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EFFECT OF SALINITY STRESS LEVELS AND SORBITOL SPRAYING ON VEGETATIVE GROWTH AND PHYSIOLOGICAL TRAITS OF LOCAL POMEGRANATE TREES (*PUNICA GRANATUM* L.)

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Abstract. A field trial was carried out in a private orchard in the district of Al-Duluiyah, Salah al-Din Governorate, over the season that ran from February to October 2026. Six-year-old pomegranate trees (*Punica granatum L.*) of a local cultivar, rooted directly in the orchard soil, were used to examine how irrigation-water salinity, foliar sprays of sorbitol, and the two combined bear upon a set of vegetative growth measures and physiological indicators. Treatments were laid out as a two-factor factorial in a randomized complete block design. Salinity was imposed through sodium chloride at 0, 2 and 3 g L^{-1}, and each level was crossed with sorbitol sprays at 0, 2 and 4 g L^{-1}. Records were subjected to analysis of variance, and treatment means were separated by Duncan's multiple range test at the 0.05 probability level, so that the separate and joint contributions of the two factors could be judged. Vegetative growth and leaf chlorophyll declined progressively as the salinity of the irrigation water rose, whereas proline concentration and peroxidase activity moved in the opposite direction and climbed with each increase in salt. Sorbitol sprays worked against this pattern: trees receiving the polyol showed better vegetative growth and higher chlorophyll, together with lower proline and peroxidase activity, than trees left unsprayed. Furthermore, the interaction between salinity and sorbitol factors revealed that foliar application of sorbitol at 4 g L^{-1} under the highest salinity level showed a better tendency to alleviate the adverse effects of salinity stress compared with the high-salinity treatment without sorbitol application. Based on these findings, it can be concluded that foliar application of sorbitol may contribute to improving the tolerance of local pomegranate trees to salinity stress by maintaining vegetative growth and leaf physiological status and reducing some indicators of oxidative stress. This suggests the potential use of sorbitol as an auxiliary substance in managing salinity stress in pomegranate trees under field conditions.

Keywords: Salinity stress; Sorbitol; Pomegranate (*Punica granatum L.*); Vegetative growth; Physiological traits.

1. Introduction

Pomegranate (*Punica granatum* L.) is an economically and nutritionally important fruit tree belonging to the family Lythraceae. It is widely cultivated in arid and semi-arid regions due to its relative ability to tolerate unfavorable environmental conditions. Its fruits are rich in sugars, organic acids, vitamins, and bioactive compounds, particularly phenolics, flavonoids, anthocyanins, punicalagin, and ellagic acid, which contribute to their antioxidant and health-promoting properties and increase their commercial and industrial value (Cheng et al., 2023).

Although pomegranate is relatively tolerant to environmental stresses, its response to salinity varies according to cultivar, genetic composition, and stress intensity and duration. Salinity stress is one of the major abiotic factors limiting plant growth and productivity, particularly in arid and semi-arid regions. It causes osmotic stress by reducing soil water potential and restricting water uptake, as well as ion toxicity resulting from the accumulation of sodium and chloride ions, which disrupts nutrient balance, particularly potassium and calcium. Apart from its impact on growth, salinity affects photosynthesis, reduces the chlorophyll content, disrupts stomatal regulation and gas exchange, and may induce oxidative stress by accumulation of reactive oxygen species leading to damage of membranes and disturbances of metabolic processes (Tang et al., 2024).

Salt treatment is not consistent across the pomegranate crop. Dichala et al. (2022) conducted saline stress comparison of 3 cultivars and observed cultivar-specific physiological and biochemical responses. Singh et al. (2023) compared 15 genotypes and found differences in ion uptake, mineral composition, chlorophyll content, some indices of oxidative damage, yield and fruit quality; with the more tolerant genotypes showing less sodium and chloride uptake, more water potential in the leaves, more chlorophyll and less oxidative damage. Tang et al. (2024) also demonstrated that salt affected the activity of antioxidant enzymes superoxide dismutase (SOD) and peroxidase (POD), and increased the level of proline and soluble sugar, indicating that physiological, biochemical and molecular processes are involved in this species' tolerance.

In recent years, attention has focused on the use of organic compounds to enhance environmental stress resistance of plants. Sorbitol (glucitol) is a water soluble sugar alcohol naturally occurring in plant tissues, and involved in metabolism, transport of carbon and growth regulation. In many species from the Rosaceae it constitutes a major product of photosynthesis. May also help osmotic adjustment, maintenance of cell water balance, membrane stability and the antioxidant defence (Li et al., 2023).

