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IRRIGATION WATER QUALITY AND ANTIOXIDANTS EFFECTS ON THE CHEMICAL COMPOSITION OF SWEET ORANGE

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SUMMARY

The existent research aimed to study the effects of irrigation with liquefied water, saline-well water, and foliar application of aspartic and ascorbic acids on the chemical properties of grafted orange seedlings in the years 2020–2021 at the Horticulture and Forestry Division, Najaf Agriculture Directorate, Iraq. The main plot was the irrigation water (liquefied and saline-well water). Meanwhile, foliar application of aspartic acid (0, 100, and 150 $mg.L^{-1}$) and ascorbic acid (0, 4000 $mg.L^{-1}$) served as the second and third factors in subplots. Compared with the saline-well water, regular liquefied water had a significant positive impact on the improvement of chemical traits, i.e., the liquefied water attained the highest rate of nitrogen content in leaves (2.600%) compared with the saline-well water (2.239%). Ascorbic acid (4000 $mg.L^{-1}$) also had a significant effect on the leaf's contents, providing the highest percentage of phosphorus (0.4060%) and reduced sodium (0.5277%) compared with the control in the leaves of orange seedlings. The saline-well water with no addition of ascorbic acid (control) provided the highest average content of the amino acid proline (132.2 $\mu g.g^{-1}$ fresh weight) in the sweet orange leaves compared with the other treatments and their interactions. Sweet orange seedlings with foliar application of aspartic and ascorbic acids authenticated that the peroxidase activity rate appeared more effective than all other treatments and the control.

Keywords: Sweet orange transplants, liquefied water, saline-well water, aspartic and ascorbic acids

Key findings: Salinity impacts agriculture, thus the need to induce crop plants with salt tolerance to sustain their economic output. Antioxidants, such as, aspartic and ascorbic acids, have auxinic action and a synergistic effect on salinity tolerance and plant growth.

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INTRODUCTION

Salinity is a growing environmental issue that has a significant impact on agriculture. The amount of salt-affected land on the planet measures 953 million ha, 7% of the total land mass and 20% of the irrigated area (Abdelfattah *et al.*, 2009). Hyperosmolarity, ion disequilibrium, nutritional imbalance, and the generation of reactive oxygen species (ROS) in soil result in the retardation of plant growth due to molecular damage (Nawaz *et al.,* 2010). Therefore, inducing crop plants to tolerate salt requires focus to sustain their economic output. Genetic changes in the host plant or pharmacologic therapies can

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accomplish this (Hamdia and Shaddad, 2010).

During stressful conditions, the endogenous levels of growth regulators were depleted, which exogenous treatment may remedy. Utilizing plant growth regulators, fertilizers, and non-enzymatic antioxidants successfully reduced the harmful effects of salinity on plant growth and output (Kaya *et al*., 2010). Antioxidants, such as ascorbic and citric acids, have auxinic action and a synergistic effect on fruit tree flowering and fruiting. Recently, employing antioxidants instead of auxins and other chemicals improved the growth and fruiting of various fruit trees (Ragab, 2002). Ascorbic acid is one of the most efficient growth regulators against abiotic stressors (Conklin, 2001; Abd-El-Aziz *et al*., 2006). Not only an antioxidant, but ascorbic acid at the cellular level also contribute to activating sophisticated biological defense mechanisms (Conklin and Barth, 2004).

In several crop plants, the widely used ascorbic acid alleviated the adverse effects of salt stress, and in plant metabolism, it has hypothesized functions (Khan *et al*., 2010). Aspartate is a precursor for the biosynthesis of multiple biomolecules required for plant growth and defense mechanisms, including nicotinamide adenine dinucleotide (NAD), amino acids, organic acids, nucleotides, and their liquefied, as well as, constituting proteins and being an active residue in many enzymes (Ali *et al.,* 2019 ; Han *et al.*, 2021). Four out of eight essential amino acids, i.e., methionine (Met), threonine (Thr), lysine (Lys), and isoleucine (Ile), are generated from aspartic via a route known as the Asp family amino acids pathway (Li *et al*., 2017). The exchange and competition for aspartic and derived intermediates substantially affected plant metabolism, which needs thorough study (Han *et al.*, 2021).

Several past studies have found a link between variations in aspartic acid concentration and plant stress conditions. During alkaline salt stress conditions, wild soybean seedlings showed a high increase (3.97-fold) in aspartic and other liquefied, such as proline (Pro), glutamine (Glu), serine (Ser), and alanine (Ala) compared with semi-wild and farmed soybean seedlings. The level of aspartic acid increased 11-fold in the roots and around 6.2-fold in the shoots of *Aeluropus lagopoides* in response to 250 mM NaCl salt stress (Shahzad *et al*., 2017). Under salty circumstances, a sizable accumulation of Asp and other amino acids, such as Pro, has played an essential function in plants by maintaining intracellular osmotic potential and liquefied membranes for proteins (Hayat *et al*., 2012).

Furthermore, the observed changes in Asp content showed linkages to changes in protein metabolism in salt-stressed plants (El-Shintinawy and El-Shourbagy, 2001). Ascorbic acid is a natural and organic antioxidant chemical (Hafez *et al*., 2010), as well as, a vital compound for plant tissues because of its antioxidant properties and ability to operate as a co-enzyme in an enzymatic cofactor and plant growth regulator (Gomez and Lajolo, 2008). Given its effects on cell division and differentiation, ascorbic acid is a plant growth and development regulator involved in a wide range of vital processes, including antioxidant defense, UV protection, photosynthesis regulation, and growth regulation (Kareem *et al.*, 2022).

