

Vermicompost leachate reduces some negative effects of salt stress in pomegranate

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Abstract

Purpose Growth and productivity of plant crops are negatively affected by salt stress. Vermicompost leachate (VCL) has beneficial effects on plant growth in several different ways. This experiment was conducted to investigate the potential of VCL for raising the tolerance of pomegranate against salinity.

Methods A pot experiment was organized to analyze some physiological attributes and antioxidant activities of two Iranian pomegranate cultivars under different concentrations of NaCl with or without VCL.

Results Salinity negatively affected the growth properties of pomegranate, while foliar spray of VCL-induced salt tolerance by reducing the accumulation of Na⁺ in the seedlings. Leaf area and shoot and root fresh and dry weight of VCL-treated pomegranate plants showed a significant increase regardless of whether the seedling growth was under non-saline or saline conditions. Chlorophyll loss and reduced photosynthesis efficiency caused by salinity were also ameliorated using VCL. Foliar application of

VCL also improved the activity of antioxidant enzymes and caused a reduction in oxidative stress and electrolyte leakage.

Conclusions These results suggested that VCL could alleviate the damage caused by salt stress in pomegranate by limiting the agglomeration of Na⁺ and amending the activities of antioxidant enzymes by which the efficiency of plant increases.

Keywords Antioxidant activities · Fruit crops · Organic wastes · Photosynthesis efficiency · Salinity · Seedlings · Sodium accumulation

Introduction

Growth and productivity of crops are negatively affected by salt stress. The entry of high amounts of salt in the soil and irrigation water disrupts all physiological and biochemical processes in the plant (Hasanuzzamaan et al. 2013). The reduced osmotic potential of soil solution, imbalance in nutritional elements, and the effect of ion toxicity are consequences of salinity which has a detrimental effect on the growth of plants (Ashraf 1994; Parvaiz and Satyawati 2008). During the onset and spread of salt stress in the plant, all physiological and biochemical processes of the plant are affected. (Parvaiz and Satyawati 2008). However, the negative effects of salt stress on plants depend on the severity and exposure time of salinity. Plant genotypes and environmental factors also play a decisive role; therefore, finding a suitable moderator or stress relievers is one of the duties of researchers (Hasanuzzamaan et al. 2013). Due to the high cost and hazardous effects of chemical fertilizers, using organic compound solutions as foliar fertilizer sprays is developing. Moreover, restricted nutrient supply and

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restriction in chemical fertilizer use in saline soils are considerable problems that should be solved by proper methods and efficient management practices (Astaraei and Ivani 2008; Chinsamy et al. 2013). Organic fertilizers improve soil structure and enhance nutrient availability, thus helping to protect the yield and quality, and are more affordable than synthetic fertilizers (Ayyobi et al. 2014). Use of organic sources of nutrients improves the soil health by sustaining the balance of organic matter, thereby helping to amend physical, chemical, and biological properties of the soil (Mir et al. 2013). Vermicomposts, which are created by crushing organic wastes from earthworm, contain nutrients in a form that is easily absorbed by the plant. Vermicompost leachate (VCL) is a liquid phase that comes from vermicompost and it has a very positive impact on soil improvement and plant growth (Chinsamy et al. 2013). Previous reports have shown that vermicompost modulates the harmful effects of salinity stress on several plant crops (Atiyeh et al. 2000; Oliva et al. 2008; Sallaku et al. 2009; Nandi et al. 2013; Ayyobi et al. 2014). Beneficial effects of vermicompost leachate have also been reported in bulbous plants and some commercially important crops such as banana and tomato (Aremu et al. 2012; Arthur et al. 2012; Aremu et al. 2014). Pomegranate (*Punica granatum*) is a strategic horticultural crop cultivated all over the world (Mirzapour and Khoshgofarmanesh 2013). It is one of the oldest fruit crops which has been cultivated since ancient times for its economic, ornamental, and medicinal properties (Pirseyyedi et al. 2010; Ramezani et al. 2009). Leaves, fruits, and bark of the pomegranate tree are used in the traditional medicine (Hulya Orak 2009). Fruits are a rich source of minerals and sugars, along with vitamin C and certain phenolic compounds which act as natural antioxidants (Zaouaya et al. 2012). The origin of pomegranate is arid and semi-arid regions of Iran, where it is seriously affected by high salinity (Okhovatian-Ardakani et al. 2010). Pomegranate trees are moderately salt tolerant under field conditions (El-Khawaga et al. 2013). Hence, an attempt has been made to examine the interactive effects of salinity and vermicompost leachate on growth indices, physiological parameters, and antioxidative enzyme activities of two commercial cultivars of pomegranate. This work was, therefore, aimed to assess the efficiency of foliar application of VCL to mitigate the negative effects of salinity on pomegranate growth and productivity.

