzymes such as superoxide dismutase, CAT, ascorbate peroxidase, GPX, and glutathione reductase to minimize the effects of oxidative stress (Shi et al. 2007).

According to the results of other researchers, the effect of VC on the activity of antioxidant enzymes in various plant species is different. Hosseinzadeh, Amiri, and Ismaili (2017) reported that in chickpea, VC treatment had no effect on CAT activity, but significantly reduced GPX activity. In contrast, Kiran (2018) showed that with the use of VC, the CAT activity was increased lettuce. Tuna et al. (2008) reported that GPX activity was increased under salt stress in maize, but the application of gibberellin reduced the activity of this enzyme in saline conditions. Therefore, since VC can improve plant growth due to its physicochemical and biological properties, including the presence of gibberellins, less ROS is likely to be produced leading to a decrease in the activity of CAT and GPX.

Concentration of minerals

The concentration of Fe, K, Ca, and Mg in the shoot of fennel landraces was significantly decreased under different salinity levels, except for Ca and Mg in Shiraz, and K in Urmia under 40 mm NaCl. Under most of the studied salinity levels, the concentration of Fe, Ca, and Mg showed a more severe decrease in the Urmia landrace compared to Shiraz one. The results showed that VC induced a significant increase in Fe, K, and Ca in the shoot of both fennel landraces, where the increase in Urmia was more than Shiraz landrace. With the application of VC in both fennel landraces, K, Fe, and Ca were significantly increased under salinity, compared to non-saline conditions (Table 3).

Tester and Davenport (2003) reported that increasing uptake and accumulation of sodium and chlorine ions reduces the uptake of essential plant elements. Decreased uptake of elements under saline conditions can be associated with reduced growth traits of fennel landraces. In this regard, Yu, Wang, and Wang (2012) stated that the activity of some cytoplasmic enzymes could be affected by some ionic levels within the cell. These enzymes are activated at high concentrations of K, but the presence of Na harms their activity. K depletion may be due to its leakage from plasma membranes or sodium competition over binding sites to membrane transporters (Ferreira-Silva et al. 2008).

Bachman and Metzger (2008) stated that VC could improve plant growth by an increase in the supply of nutrients such as N, P, K, Ca, Mg, as well as trace elements. An increase in K and Ca levels in chickpea under VC treatment has also been shown (Hosseinzadeh, Amiri, and Ismaili 2017). Under salinity conditions, VC treatment in blessed thistle and peppermint induced an increase in K/Na, and Ca/Na ratios, and reduced the effect of salinity stress on these plants (Li et al. 2016). Beyk-Khormizi et al. (2016) also reported that salinity stress reduced K and Ca in bean, where with the use of VC, these elements were increased. They attributed this effect to the abundance of nutrients, plant hormones such as cytokinin (which can increase K uptake), and the high water storage capacity of VC.

Membrane stability (MSI and MDA)

Under different levels of salinity stress, a significant decrease in MSI, and a significant increase in MDA were observed in both fennel landraces. VC treatment significantly increased MSI of Urmia landrace but did not affect the later trait in Shiraz one. With the use of VC, MDA was decreased in both landraces by 45% and 42.8%, respectively. With the application of VC in Urmia landrace, MSI was increased under salinity levels of 40 and 80 mM NaCl, and MDA was decreased under 80 mM NaCl. Also, in Shiraz landrace under 80 and 120 mM NaCl levels, VC increased MSI and decreased MDA (Figure 1A and B).

Consistent with the present study, Kabiri, Naghizadeh, and Hatami (2016) reported that MDA, which is a product of lipid peroxidation and an indicator of membrane damage, was significantly increased in ajwain under osmotic stress. Farooq and Azam (2006) stated that decreasing leaf water content and increasing osmotic potential, along with increasing sodium ion concentration, induces lipid peroxidation, and disruption of the function and structure of cell membranes. Consistent with the present study, reduced MDA in lettuce (Kiran 2018), and increased MSI in bean (Beyk-Khormizi et al. 2016) have been reported. Shirani Bidabadi, Dehghanipoodeh, and Wright (2017) also showed that in salinity stress, MDA and electrolyte leakage of two pomegranate cultivars were increased, while the presence of VC leachate in these conditions reduced these traits. It has been shown that the structure and function of plant cell membranes are strongly affected by Zn deficiency (Daneshbakhsh et al. 2013). Studies have indicated that Zn nutrition reduces the permeability of the root membrane as well as the free radicals-induced lipid peroxidation by increasing the concentration of sulfhydryl in the roots (Sanaeiostovar et al. 2012). Bandeoglu et al. (2004) also reported that GA reduces the level of hydrogen peroxide in the cell by altering the activity of relevant enzymes and thus reduces lipid peroxidation and ion leakage of the membrane. Therefore, VC with high levels of minerals such as Zn and plant hormones such as GA can reduce lipid peroxidation and increase the stability of fennel leaf cell membranes.

Total phenol (TP)

The results showed that under salinity stress, VC, and their combination, TP showed a significant increase in both fennel landraces, where the increase under different salinity levels and VC alone was higher in Urmia and Shiraz landrace, respectively (Figure 1C). TP plays a key role as one of the non-enzymatic defense mechanisms in plants. These compounds are one of the indicators sensitive to environmental changes and also as one of the biochemical indicators of plant defense against environmental stresses. They help to scavenge hydrogen peroxide in plant cells (Vogt 2010). Plants under stress use defense mechanisms, including the increase in TP concentration, to counteract oxidative stress (Apel and Hirt 2004). Connor et al. (2002) considered access to nutrients as one of the important factors in TP production.