Plants that get amino acids ably withstand harsh climatic circumstances, such as drought, salt, and heavy metal toxicity (Rai, 2002). Aspartate is the precursor to several amino acids in plants, including threonine, methionine, and isoleucine (Rawia *et al*., 2011). Employing the three amino acids—glutamic acid, aspartic acid, and alanine—helps in the creation of bio-stimulant compounds (Colla *et al*., 2015). Adding these amino acids can become part of the raw ingredients from hydrolyzed proteins. In plants, a transamination event between glutamate and oxaloacetate produced aspartic acid. Then, it gets liquefied to create the amino acids lysine, threonine, methionine, and isoleucine via the aspartic acid liquefied pathway. Plants getting these three amino acids better withstand severe climatic circumstances, such as drought, salinity, and toxicity of heavy metals (Rai, 2002). The problem of irrigating citrus seedlings grown in various research stations and nurseries with saline well water is one of the determinants of the growth, reproduction, and spread of citrus in Iraq. As a result of the above discussion, the immediate study aimed to determine the impact of irrigation water quality and foliar application of antioxidants (ascorbic and aspartic acids) on the chemical composition of the budded sweet orange seedlings.

MATERIALS AND METHODS

The local citrus of homogeneous age and size, selected at the Citrus Production Nursery, belonged to the General Directorate of Horticulture and Forests, Holy Karbala

Governorate, Iraq, which served as original stocks and grafted in autumn with local sweet orange buds as a graft in May 2020. For the three factors effect determination, the grafted orange seedlings proceeded to transport and distribution in the canopy of the Horticulture and Forestry Division, Najaf Agriculture Directorate, Iraq. The irrigation water consisted of two types (regular liquefied water and saline well water) in the main plots. Meanwhile, the second and third factors comprised the foliar application of aspartic acid at a concentration (0, 100, and 150 mg L^{-1}) and ascorbic acid at a concentration (0 and 4000 mg L^{-1}) as subplots. Irrigating the orange seedlings with the quality factor of irrigation water started on 1 May 2020. Likewise, the foliar application of both aspartic and ascorbic acids by spraying occurred every first of April to September 2021. The results of some vegetative and chemical traits studied in the orange grafts seedlings grown in the original underwent statistical analysis according to ANOVA using the statistical program Genstat Ver. 2012.

Chemical characteristics

The chemical composition of various elements studied in sweet orange leaves consisted as follows:

Nitrogen (N) content (%)

In the sweet orange leaves, the nitrogen (N) estimation used the Micro Kjeldahl apparatus by taking 10 ml of each sample and adding 10 ml of sodium hydroxide. At 40% concentration, the distillation continued, with the released ammonia collected in a glass beaker containing 20 ml of 2% boric acid with a mixture of Methyl Red and Bromocresol Green. Total nitrogen calculation used the following equation:

$$\frac{\text{The volume of acid consumed by grinding} \times \text{acidity standard} \times 14 \times \text{dilution volume} \times 100}{\text{The volume of a sample taken at distillation} \times \text{weight of digested sample} \times 1000}$$

Phosphorus (P) content (%)

The phosphorus content in the sweet orange leaves estimation used the formula of ammonium molybdate and ascorbic acid according to the method of Self-Davis *et al*. (2000).

Sodium (Na) content (%)

In the sweet orange leaves, the sodium element estimation used an atomic absorption spectrophotometer with the standard sodium curve as the basis to show the results.

Magnesium (Mg) content (%)

Estimating the magnesium content in the sweet orange leaves ensued by grinding, with the digested sample transferred to a volumetric flask of 100 ml capacity and completed to the mark with distilled water. A 25 ml digestion solution was taken and placed in a standard flask of 100 ml capacity, with 5 ml of 5% iron chloride added. Mixing standard sodium followed until it changed color and became turbid brown, with no observed precipitate. The process continued by adding 20 ml of standard acid solution (Standard Acetate pH 4.63), then placing the sample in a water bath for 15 min shaking from time to time, then cooling and completing to the mark. Filtering through ordinary filter paper, magnesium estimation progressed by taking 25 ml of the final filtrate and adding 10 ml of NaOH with a small amount of peroxide in a 300 ml conical flask, then flattened with EDTA-Na2 (0.5 N) until its color changed to brown. The computation followed using the succeeding equation.

$$\text{Magnesium (\%)} = 100 \times (100 \times 100 \times 100 \times h) / (1000 \times 25 \times 25 \times y),$$

Where h = Mg (mg) for 25 ml of sample and y = weight of the sample.

Chloride ions (%)

Estimating chloride content in the digested and diluted leaf samples of sweet orange employed titration and smearing with silver nitrate (0.05 N) using the potassium chromate index as described in Kalra and Maynard, 1991.

Proline content

In the sweet orange leaves, the proline content measurement used a light spectrum meter as follows: Pouring in 1.5 mL hermetically sealed test tubes 1000 µl of the reaction solution (ninhydrin 1% [w/v] in 60% [v/v] acetic acid and 20% [v/v] ethanol), then adding 500 µl of plant extract (100 µl of standard proline to

contain different dilutions of standard proline [0.2, 0.5, 1, 2.5 mmol of proline]) drew the proline curve (Fig. 8.3). In completing the volume added 400 µl using Ethanol: water (40:60 v/v). Closing the pipes, mixing, and heating them at 95 °C in a water bath went on for 20 min. Using a centrifuge (1 min, 10,000 rpm) removes a precipitate. Afterward, the transfer of the candidate to a 1.5 ml glass cell went on to read at a wavelength of 520 nm. The following equation calculated the amount of proline in extracts:

Proline (nmol.mg-1 FW or in µmol.g-1 FW) = (Abstract - blank)/slope × Vol extract / Vol aliquot × 1/FW

Where:
FW = Fresh weight and
Abstract - blank = (excluded - blank).