Materials and methods

Plant material and growing conditions

Seed extraction and germination was achieved according to the method recommended by Rawat et al. (2010). Ripe fruits of *Punica granatum* cultivars “Rabab” and “Malas

Daneh Ghermez” were harvested in the month of August 2013. Then, their seeds were collected and dried in the shade. Afterwards, the imbibed seeds were placed between layers of moist peat moss and exposed to chilling (5 °C) for up to 30 days. Following germination under greenhouse conditions, 60-day-old seedlings were transplanted into black plastic pots 10 cm in diameter (one plant per pot) filled with soil garden having loamy texture, pH 7.8, with an EC of 1.7 ds cm⁻¹, perlite, and sand with a ratio of 2:1:1, respectively, and grown under glasshouse conditions. Plants were irrigated and fertilized with a complete floral fertilizer 20-20-20 (a product of CIFO S.P.A., Bologna, Italy.), to promote strong growth until 30 July 2014, when salinity and VCL treatments were imposed (seedlings were 30–40 cm in height).

Treatments and experimental design

On 1 August 2014, 36 pomegranate seedlings that were used for the experiment were arranged in a completely randomized factorial design of six treatments with three single-seedling replications along a glasshouse bench under 28 °C daytime and 18 °C nighttime greenhouse temperatures and 250 μmol m⁻² S⁻¹ light intensity. The salinity of the irrigation water was 0, 30, and 60-mM NaCl. Eighteen plants, 12 of each cultivar, received each salinity treatment. NaCl treatments started in 1-year-old seedlings. Plants were irrigated with 1 L of salt solution as needed and leached with tap water, after every fourth irrigation to prevent salt accumulation. Salt treatments were continued for 3 months, and plants were destructively harvested on 1 November 2014. VCL treatment was applied along with saline treatments, beginning on 1 August 2014 using VCL as spray and tap water as a control. A hand sprayer was used to apply VCL to run off. Seedlings that were grown under normal or saline conditions sprayed with VCL and tap water.

VCL preparation and specification

Vermicompost (VC) and vermicompost leachate (VCL) were prepared according to the approach of Ayyobi et al. (2014). Vermicompost was processed by Earthworms (*Eisenia fetida*) in proportion to 25 g of earthworms per 1 kg of cow manure for 2 months. The amount of 50 kg of obtaining vermicompost was squeezed by adding 25 L of water and allowed the leachate to be extracted for 48 h and then gathered in a tank. Table 1 shows the characteristics of VCL derived from cow manure.

Analysis of growth parameters

Leaf area was measured in cm² using a leaf area meter (WinArea- UT- 11, Iran). After washing the plants for the

separation of mud and other foreign particles sticking to the plants, fresh mass of shoots and roots was recorded. The shoots and the roots were then dried in an oven at 80 °C for 48 h to specify a fixed dry weight. The leaves were collected and frozen in liquid nitrogen and kept at -80 °C until used for biochemical and physiological measurements.

Chlorophyll fluorescence, chlorophyll, and proline measurements

Chlorophyll fluorescence (Fv/Fm) was measured applying a fluorometer (Walz, Effeltrich, Germany). The chlorophyll content was measured with a portable leaf chlorophyll meter (SPAD 502, Minolta Co., Osaka, Japan). The proline content was measured according to the method of Bates et al. (1973).