Due to its properties such as high water holding capacity and having large amounts of macro and microelements, VC probably improves the conditions of the root environment resulted in a

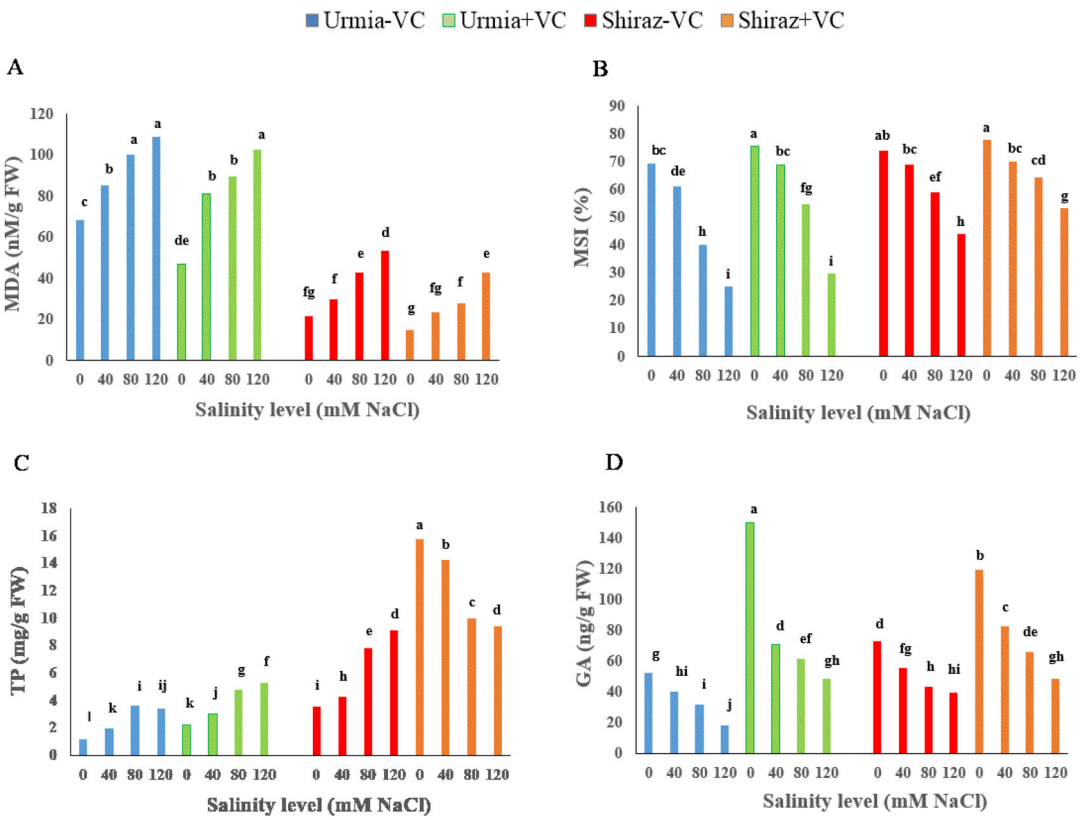

Figure 1. Interaction of VC and salinity stress on malondialdehyde (MDA), membrane stability index (MSI), total phenol (TP), and gibberellin (GA) levels of two fennel landraces. The means that have at least one common letter are not significantly different according to Duncan's multiple range test ($p \leq 0.05$). Urmia-VC: Urmia landrace without VC treatment; Urmia + VC: Urmia landrace with VC treatment; Shiraz-VC: Shiraz landrace without VC treatment; Shiraz + VC: Shiraz landrace with VC treatment.

decrease in ROS. Therefore, the need for antioxidants to neutralize these reactive species is reduced. This is consistent with the reduction of enzymatic antioxidants such as CAT and GPX in the present study.

Concentration of GA

The results showed that in both fennel landraces, the concentration of GA in the shoot was decreased under all salinity levels. VC treatment alone caused a significant increase in the concentration of GA in Urmia (2.8 times) and Shiraz (63%) landraces. Also, with the use of VC, the concentration of GA in Urmia landrace at all salinity levels and in Shiraz landrace at 40 and 80 mM NaCl levels were significantly increased, compared to non-saline condition (Figure 1D).

GAs are the phytohormones that control many aspects of plant growth, including seed germination, leaf spread, stem longitudinal growth, and flowering (Fleet and Sun 2005). Leitao and Enguita (2016) reported that the level of this hormone in plants under saline stress is reduced. Under saline condition, the level of endogenous GA in Arabidopsis is reduced by inducing GA2 oxidase, an enzyme that converts active forms of GA into inactive forms (Magome et al. 2008).

The results of the present study showed that with the use of VC, GA was increased in both fennel landraces. Plants can absorb PGR present in VC to affect plant growth (Edwards 2004). Oliva et al. (2008) reported that VC contains biological factors such as mycorrhizal fungi. Hassan (2002) stated that many fungi can form symbiosis with plant roots. Fungi may be responsible for

maintaining soil fertility by producing GA. Production of GA by fungi was decreased under high salinity levels, but adding Ca to the environment increased GA level (Hassan 2002). Therefore, it seems that salinity stress prevents the increase of GA in the plant due to the limitation of soil microbial fauna and inhibition of mineral uptake. However, VC contains microorganisms that can produce GA, and on the other hand, it contains minerals such as calcium. Ca can probably prevent the negative effect of salinity on hormone production by microorganisms and plants in saline environments.

Conclusions

Salinity stress has an adverse effect on physiological and biochemical traits of Shiraz and Urmia fennel landraces and finally reduces their biomass and leaf area. Shiraz landrace is more resistant to salinity stress than Urmia one. The use of VC limits the adverse effect of salinity stress in both studied landraces, especially Urmia.

Disclosure statement

The authors declare that they have no conflict of interest.

Funding

This work was supported by the Urmia University.

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