Total activity of peroxidase enzyme - POX

Picking fresh sweet orange leaves took place in the early morning after six months of the experiment on 1 November 2021. Then placing in transparent polyethylene bags, keeping the bags in a cool cork box containing ice to preserve the samples from wilting before transferring them directly to the freezer at -18 °C until analyses. Materials and solutions used: 1- Guaicaol solution: Prepared by mixing 1.36 ml of guaiacol in a volumetric flask, then completing the volume with 250 ml of distilled water; Hydrogen peroxide solution H_2O_2 at a concentration of 0.1%: Prepared a volume of 0.4 ml of 30% H_2O_2 and completing the volume to 120 ml of distilled water; The method of work: Mix 1 ml of H_2O_2 + 1 ml of Guaicaol, then placing with the samples in a centrifuge at a rotation speed of 12000 rpm for 15 min. The absorbance reading used a spectrophotometer with a wavelength of 240 nm. The enzymatic activity estimation occurred by adding 2 ml of the reaction mixture to the spectrophotometer cell, then adding 0.1 ml of the sample, where the change in light absorption was followed every 30 sec for 3 min at a wavelength of 240 nm.

RESULTS

Nitrogen (%) in the leaves

Results confirmed that the irrigation water quality positively affected the percentage of nitrogen in the leaves of orange seedlings (Table 1). The treatment of regular liquefied water recorded the highest nitrogen content in the orange leaves (2.600%) compared with the saline well water treatment (2.239%). The foliar application of two other factors (whether aspartic and ascorbic acids spraying on the orange seedlings individually or with interaction [combined], bilateral with each other or with irrigation water, and/or combined in a triple interaction manner) gave no relevant effect on the percentage of nitrogen in sweet orange seedling leaves.

Phosphorus (%) in leaves

The percentage of phosphorus in the leaves of sweet orange seedlings with normal liquefied water and saline irrigation water did not have a significant effect (Table 2). Also, the aspartic acid concentrations did not have a significant impact on the percentage of phosphorus in the leaves of the orange seedlings. However, foliar application of ascorbic acid to the orange leaves had a significant effect on the percentage of phosphorus. The ascorbic acid at a concentration of 4000 mg. L^{-1} gave the leaves an increase in the percentage of phosphorus that reached 0.406% compared with the control treatment (0.338%). The bilateral interaction between irrigation water quality and aspartic acid concentrations revealed an insignificant impact on the percentage of phosphorus in the leaves of orange seedlings. Although, the interaction between irrigation water quality and ascorbic acid positively affected the phosphorus content. The sweet orange seedlings with combination of saline well water and ascorbic acid at a concentration of 4000 mg. L^{-1} displayed significant superiority for the phosphorus content (0.420%) compared with the treatment with saline well water alone (0.291%). The bilateral interaction between aspartic and ascorbic acids also exhibited a nonsignificant effect on the phosphorus content in the leaves of sweet orange seedlings. Likewise, the triple interaction of the three experimental factors proved insignificant on the phosphorus percentage in the leaves of sweet orange seedlings.

Sodium (%) in the leaves

The irrigation water quality significantly affected the percentage of sodium in the leaves of sweet oranges (Table 3). The saline well water irrigation recorded the highest rate of sodium element (0.5777%) compared with the normal liquefied water (0.5324%) in the sweet orange leaves. Aspartic acid individually had a

Table 1. Effect of regular liquefied water and saline well water in addition to aspartic and ascorbic acids on the average Nitrogen (%) in the leaves of grafted sweet orange seedlings.

Average of Irrigation Water Quality	Ascorbic Acid Concentration		Aspartic Acid Concentration	Irrigation Water Quality
	mg.L^{-1}4000	0 mg.L^{-1}		
2.600	2.680	2.497	0 mg.L^{-1}	Liquefied irrigation water
	2.397	2.748	100 mg.L^{-1}	
	2.665	2.613	150 mg.L^{-1}	
2.239	2.281	2.217	0 mg.L^{-1}	Well irrigation water
	2.190	2.315	100 mg.L^{-1}	
	2.262	2.169	150 mg.L^{-1}	
0.0807= $LSD_{0.05}$ Irrigation Water Quality	2.413	2.426	Average of ascorbic acid	
$LSD_{0.05}$ Aspartic × Ascorbic × Irrigation Water Quality = N.S				
Average of Irrigation Water Quality	Aspartic Acid Concentration			Irrigation Water Quality
	150 mg.L^{-1}	100 mg.L^{-1}	0 mg.L^{-1}	
2.600	2.639	2.573	2.589	Liquefied irrigation water
2.239	2.215	2.252	2.249	Well irrigation water
	2.427	2.412	2.419	Average of aspartic acid
$LSD_{0.05}$ Irrigation Water Quality × Aspartic Acid = N.S.				
Average of Irrigation Water Quality	Ascorbic Acid Concentration		Irrigation Water Quality	
	mg.L^{-1}4000	0 mg.L^{-1}		
2.600	2.581	2.619	Liquefied irrigation water	
2.239	2.244	2.234	Well irrigation water	
	2.413	2.426	Average of ascorbic acid	
$LSD_{0.05}$ Irrigation Water Quality × Ascorbic Acid = N.S.				
Average of Aspartic acid	Ascorbic Acid Concentration		Aspartic Acid Concentration	
	mg.L^{-1}4000	0 mg.L^{-1}		
2.419	2.481	2.357	0 mg.L^{-1}	
2.412	2.294	2.531	100 mg.L^{-1}	
2.427	2.464	2.391	150 mg.L^{-1}	
$LSD_{0.05}$ Aspartic acid = N.S.	2.413	2.426	Average of Ascorbic acid	
$LSD_{0.05}$ Ascorbic acid = N.S.				
N.S. =Aspartic*Ascorbic $LSD_{0.05}$				

Table 2. Effect of regular liquefied water and saline well water in addition to aspartic and ascorbic acids on the average percentage of phosphorus (P) in the leaves of grafted sweet orange seedlings.