Determination of endogenous MDA and H₂O₂ concentrations and electrolyte leakage (EL) of pomegranate leaves

Lipid peroxidation indicated by accumulated malondialdehyde (MDA) was assessed according to Wang et al. (2009). After recording the absorbances of the supernatant at 450-, 532-, and 600-nm wavelengths, MDA content was calculated on a fresh weight basis using the following formula:

$$(\text{nmol MDA g}^{-1}\text{FW}) = 6.45(\text{OD}_{532} - \text{OD}_{600}) - 0.56(\text{OD}_{450}) \times 1000.$$

Hydrogen peroxide (H₂O₂) was measured according to method of Velikova and Loreto (2005). Electrolyte leakage (EL) was measured by applying a conductivity meter according to Ozden et al. (2009).

Determination of sodium (Na⁺) concentration

For measurement of Na⁺ concentration, the leaf samples were dried in 60 °C for 48 h. Then, 1 g of leaves was ground using an electric grinder and scorched in 560 °C. The obtained ashes were digested in 10 ml of 1 N HCl. Then, the concentration of Na⁺ in the digested samples was determined using a flame photometer (Model CL 378, Nanolytik, Germany).

Enzyme extractions and assays

Three months after salinity and VCL treatments, the fresh leaves were collected and enzyme activities were assayed. The samples were prepared as described by Azooz et al. (2012) for CAT (catalase): APX (ascorbate peroxidase) and SOD (superoxide dismutase) activity assay. The activity of SOD was estimated by recording the decrease in absorbance of superoxide-nitroblue tetrazolium complex by the enzyme (Cavalcanti et al. 2004). In addition, then, one SOD unit was defined as the amount of enzyme required to inhibit 50% of the NBT photo-reduction compared to the tubes without the plant extract and expressed as a unit of enzyme activity per mg of protein. Catalase (CAT) activity was measured according to Aebi (1984). The decrease of H₂O₂ was monitored at 240 nm and quantified by its molar extinction coefficient (36 M⁻¹ cm⁻¹) and the results expressed as CAT units per mg of leaf fresh weight.

Ascorbate peroxidase (APX) activity was determined by the method of Nakano and Asada (1981). One unit of APX activity was defined as 1-M ascorbate oxidized per minute at 290 nm and the specific activity was expressed as U mg⁻¹ protein.

The statistical procedure was performed by SAS 9.1 and means were compared with the LSD multiple range test.

Results and discussion

Effect of foliar application of VCL on leaf area, fresh and dry weight of shoots and roots of pomegranate seedlings under different NaCl levels is presented in Table 2. Leaf area, fresh and dry weight of shoots and roots of both cultivars were significantly reduced by increasing salinity, whereas in cultivar Rabab, leaf area, fresh and dry weight of shoots and roots were reduced by 32.86, 37.48, 50.19, 53.33, and 50.97%, respectively, while in the Malas Daneh Ghermez cultivar, leaf area, fresh and dry weight of shoots and roots were reduced by 46.90, 37.80, 49.34, 50.92, and 52.13%, respectively, at the highest salinity level (60 mM NaCl) in comparison with non-saline controls (Table 2). The negative impacts of salinity on the plants growth have been reported by many researchers (Parvaiz

Table 1 Characteristic of vermicompost leachate obtained from cattle manure

Characteristic	Vermicompost leachate
pH	7.56
Electrical conductivity (ds m ⁻¹)	5.42
N (%)	1.3
P ₂ O ₅ (%)	0.7
K ₂ O (%)	0.9
Cu (%)	0.05
Zn (%)	0.09
Mn (%)	0.18

These values are subjected to variations depending on the cattle feeding



Table 2 Effects of VCL treatments on shoot fresh and dry weight, root, fresh and dry weight and leaf area of pomegranate seedlings under NaCl-induced salt stress