Average of Irrigation Water Quality	Ascorbic Acid Concentration		Aspartic Acid Concentration	Irrigation Water Quality
	mg.L^{-1}4000	0 mg.L^{-1}		
0.389	0.418	0.369	0 mg.L^{-1}	Liquefied irrigation water
	0.355	0.409	100 mg.L^{-1}	
	0.406	0.376	150 mg.L^{-1}	
0.356	0.383	0.278	0 mg.L^{-1}	Well irrigation water
	0.436	0.354	100 mg.L^{-1}	
	0.441	0.242	150 mg.L^{-1}	
N.S. = Irrigation Water Quality $LSD_{0.05}$	0.406	0.338	Average of Ascorbic acid	
$LSD_{0.05}$ Aspartic × Ascorbic × Irrigation Water Quality = N.S.				
Average of Irrigation Water Quality	Aspartic Acid Concentration			Irrigation Water Quality
	150 mg.L^{-1}	100 mg.L^{-1}	0 mg.L^{-1}	
0.389	0.391	0.382	0.394	Liquefied irrigation water
0.356	0.341	0.395	0.330	Well irrigation water
	0.366	0.388	0.362	Average of Aspartic acid
$LSD_{0.05}$ Irrigation Water Quality × Aspartic Acid = N.S.				
Average of Irrigation Water Quality	Ascorbic acid concentration		Irrigation Water Quality	
	mg.L^{-1}4000	0 mg.L^{-1}		
0.389	0.393	0.385	Liquefied irrigation water	
0.356	0.420	0.291	Well irrigation water	
	0.406	0.338	Average of Ascorbic acid	
$LSD_{0.05}$ Irrigation Water Quality × Ascorbic Acid = 0.0955				
Average of Aspartic acid	Ascorbic acid concentration		Aspartic acid concentration	
	mg.L^{-1}4000	0 mg.L^{-1}		
0.362	0.400	0.324	0 mg.L^{-1}	
0.388	0.395	0.382	100 mg.L^{-1}	
0.366	0.424	0.309	150 mg.L^{-1}	
$LSD_{0.05}$ Aspartic acid = N.S.	0.406	0.338	Average of Ascorbic acid	
$LSD_{0.05}$ Ascorbic acid = 0.045				
N.S.=Aspartic×Ascorbic $LSD_{0.05}$				

Table 3. Effect of regular liquefied water and saline well water in addition to aspartic and ascorbic acids on the average percentage of sodium (Na) in the leaves of grafted sweet orange seedlings.

Average of Irrigation Water Quality	Ascorbic Acid Concentration		Aspartic Acid Concentration	Irrigation Water Quality
	$mg.L^{-1}$4000	0 $mg.L^{-1}$		
0.5324	0.5453	0.5053	0 $mg.L^{-1}$	Liquefied irrigation water
	0.5307	0.5013	100 $mg.L^{-1}$	
	0.5767	0.5353	150 $mg.L^{-1}$	
0.5777	0.5113	0.9287	0 $mg.L^{-1}$	Well irrigation water
	0.5120	0.5067	100 $mg.L^{-1}$	
	0.4900	0.5173	150 $mg.L^{-1}$	
0.03347= Irrigation Water Quality $LSD_{0.05}$	0.5277	0.5824	Average of Ascorbic acid	

$LSD_{0.05}$ Aspartic × Ascorbic × Irrigation Water Quality = 0.04487

Average of Irrigation Water Quality	Aspartic Acid Concentration			Irrigation Water Quality
	150 $mg.L^{-1}$	100 $mg.L^{-1}$	0 $mg.L^{-1}$	
0.5324	0.5560	0.5160	0.5253	Liquefied irrigation water
0.5777	0.5037	0.5093	0.7200	Well irrigation water
	0.5298	0.5127	0.6227	Average of Aspartic acid

$LSD_{0.05}$ Irrigation Water Quality × Aspartic Acid = 0.02744

Average of Irrigation Water Quality	Ascorbic Acid Concentration		Irrigation Water Quality
	$mg.L^{-1}$4000	0 $mg.L^{-1}$	
0.5324	0.5509	0.5140	Liquefied irrigation water
0.5777	0.5044	0.6509	Well irrigation water
	0.5277	0.5824	Average of Ascorbic acid

$LSD_{0.05}$ Irrigation Water Quality × Ascorbic Acid = 0.02882

Average of Aspartic acid	Ascorbic Acid Concentration		Aspartic Acid Concentration
	$mg.L^{-1}$4000	0 $mg.L^{-1}$	
0.6227	0.5283	0.7170	0 $mg.L^{-1}$
0.5127	0.5213	0.5040	100 $mg.L^{-1}$
0.5298	0.5333	0.5263	150 $mg.L^{-1}$
$LSD_{0.05}$ Aspartic acid = 0.01748	0.5277	0.5824	Average of Ascorbic acid
$LSD_{0.05}$ Ascorbic acid = 0.02256			

0.03098 =Aspartic × Ascorbic $LSD_{0.05}$

significant effect on the sodium content in the leaves of treated orange seedlings with the spraying of 100 mg. L^{-1}, revealing the lowest possible percentage of sodium (0.5127%) compared with the control with a high sodium percentage (0.6227%) in the sweet orange leaves. However, the aspartic acid spray treatment at a concentration of 150 $mg.L^{-1}$ mediated the highest and lowest values of sodium content in the leaves. The foliar application of ascorbic acid also revealed a significant impact on the percentage of sodium in the leaves, with the control treatment giving the highest sodium percentage (0.5824%) compared with the ascorbic acid concentration of 4000 mg. L^{-1}, effectively reducing the sodium percentage (0.5277%) in the leaves of orange seedlings.