Cult.	VCL treatment	Salinity (mM)	Shoot fresh weight (g)	Shoot dry weight (g)	Leaf area (cm ²)	Root fresh weight (g)	Root dry weight (g)
"Rabab"	Treated	0	5.84 ± 0.18 ^a	2.84 ± 0.07 ^a	2.84 ± 0.07 ^a	2.93 ± 0.22 ^a	1.62 ± 0.33 ^a
		30	4.90 ± 0.17 ^{bc}	2.44 ± 0.11 ^{bc}	2.58 ± 0.11 ^{ab}	2.15 ± 0.21 ^b	1.47 ± 0.33 ^a
		60	3.80 ± 0.10 ^{de}	2.01 ± 0.16 ^d	2.30 ± 0.07 ^{cd}	1.86 ± 0.20 ^c	1.19 ± 0.06 ^b
	Untreated	0	5.63 ± 0.37 ^a	2.65 ± 0.18 ^b	2.80 ± 0.10 ^a	2.85 ± 0.10 ^a	1.55 ± 0.08 ^a
		30	4.06 ± 0.14 ^d	1.91 ± .013 ^d	2.21 ± 0.10 ^d	1.64 ± 0.15 ^c	1.16 ± 0.09 ^b
		60	3.52 ± 0.18 ^{ef}	1.32 ± 0.08 ^f	1.88 ± 0.08 ^c	1.33 ± 0.12 ^d	0.76 ± 0.06 ^{cd}
"Malas Daneh Ghermez"	Treated	0	5.17 ± 0.33 ^b	2.37 ± 0.18 ^{bc}	2.51 ± 0.39 ^{bc}	2.27 ± 0.14 ^b	0.99 ± 0.19 ^{bc}
		30	4.67 ± 0.14 ^c	1.99 ± 0.13 ^d	2.50 ± 0.12 ^{bc}	1.84 ± 0.09 ^c	0.74 ± 0.12 ^{cd}
		60	3.29 ± 0.17 ^{fg}	1.55 ± 0.13 ^e	2.10 ± 0.20 ^{de}	1.28 ± 0.15 ^{de}	0.53 ± 0.08 ^{de}
	Untreated	0	4.92 ± 0.36 ^{bc}	2.29 ± 0.16 ^c	2.58 ± 0.16 ^{ab}	2.18 ± 0.14 ^b	0.94 ± 0.13 ^{bc}
		30	3.43 ± 0.25 ^{efg}	1.33 ± 0.09 ^{ef}	2.22 ± 0.08 ^d	1.24 ± 0.06 ^{de}	0.60 ± 0.07 ^{de}
		60	3.06 ± 0.22 ^g	1.16 ± 0.10 ^f	1.37 ± 0.11 ^f	1.07 ± 0.11 ^e	0.45 ± 0.09 ^e
LSD _(0.05)			0.40	0.23	0.26	0.25	0.27

Means followed by the same letter are not significantly different by the LSD Multiple Range test at $P \leq 0.05$

and Satyawati 2008; Chinsamy et al. 2013; El-Khawaga et al. 2013 and Hasanuzzamaan et al. 2013). Naeini et al. (2006) reported that pomegranate cuttings tolerate salinity up to 40 Mm. Leaf area, fresh and dry weight of shoots and roots of both cultivars were increased by foliar spray of VCL irrespective to the seedling growth under non-saline or saline conditions. In cultivar Rabab treated with VCL, leaf area, fresh and dry weight of shoots and roots increased by 22.34, 7.95, 52.27, 39.85, and 56.58%, while in VCL-treated Malas Daneh Ghermez cultivar, these attributes were increased 53.28, 7.52, 33.62, 19.62, and 17.78%, respectively, at the highest salinity level (60 mM NaCl) in comparison with their respective controls (Table 2). Similar results in improving morphological attributes of VCL-treated tomato seedlings such as leaf area and overall aboveground growth were observed in tomato seedlings under a high NaCl-tested concentration (100 mM) (Chinsamy et al. 2013). The lack of sensitivity of all morphological attributes to salinity for VCL-treated seedlings was largely caused by a significant reduction in these attributes for non-VCL-treated seedlings under stressed conditions. The prolonged availability of nutrients during the crop growth provided by the application of enriched vermicomposts might have enhanced the plant growth (Nandi et al. 2013). Ability to high water absorption in vermicompost associated with high amount of macro- and micronutrients results in improving photosynthesis (Fathi et al. 2012). Arthur et al. (2012) reported that the presence of VCL relieved the negative effects caused by shortage of P and K during the growth tomato seedlings under nutrient stress. Improvement of plant growth using

organic fertilizer such as vermicompost is reported by Ayyobi et al. (2014) in peppermint plants and Nandi et al. (2013) in pomegranate.

For the interaction between the two levels of salinity (30 and 60 mM) and the two pomegranate cultivars, the best results in terms of morphological characteristics were noticed with cultivar Rabab (Table 2). Accordingly, El-Khawaga et al. (2013) concluded that these differences may be attributed to the various responses of some pomegranate cultivars to environmental conditions. Although the application of NaCl decreased the content of relative chlorophyll and photosynthesis efficiency of pomegranate seedlings, the foliar application of VCL limited the decreases. VCL increased photosynthesis efficiency in both saline treatments in comparison with the untreated counterparts for both cultivars (Table 3). A reduced amount of chlorophyll and photosynthesis efficiency has also been observed in several species of plants grown in saline conditions (Okhovatian-Ardakani et al. 2010; Hasanpour et al. 2014). Similarly, leaf chlorophyll increased following the application of VCL to tomato seedlings and some bulbous plants under salinity, temperature, water, and nutrient stresses (Chinsamy et al. 2013; Aremu et al. 2014; Chinsamy et al. 2014). VCL may have an important role in alleviating the salinity-induced damage to the chloroplasts by reducing chlorophyllase activity (Chinsamy et al. 2013). Chlorophyll fluorescence provides useful information about primary reactions of photosynthesis and has been widely used in assessing plant responses to environmental stress (Schnabel et al. 1998). Reducing chlorophyll pigments of leaf in response to



Table 3 Effects of VCL treatments on chlorophyll, photosynthesis efficiency, and proline of pomegranate seedlings under NaCl-induced salt stress

Cult.	VCL treatment	Salinity (mM)	Relative chlorophyll	Photosynthesis efficiency (Fv/Fm)	Proline ($\mu\text{mol g}^{-1}$ FW)
"Rabab"	Treated	0	13.76 ± 1.35^a	0.27 ± 0.04^a	6.69 ± 0.57^{cde}
		30	11.78 ± 0.56^c	0.21 ± 0.02^b	6.90 ± 0.46^{bcde}
		60	9.39 ± 0.46^{de}	0.13 ± 0.03^{cde}	8.71 ± 0.15^a
	Untreated	0	13.85 ± 0.36^a	0.16 ± 0.04^c	6.76 ± 0.39^{cde}
		30	10.02 ± 0.67^d	0.09 ± 0.02^{efg}	6.49 ± 0.50^{de}
		60	7.87 ± 0.45^{fg}	0.06 ± 0.01^{fg}	6.71 ± 0.38^{cde}
"Ghermez" Malas Daneh	Treated	0	13.26 ± 0.85^{ab}	0.25 ± 0.04^{ab}	6.73 ± 0.65^{cde}
		30	10.30 ± 0.50^d	0.14 ± 0.03^{cd}	6.28 ± 0.22^e
		60	8.17 ± 0.32^f	0.10 ± 0.02^{def}	7.00 ± 0.28^{bcd}
	Untreated	0	12.35 ± 0.44^{bc}	0.23 ± 0.02^{ab}	6.87 ± 0.23^{cde}
		30	8.55 ± 0.82^{ef}	0.11 ± 0.02^{de}	7.17 ± 0.22^{bc}
		60	6.83 ± 0.50^g	0.05 ± 0.02^g	7.55 ± 0.21^b
	LSD _(0.05)		1.12	0.05	0.65

Means followed by the same letter are not significantly different by the LSD Multiple Range test at $P \leq 0.05$

salinity results in the decline in the values of Fv/Fm ratio (Hasanpour et al. 2014). The results of significant reduction in the photosynthetic efficiency (Fv/Fm) by the higher doses of vermicompost agree with the findings of Lazcano and Dominguez (2010) on the primula plant.