The effect of the bilateral interaction between irrigation water quality and aspartic acid became a concern for its significant outcome. The treatment of saline well water with control of aspartic acid (0 $mg.L^{-1}$) had the highest percentage of sodium (0.7200%) (Table 3). The saline well water and aspartic spray at a concentration of 150 $mg.L^{-1}$ gave a significantly low value for the sodium percentage (0.5037%) in the sweet orange leaves. The interaction between irrigation water quality and ascorbic acid concentrations also resulted as significant, with saline well water and ascorbic acid (0 mg. L^{-1}) providing the highest sodium percentage (0.6509%) in the orange leaves. Ascorbic at a concentration of 4000 $mg.L^{-1}$ with saline well water also significantly reduced the percentage of sodium in the leaves (0.5044%). The interaction between the three experimental factors was feasible in reducing sodium in the orange leaves. The control treatments of both acids with saline water irrigation had the highest percentage of sodium (0.9287%), and the saline well water with foliar application of aspartic acid (150 $mg.L^{-1}$) and ascorbic acid (4000 $mg.L^{-1}$) significantly reduced the sodium percentage (0.4900%) in the grafted sweet orange leaves.

Magnesium (%) in leaves

The irrigation water treatments revealed a significant effect on the magnesium content in the leaves of orange seedlings (Table 4). The normal liquefied water recorded the highest percentage of magnesium in the leaves (1.247%) compared with the treatment of seedlings with saline well water irrigation, which scored less magnesium (1.018%). The

Table 4. Effect of regular liquefied water and saline well water in addition to aspartic and ascorbic acids on the average percentage of magnesium (Mg) in the leaves of grafted sweet orange seedlings.

Average of Irrigation Water Quality	Ascorbic Acid Concentration		Aspartic Acid Concentration	Irrigation Water Quality
	$mg.L^{-1}$4000	0 $mg.L^{-1}$		
1.237	1.158	1.019	0 $mg.L^{-1}$	Liquefied irrigation water
	1.353	1.176	100 $mg.L^{-1}$	
	1.544	1.174	150 $mg.L^{-1}$	
1.018	1.205	0.540	0 $mg.L^{-1}$	Well irrigation water
	1.133	0.908	100 $mg.L^{-1}$	
	1.287	1.037	150 $mg.L^{-1}$	
0.091 = $LSD_{0.05}$ Irrigation water quality	1.280	0.976	Average of Ascorbic acid	

$LSD_{0.05}$ Aspartic × Ascorbic × Irrigation Water Quality = 0.123

Average of Irrigation Water Quality	Aspartic Acid Concentration			Irrigation Water Quality
	150 $mg.L^{-1}$	100 $mg.L^{-1}$	0 $mg.L^{-1}$	
1.237	1.359	1.264	1.089	Liquefied irrigation water
1.018	1.162	1.020	0.872	Well irrigation water
	1.260	1.142	0.980	Average of Aspartic acid

$LSD_{0.05}$ Irrigation Water Quality × Aspartic Acid = N.S.

Average of Irrigation Water Quality	Ascorbic Acid Concentration		Irrigation Water Quality
	$mg.L^{-1}$4000	0 $mg.L^{-1}$	
1.237	1.352	1.123	Liquefied irrigation water
1.018	1.208	0.828	Well irrigation water
1.260	1.280	0.976	Average of Ascorbic acid

$LSD_{0.05}$ Irrigation Water Quality × Ascorbic Acid = 0.075

Average of Aspartic acid	Ascorbic Acid Concentration		Aspartic Acid Concentration
	$mg.L^{-1}$4000	0 $mg.L^{-1}$	
0.980	1.181	0.779	0 $mg.L^{-1}$
1.142	1.243	1.042	100 $mg.L^{-1}$
1.260	1.415	1.105	150 $mg.L^{-1}$
$LSD_{0.05}$ Aspartic acid = 0.068	1.280	0.976	Average of Ascorbic acid
$LSD_{0.05}$ Ascorbic acid =0.055			
0.089 =Aspartic × Ascorbic $LSD_{0.05}$			

aspartic acid application also significantly impacted the magnesium percentage in the leaves of orange seedlings, with aspartic acid at a concentration of 150mg. L^{-1} increased magnesium (1.260%) compared with the control treatment, which showed the lowest value (0.980%). The ascorbic acid also had a significant effect on the magnesium content in the orange leaves with a concentration of 4000 $mg.L^{-1}$ showing an increase in magnesium percentage (1.280%) as compared with the average value of ascorbic acid treatments (0.976%) and control (0.779%).

The bilateral interaction between the two types of irrigation water and spraying with ascorbic acid provided a significant effect (Table 4). The ordinary liquefied water with ascorbic acid at a concentration of 4000 $mg.L^{-1}$ led to the highest average percentage of magnesium (1.352%) compared with saline well water and ascorbic acid control (0 mg. L-1), which recorded a significant decrease in the magnesium percentage (0.828%). The triple interaction between the irrigation water quality and both acids significantly affected the percent magnesium. The normal liquefaction of water with aspartic acid at a concentration of 150 $mg.L^{-1}$ and ascorbic acid at a concentration of 4000 $mg.L^{-1}$ recorded the highest percentage of magnesium (1.544%) compared with the lowest value (0.540%) obtained in the interaction of saline well water with control treatments of both acids in the leaves of sweet orange seedlings.

Chloride (%) in leaves

The irrigation water quality remarkably affected the percentage of chloride in the leaves of sweet orange seedlings (Table 5). Irrigation with saline well water delivered the highest percent chloride (1.023%) compared with irrigation with normal liquefied water, which gave a record lowest value (0.500%). The foliar application of aspartic and ascorbic acids individually and the control treatment (no spray) of aspartic acid led to the highest percentage of chloride (0.951%) compared with the lowest value (0.656%) obtained with the aspartic acid application at a concentration of 150 $mg.L^{-1}$ in the leaves of sweet orange. Ascorbic acid also contributed effectively in reducing the percentage of chloride (0.484%) compared with the control treatment, which recorded the highest value (1.039%) in the sweet orange leaves.