Although VCL-sprayed seedlings of the Malas Dane Ghermez cultivar treated with 60 mM salt had almost similar proline content to untreated counterparts with no significant difference, the highest content of proline was recorded with VCL-treated seedlings of cultivar Rabab under the highest level of NaCl (60 mM)-induced salt stress (Table 3). Among other roles, proline acts as an osmolyte under conditions of water deficit and salinity-induced stress and it is recognized as an osmo-protectant compound, stabilizing proteins, inhibiting lipid peroxidation and scavenging of ROS (Garcial et al. 2012). Chinsamy et al. (2013) showed a significant increase in proline concentration in salt-stressed tomato seedlings by VCL treatment. A similar effect was observed with humic acids of vermicompost as reported by Garcial et al. (2012) in rice seedlings under water stress.

The contents of H_2O_2 and MDA in pomegranate leaves were strongly affected by both salinity and VCL (Table 4). As expected, salinity increased H_2O_2 and MDA contents of the leaves of both cultivars. In Males Dane Ghermez, the content of H_2O_2 and MDA was 16.67 and 6.13% greater than in cultivar Rabab under the highest level of NaCl (60 Mm) (Table 4). The H_2O_2 and MDA contents of VCL-treated seedlings receiving salts were less than those of the untreated seedlings under all levels of NaCl-induced salt stress (Table 4). Clearly, VCL improved the quality of

growth, as salt-stressed seedlings showed less foliar necrosis, and less defoliation when treated with VCL. An adaptive mechanism in VCL-treated tomato seedlings reported by Chinsamy et al. (2013) indicates the positive effects of VCL on physiological attributes of plants under salinity. Electrolyte leakage (EL) of leaves in both cultivars increased significantly with increasing levels of NaCl-induced salt stress (Table 4). Malas Daneh Ghermez cultivar showed significantly higher EL (35.33%) resulting from the highest level of NaCl (60 mM)-induced salt stress without VCL treatment. Meanwhile, foliar application of VCL caused a reduction in electrolyte leakage when seedlings subjected to salt stress. According to the results of this investigation, under salinity stress, Malas Daneh Ghermez was more sensitive than Rabab.

The Na^+ concentration of seedlings receiving no vermicompost leachate increased with increasing salinity, as the highest sodium concentration was recorded at 60-mM NaCl (Table 5). Accumulation of Na^+ in both cultivars of pomegranate seedlings under salinity and the tendency of Malas Daneh Ghermez to accumulate more Na^+ than Rabab (Table 5) might partly clarify the apparent greater salt sensitivity of Malas Daneh Ghermez. Naeini et al. (2004) reported that pomegranate accumulates sodium in salt concentrations greater than 40 mM. When subjected to increasing salt levels, the seedlings of both cultivars receiving VCL showed a significant decrease in value of Na^+ compared to the seedlings receiving no VCL and there was no significant difference between two cultivars at 60-mM NaCl (Table 5). Our present results are consistent with the findings of Maie Mohsen et al. (2016) on

Table 4 Effects of VCL treatments on H₂O₂ content, MDA, and EL of pomegranate seedlings under NaCl-induced salt stress

Cult.	VCL treatment	Salinity (mM)	H ₂ O ₂ content (μmol g ⁻¹ FW)	MDA (nmol g ⁻¹ FW)	EL (%)
"Rabab"	Treated	0	20.92 ± 1.42 ^g	7.71 ± 0.63 ^f	20.33 ± 1.53 ^{fg}
		30	24.90 ± 1.40 ^e	9.74 ± 0.35 ^e	22.67 ± 1.15 ^{ef}
		60	29.53 ± 0.72 ^d	12.08 ± 0.35 ^c	26.33 ± 0.58 ^{cd}
	Untreated	0	20.34 ± 0.69 ^g	8.02 ± 0.72 ^f	22.67 ± 1.53 ^{ef}
		30	30.48 ± 1.18 ^d	11.18 ± 0.35 ^d	28.33 ± 1.53 ^c
		60	35.76 ± 0.89 ^b	13.86 ± 0.31 ^{ab}	32.67 ± 1.53 ^b
"Malas Daneh Ghermez"	Treated	0	21.31 ± 1.36 ^{fg}	8.11 ± 0.81 ^f	19.33 ± 1.53 ^g
		30	29.42 ± 0.73 ^d	9.67 ± 0.34 ^e	24.67 ± 0.58 ^{de}
		60	33.10 ± 0.92 ^c	13.14 ± 0.33 ^b	28.67 ± 1.53 ^c
	Untreated	0	23.23 ± 0.47 ^{ef}	9.28 ± 0.48 ^e	21.00 ± 2.00 ^{fg}
		30	37.38 ± 1.43 ^b	13.06 ± 0.73 ^b	28.33 ± 1.16 ^c
		60	41.72 ± 1.81 ^a	14.71 ± 0.60 ^a	35.33 ± 1.53 ^a
	LSD _(0.05)		1.94	0.89	2.37

Means followed by the same letter are not significantly different by the LSD Multiple Range test at $P \leq 0.05$

Table 5 Effects of VCL treatments on Na⁺ content of pomegranate leaf samples under NaCl-induced salt stress

Cult.	VCL treatment	Salinity (mM)	Na ⁺ (%)
"Rabab"	Treated	0	0.055 ± 0.004 ^f
		30	0.099 ± 0.013 ^e
		60	0.174 ± 0.005 ^c
	Untreated	0	0.055 ± 0.002 ^f
		30	0.104 ± 0.011 ^e
		60	0.187 ± 0.004 ^b
"Malas Daneh Ghermez"	Treated	0	0.062 ± 0.005 ^f
		30	0.117 ± 0.003 ^d
		60	0.180 ± 0.002 ^{bc}
	Untreated	0	0.064 ± 0.004 ^f
		30	0.122 ± 0.008 ^d
		60	0.205 ± 0.008 ^a
	LSD _(0.05)		0.012

Means followed by the same letter are not significantly different by the LSD Multiple Range test at $P \leq 0.05$

Majorana hortensis L. They reported that vermicompost reduced Na⁺ content of the shoots.

CAT, APX, and SOD activity data presented in Table 6 revealed that a significant effect was observed in enzyme activity. CAT activity showed significant negative correlation with hydrogen peroxide content, in the presence of increasing salinity (Table 7). Following application of a VCL treatment, the highest activity of CAT (0.51) was found in Rabab under 0 mM NaCl (control conditions) (Table 6). The VCL-treated seedlings of cultivar Rababs had greater CAT activity under 60-mM NaCl-induced salt stress, but there was no significant difference in both cultivars. Results in Table 6 showed that different salinity levels exhibited variable effects on increasing APX activity

and spraying VCL caused no significant changes of APX activity in response to the varying levels of NaCl-induced salinity (Table 6). Applying the VCL-induced noticeable changes in SOD activity among both cultivars under different levels of salinity (Table 6). The VCL-treated plants of both cultivars had significantly greater SOD activity compared with non-VCL-treated plants under the highest level (60 mM NaCl) of salinity (Table 6). Garcia et al. (2012) reported that vermicompost enhanced the activity of POX resulted in a reduction of H₂O₂ content and greater conservation of membrane permeability in rice seedlings under water stress. Aremu et al. (2014) showed that increased antioxidant activities of some bulbous plants detected when VCL was applied under nutrient stress.