Table 5. Effect of regular liquefied water and saline well water in addition to aspartic and ascorbic acids on the average percentage of chloride in the leaves of grafted sweet orange seedlings.

Average of Irrigation Water Quality	Ascorbic Acid Concentration		Aspartic Acid Concentration	Irrigation Water Quality
	$mg.L^{-1}$4000	0 $mg.L^{-1}$		
0.500	0.240	0.883	0 $mg.L^{-1}$	Liquefied irrigation water
	0.203	0.619	100 $mg.L^{-1}$	
	0.386	0.667	150 $mg.L^{-1}$	
1.023	0.742	1.938	0 $mg.L^{-1}$	Well irrigation water
	0.722	1.164	100 $mg.L^{-1}$	
	0.608	0.964	150 $mg.L^{-1}$	
0.121Irrigation Water Quality= $LSD_{0.05}$	0.484	1.039	Average of Ascorbic acid	
$LSD_{0.05}$ Aspartic × Ascorbic × Irrigation Water Quality = 0.115				

Average of Irrigation Water Quality	Aspartic Acid Concentration			Irrigation Water Quality
	150 $mg.L^{-1}$	100 $mg.L^{-1}$	0 $mg.L^{-1}$	
0.500	0.527	0.411	0.562	Liquefied irrigation water
1.023	0.786	0.943	1.340	Well irrigation water
	0.656	0.677	0.951	Average of Aspartic acid
$LSD_{0.05}$ Irrigation Water Quality × Aspartic Acid = 0.093				

Average of Irrigation Water Quality	Ascorbic Acid Concentration		Irrigation Water Quality
	$mg.L^{-1}$4000	0 $mg.L^{-1}$	
0.500	0.277	0.723	Liquefied irrigation water
1.023	0.691	1.355	Well irrigation water
	0.484	1.039	Average of Ascorbic acid
$LSD_{0.05}$ Irrigation Water Quality × Ascorbic Acid = 0.093			

Average of Aspartic acid	Ascorbic Acid Concentration		Aspartic Acid Concentration
	$mg.L^{-1}$4000	0 $mg.L^{-1}$	
0.951	0.491	1.410	0 $mg.L^{-1}$
0.677	0.463	0.891	100 $mg.L^{-1}$
0.656	0.497	0.816	150 $mg.L^{-1}$
$LSD_{0.05}$ Aspartic acid = 0.041	0.484	1.039	Average of Ascorbic acid
$LSD_{0.05}$ Ascorbic acid = 0.052			
0.071=Aspartic × Ascorbic $LSD_{0.05}$			

The bilateral interaction between the irrigation water quality and aspartic acid positively influenced the chloride percentage in the orange leaves (Table 5). The saline well water and aspartic acid control (0 $mg.L^{-1}$) gave the highest percentage of chloride (1.340%) compared with the liquefied water. The regular liquefied water and aspartic (100 $mg.L^{-1}$) provided the lowest percent chloride (0.411%). The interaction between irrigation water and spraying with ascorbic acid had a relevant role in reducing the percentage of chloride, and the irrigation with saline well water and ascorbic control (0 $mg.L^{-1}$) gave the highest percentage of chloride in the leaves (1.355%). The irrigation with normal liquefied water and the ascorbic acid (4000 $mg.L^{-1}$) reduced the percent chloride (0.277%). The interaction between aspartic acid and ascorbic acid displayed a crucial role in reducing the percentage of chloride compared with the control. Ascorbic acid (0 $mg.L^{-1}$) gave the highest percentage of chloride in leaves (1.410%), whereas aspartic acid (100 $mg.L^{-1}$) and ascorbic acid (4000 $mg.L^{-1}$) provided the lowest chloride percentage (0.463%). The triple interaction between the three experimental factors positively affected the chloride percentage in the sweet orange leaves, as shown by the saline well water and control treatments of both acids providing the highest percent chloride (1.938%). However, the liquefied water, aspartic acid (100 $mg.L^{-1}$), and ascorbic acid (4000 $mg.L^{-1}$) revealed the lowest chloride percent content in the orange leaves (0.203%).

Proline content in leaf

The three factors applied to the orange seedlings showed significant differences in proline content in the leaves (Table 6). Irrigation with saline water obtained the highest value of proline content (108.5 $\mu g.g^{-1}$ fresh weight) compared with the normal liquefied water, which recorded the lowest value (75.3 $\mu g.g^{-1}$ fresh weight). Aspartic acid provided a nonsignificant effect on the proline acid, whether applied individually or in interaction with other experimental factors. However, ascorbic acid scored superiority by reducing the proline content and the effects of salt water, compared with the control treatment of ascorbic acid (0 $mg.L^{-1}$), which significantly enhanced the proline content in the leaves of the sweet orange seedlings. Also,

Table 6. Effect of regular liquefied water and saline well water in addition to aspartic and ascorbic acids on the average content of proline (µg.gm^{-1} fresh weight) in the leaves of grafted sweet orange seedlings.

Average of Irrigation Water Quality	Ascorbic Acid Concentration		Aspartic Acid Concentration	Irrigation Water Quality
	mg.L^{-1}4000	0 mg.L^{-1}		
75.3	75.8	82.5	0 mg.L^{-1}	Liquefied irrigation water
	65.2	83.6	100 mg.L^{-1}	
	72.0	72.8	150 mg.L^{-1}	
108.5	93.8	148.5	0 mg.L^{-1}	Well irrigation water
	80.7	127.7	100 mg.L^{-1}	
	79.9	120.5	150 mg.L^{-1}	
18.86 = Irrigation Water Quality $LSD_{0.05}$	77.9	105.9	Average of Ascorbic acid	
$LSD_{0.05}$ Aspartic × Ascorbic × Irrigation Water Quality = N.S.				