Table 6 Effects of VCL treatments on the activity of CAT, APX, and SOD of pomegranate seedlings under NaCl-induced salt stress

Cult.	VCL treatment	Salinity (mM)	Activity of CAT (U/mg protein)	Activity of APX (U/mg protein)	Activity of SOD (U/mg protein)
"Rabab"	Treated	0	0.51 ± 0.08 ^a	3.19 ± 0.39 ^b	230.90 ± 24.27 ^c
		30	0.31 ± 0.07 ^c	3.71 ± 0.43 ^{ab}	256.45 ± 9.47 ^{ab}
		60	0.19 ± 0.02 ^{de}	3.77 ± 0.41 ^{ab}	204.43 ± 6.27 ^{def}
	Untreated	0	0.42 ± 0.05 ^b	3.19 ± 0.51 ^b	224.32 ± 18.06 ^{cd}
		30	0.24 ± 0.03 ^{cd}	3.89 ± 0.35 ^a	185.00 ± 6.00 ^{fg}
		60	0.13 ± 0.03 ^{ef}	2.20 ± 0.27 ^c	176.45 ± 10.29 ^g
"Malas Daneh Ghermez"	Treated	0	0.30 ± 0.02 ^c	3.18 ± 0.25 ^b	237.23 ± 9.58 ^{bc}
		30	0.13 ± 0.02 ^{ef}	3.89 ± 0.23 ^a	262.62 ± 12.71 ^a
		60	0.11 ± 0.03 ^f	3.57 ± 0.51 ^{ab}	271.29 ± 10.79 ^a
	Untreated	0	0.46 ± 0.05 ^{ab}	3.20 ± 0.30 ^b	221.03 ± 8.75 ^{cde}
		30	0.19 ± 0.02 ^{de}	3.93 ± 0.11 ^a	240.51 ± 16.04 ^{bc}
		60	0.11 ± 0.03 ^f	3.38 ± 0.41 ^{ab}	202.21 ± 12.83 ^{ef}
LSD _(0.05)			0.08	0.61	22.05

Means followed by the same letter are not significantly different by the LSD Multiple Range test at $P \leq 0.05$

Table 7 Correlation between measured traits of pomegranate seedlings

	1	2	3	4	5	6	7	8	9	10	11	12
1. Shoot dry weight	1	0.76**	0.86**	0.90**	0.81**	-0.17 ^{ns}	-0.94**	-0.92**	-0.87**	0.90**	0.02 ^{ns}	0.21 ^{ns}
2. Root dry weight		1	0.71**	0.74**	0.55**	-0.001 ^{ns}	-0.75**	-0.70**	-0.61**	0.58**	-0.004 ^{ns}	-0.07 ^{ns}
3. Leaf Area			1	0.84**	0.75**	-0.22 ^{ns}	-0.85**	-0.87**	-0.88**	0.77**	0.05 ^{ns}	0.35*
4. Chlorophyll content				1	0.82**	-0.30*	-0.95**	-0.94**	-0.90**	0.90**	-0.10 ^{ns}	0.22 ^{ns}
5. chlorophyll fluorescence					1	-0.16 ^{ns}	-0.81**	-0.83**	-0.83**	0.86**	-0.04 ^{ns}	0.35*
6. Proline						1	0.27 ^{ns}	0.38*	0.21 ^{ns}	-0.38*	0.98 ^{ns}	-0.25 ^{ns}
7. H ₂ O ₂							1	0.94**	0.91**	-0.88**	0.06 ^{ns}	-0.24 ^{ns}
8. MDA								1	0.90**	-0.93**	-0.03 ^{ns}	-0.31*
9-EL									1	-0.86**	-0.03 ^{ns}	-0.37*
10. Activity of CAT										1	-0.05 ^{ns}	0.22 ^{ns}
11. Activity of APX											1	0.36*
12. Activity of SOD												1

*, ** and ns: significant at 0.05 and 0.01 probability levels and no significant, respectively

These results confirm the effectiveness of VCL in stimulating antioxidant mechanisms in plants.

Conclusion

The results of the present research strongly proved that foliar application of VCL improved growth parameters and physiological factors, enabling VCL-treated pomegranate seedlings to perform better. Therefore, application of VCL is recommended under salt-stress conditions.

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