Average of Irrigation Water Quality	Aspartic Acid Concentration			Irrigation Water Quality
	150 mg.L^{-1}	100 mg.L^{-1}	0 mg.L^{-1}	
75.3	72.4	74.4	79.2	Liquefied irrigation water
108.5	100.2	104.2	121.1	Well irrigation water
	86.3	89.3	100.1	Average of Aspartic acid
$LSD_{0.05}$ Irrigation Water Quality × Aspartic = N.S.				

Average of Irrigation Water Quality	Ascorbic Acid Concentration		Irrigation Water Quality
	Mg.L^{-1}4000	0 mg.L^{-1}	
75.3	71.0	79.7	Liquefied irrigation water
108.5	84.8	132.2	Well irrigation water
	77.9	105.9	Average of Ascorbic acid
$LSD_{0.05}$ Irrigation Water Quality × Ascorbic Acid = 14.58			

Average of Aspartic acid	Ascorbic Acid Concentration		Aspartic Acid Concentration
	mg.L^{-1}4000	0 mg.L^{-1}	
100.1	84.8	115.5	0 mg.L^{-1}
89.3	72.9	105.7	100 mg.L^{-1}
86.3	75.9	96.7	150 mg.L^{-1}
$LSD_{0.05}$ Aspartic acid = N.S.	77.9	105.9	Average of Ascorbic acid
$LSD_{0.05}$ Ascorbic acid = 9.02			
N.S.=Aspartic × Ascorbic $LSD_{0.05}$			

the saline well water with ascorbic acid (4000 mg.L^{-1}) decreased the proline content (84.8 µg.g^{-1} fresh weight).

Peroxidase activity rate

The quality of the water used in the irrigation of orange seedlings had a significant effect on the activity of the peroxidase enzyme (Table 7). The peroxidase enzyme activity exhibited high when orange seedlings received saline water (8.34 IU.g^{-1} fresh weight) compared with the liquefied water (5.41 IU.g^{-1} fresh weight). Similarly, ascorbic acid significantly affected the peroxidase enzyme activity in orange leaves. The influence of the control treatment of ascorbic acid (0 mg. L^{-1}) resulted in a high rate of peroxidase enzyme activity (7.79 IU.g^{-1} fresh weight) in the orange leaves. On the other hand, the ascorbic acid (4000 mg.L^{-1}) significantly reduced the rate of peroxidase enzyme activity (5.96 IU.g^{-1} fresh weight)in sweet orange leaves. Ascorbic acid and its interaction with the irrigation water quality established significant reactions. The saline well water with the ascorbic acid control treatment influenced the highest activity rate of peroxidase enzyme (9.68 IU.g^{-1} fresh weight). In comparison, the liquefied water and ascorbic (4000 mg.L^{-1}) caused a reduction in the activity rate of peroxidase enzyme (4.91 IU.g^{-1} fresh weight) in the sweet orange leaves.

DISCUSSION

The study discovered that foliar application of ascorbic acid positively affected the chemical composition of grafted local orange seedlings, with a significant increase in crucial chemical characteristics (nitrogen and phosphorus). It can refer to ascorbic acid's role in increasing the chlorophyll content in leaves and its function as a co-enzyme for several enzymes responsible for the metabolism of carbohydrates and proteins, as well as, regulation of cell division and expansion processes (Blokhina *et al*., 2003; Rawia *et al*., 2011). The low percentage of nitrogen results from the salinity effects on all vital activities in plants, such as, photosynthesis, transport of liquefied substances, absorption, and transport across cell membranes, which weakens the ability of the plant to accumulate nutrients (Han *et al*., 2021).

Table 7. Effect of regular liquefied water and saline well water in addition to aspartic and ascorbic acids on the average peroxidase activity in the leaves of grafted sweet orange seedlings.

Average of Irrigation Water Quality	Ascorbic Acid Concentration		Aspartic Acid Concentration	Irrigation Water Quality
	mg.L^{-1}4000	0 mg.L^{-1}		
5.41	5.33	4.92	0 mg.L^{-1}	Liquefied irrigation water
	4.71	6.06	100 mg.L^{-1}	
	4.69	6.73	150 mg.L^{-1}	
8.34	7.57	12.67	0 mg.L^{-1}	Well irrigation water
	7.28	8.47	100 mg.L^{-1}	
	6.17	7.90	150 mg.L^{-1}	
2.586 = Irrigation Water Quality $LSD_{0.05}$	5.96	7.79	Average of Ascorbic acid	
$LSD_{0.05}$ Aspartic × Ascorbic × Irrigation Water Quality = 2.134				
Average of Irrigation Water Quality	Aspartic Acid Concentration			Irrigation Water Quality
	150 mg.L^{-1}	100 mg.L^{-1}	0 mg.L^{-1}	
5.41	5.71	5.39	5.13	Liquefied irrigation water
8.34	7.03	7.87	10.12	Well irrigation water
	6.37	6.63	7.63	Average of Aspartic acid
$LSD_{0.05}$ Irrigation Water Quality × Aspartic Acid = 1.997				
Average of Irrigation Water Quality	Ascorbic Acid Concentration		Irrigation Water Quality	
	mg.L^{-1}4000	0 mg.L^{-1}		
5.41	4.91	5.91	Liquefied irrigation water	
8.34	7.01	9.68	Well irrigation water	
	5.96	7.79	Average of Ascorbic acid	
$LSD_{0.05}$ Irrigation Water Quality × Ascorbic Acid = 2.061				
Average of Aspartic acid	Ascorbic Acid Concentration		Aspartic Acid Concentration	
	mg.L^{-1}4000	0 mg.L^{-1}		
7.63	6.45	8.80	0 mg.L^{-1}	
6.63	5.99	7.27	100 mg.L^{-1}	
6.37	5.43	7.32	150 mg.L^{-1}	
$LSD_{0.05}$ Aspartic acid = 1.011			Average of Ascorbic acid	
$LSD_{0.05}$ Ascorbic acid = 0.720				
N.S.=Aspartic × Ascorbic $LSD_{0.05}$				

The enhanced content of sodium and chloride in the orange leaves resulted from their high percentage in the irrigation water, which caused their accumulation in the soil as a result of frequent irrigation and evaporation resulting from high temperatures, which led to the absorption of these salts by the roots of the plants (Yeo and Flowers, 1989). The increase in proline acid and activity of peroxidase and catalase enzymes in the leaves of orange seedlings revealed an adverse effect of salinity as the plant gets exposed to the induced oxidative stress for some time. Stagnant salt concentration in the external surroundings of crop plants adversely affects the crops, which leads to the closing of the stomata, reduction of the available CO_2 in the leaves, and inhibited carbon fixation process, exposing the chloroplast to excessive excitation energy, subsequently increasing the active oxygen species (ROS) generation, such as, superoxide (O_2*), hydrogen peroxide (H_2O_2), hydroxyl radical (OH*), and single and/or bonded oxygen (Parida and Das, 2005; Jaspers and Kanjasjarvi, 2010).

The increase in free radicals resulted in reducing the activity of peroxidase, catalase, and other anti-enzymes, and then causes an increase in dissolution and cellular toxic effects by oxidation of membrane lipids and cellular proteins. Highly reactive, it can cause cell damage through the oxidation of lipids, proteins, and nucleic acids (Apel and Hirt, 2004). Several studies reported that proline as an osmotic protector increases its concentration in plant tissues with the increase in the intensity and duration of exposure to salt stress (Mostafakamal *et al*., 2016; Barickman and Sams, 2020). However, severe salt stress inhibited the activities of enzymes, such as, catalase and peroxidase, in *Pancratium maritimum* plants, but the presence of proline showed the reactions of these enzymes were significantly higher (Jaspers and Kanjasjarvi, 2010). The study results related to the effect of osmotic protectors in increasing the concentration of antioxidants agree with these past studies.

Earlier reports stated that super oxidase dismutase (SOD) enzymes (Muytova and Volakit, 2004), CAT, and APX (Ozden *et al*., 2009; Aktas and Guven, 2014) increased in leaves when sprayed with proline. Also, other study findings reported that spraying with glycine betaine k caused significant increases in several enzymatic antioxidants, such as APX, POD, and SOD (Zhimin *et al*., 2012), APX enzyme (Cruz *et al*., 2013), and CAT, POD, and

APX enzymes (Mostafakamal *et al*., 2016). Proline is a scavenger of free radicals and rids cells of their destructive effects, as it generates an osmotic effort, which leads to a decline in the activity of the enzyme SOD, maintaining the process of photosynthesis, preventing lipid oxidation in the cell membrane, and increasing protein degradation (Tan *et al*., 2008). Proline acts as a defense mechanism, as it collects harmful amino acids, such as aspartic acid and glutamic acid, as well as antioxidant enzymes, ATP (Behnassi *et al*., 2011).

Aspartic acid is one of the essential amino acids found when there is a lack of nitrogen in the plant's environment, and it gets transported through the bark to the plant roots and then converted to the ionic form (aspartate) (Vitor *et al*., 2018; Tiwari *et al*., 2020). Marked stress-related responses of aspartic acid come out with acute changes and environmental stresses on plants, indicating the essential role of aspartic acid in response to stress conditions. Aspartic acid limits the availability of the final liquefied that are indispensable to plants for growth and resistance to various stresses. Aspartic acid also modulates central metabolism using glycolysis (sucrose, hexose, and pyruvate), the citric acid cycle, NAD, and nucleotides to support cell survival and tolerance (AL-Hadrawi and AL-Janabi, 2020 ; Han *et al*., 2021).

Salinity affects the plant's productive capacity, especially in the pre-flowering stage, which leads to a partial deficit in the production of fruits, reducing their size, number, and weight (Al-Janabi *et al*., 2021a). The obtained results indicated that the plants grown in the saline medium displayed a decrease in the fresh weight of the leaves due to the prevention of cambium activity in both the stem and the root, which causes the thickness of each of them, while the new meristematic cells do not increase, with their transformation into cells prevented (Ahmad *et al*., 2009 ; Al-Janabi *et al*., 2021b, c). The role of ascorbic acid directly causes stimulating and regulating the various vital processes inside the cell as it donates electrons to a wide range of reactions in the cell and also functions as a booster to some enzymes related to the metabolism of carbohydrates and proteins, with their auxin effects supporting growth processes (Blokhina *et al*., 2003).

Results revealed that the magnesium content in the leaves indicates that the foliar spray with ascorbic acid corrects the nutritional balance and increases the absorption of calcium and magnesium involved in the cell formation of walls (Bassuony *et al*., 2008). The role of ascorbic acid in reducing the effects of environmental stresses, such as, salt stress, emerged through the active cycle as an antioxidant cofactor, as well as, regulating liquefied processes and mitigating the harmful effects of stress by increasing the photosynthetic pigments and increasing the efficiency of the photosynthesis process. These processes make plants withstand environmental stress conditions, which positively reflects in plant growth stimulation (Allen and David, 2007; Dolatapian *et al*., 2010).

CONCLUSIONS

Irrigation with regular liquefied water and saline well water significantly affected the chemical characteristics of the local orange seedlings. Aspartic and ascorbic acids minimized the detrimental effects of salts generated by salty irrigation water by enhancing chemical characteristics. Ascorbic acid, individually, in binary, and triple factor interactions proved superior, significantly affecting the chemical characteristics of sweet orange seedlings by enhancing seedling resistance to the harmful effects of irrigation water salinity.

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