Some crops have provided evidence for a protective role. Wheat cultivars were sprayed with sorbitol and were observed to improve the morphological and physiological traits, and yield and relieve the salinity induced damage, by Khaliq et al. (2023), where the concentrations of 50 and 75 mmol L-1 were found to be favorable in several traits. Li et al. (2023) observed that 10 mmol L-1 exogenous sorbitol enhanced the growth, water status, proline and soluble compounds, enhanced catalase, peroxidase and superoxide dismutase activities and reduced the oxidative damage markers in maize seedlings under drought. These findings indicate that sorbitol will increase environmental stress tolerance but the extent to which this is achieved depends upon species and the concentration used.

Despite this, the response of perennial fruit trees (especially pomegranate) to exogenous sorbitol under saline conditions has not been adequately studied, as field crop data is not necessarily transposable to woody perennials. Therefore, the present investigation was planned to investigate the response of vegetative growth and physiological and biochemical attributes of pomegranate trees (Punica granatum L.) to various salinity levels and to the foliar application of sorbitol alone and in combination to evaluate its ability in mitigating the salinity effects to maintain the plant growth on saline soil.

2. Materials and Methods

2.1. Experimental Site

The trial was conducted in a farm orchard in Al-Dibsa area of Tikrit in Salah al-Din Governorate, Iraq during the season from February to October. Six-year-old un-grafted pomegranate (Punica granatum L.) trees were used for the experiments, which were planted directly in the orchard soil. The object of the work was to see how various levels of salt stress and foliar application of sorbitol affect some vegetative and physiological tree features.

2.2. Materials Used in the Experiment

The following materials were employed:

1. Local pomegranate trees, six years of age.
2. Sodium chloride (NaCl), added to the irrigation water to establish the salinity levels.
3. Sorbitol, applied to the foliage as a spray.
4. Water, used in preparing both the saline solutions and the spray solutions.
5. Laboratory instruments and apparatus needed to measure the vegetative, physiological and biochemical characters.

2.3. Experimental Factors and Treatments

Two factors were examined.

First factor: salinity stress, imposed with sodium chloride (NaCl) at three concentrations, as listed below.

NaCl concentration (g L^{-1}) | Treatment code

Three sodium chloride concentrations were used, as follows:

Treatment code	NaCl concentration(g L^{-1})
N0	0
N1	2
N2	3

Second factor: Sorbitol concentrations applied as foliar sprays

Three sorbitol concentrations were used, as follows:

Treatment code	Sorbitol concentration (g L^{-1})
S0	0
S1	2
S2	4

Accordingly, nine experimental treatments were obtained from the interaction between salinity levels and sorbitol concentrations, as presented in the following table:

Treatment code	Salinity (g L^{-1})	Sorbitol (g L^{-1})
S0N0	0	0
S1N0	0	2
S2N0	0	4
S0N1	2	0
S1N1	2	2
S2N1	2	4
S0N2	3	0
S1N2	3	2
S2N2	3	4

2.4. Experimental Design and Statistical Analysis

The experiment was laid out as a two factor factorial experiment in Randomized Complete Block Design (RCBD). The first factor comprised of three salinity stress levels and the second factor comprised of three sorbitol concentrations.

The experiment consisted of nine (9) treatments replicated three (3) times making a total of twenty seven (27) experimental units. These treatments were randomly allotted within each replicate.

Analysis of variance (ANOVA) was used to analyze the data, and significance of the salinity, sorbitol and salinity x sorbitol interactions tested using Duncan's Multiple Range Test at the 0.05 level of significance.

2.5. Selection and Preparation of Trees for the Experiment

Local pomegranates were selected which were six years old with similar age, size and health status and planted in the soil directly. The selected trees were kept as uniform as possible with regard to vegetative growth and vigor of root system and free of disease and insect infestation to minimize variations other than those that occurred as a result of the experimental treatment.

In addition, the conventional agronomic practices such as irrigation, pruning and pest control were performed consistently for all trees with the exception of the imposed experimental treatments.

2.6. Preparation and Application of Salinity Stress Solutions Through Irrigation

The appropriate amounts of sodium chloride (NaCl) were dissolved in the irrigation water to achieve three concentrations (0, 2, and 3 g L^{-1}).

These concentrations are equivalent to about 0, 34.2, and 51.3 mmol L -1 of sodium chloride, based on the molecular mass of sodium chloride (58.44 g mol -1).

The trees of each salinity level were irrigated with the prepared saline solutions while the control trees were irrigated with water without the addition of sodium chloride. Irrigation was kept as uniform as possible for all treatments.

2.7. Preparation of Sorbitol Solutions and Foliar Application

Sorbitol solutions were made by adding the appropriate amounts of compound to water to give the desired final concentrations (0, 2 and 4 g L-1).

The solutions were sprayed onto the trees and applied as foliar treatment with uniform distribution of solution on leaves as per treatments. The zero concentrations were water without sorbitol.

2.8. Studied Characteristics and Measurement Methods

First: Vegetative Growth Characteristics

1. Length of New Branches (cm)

The length of new branches were measured with a measuring tape. A representative sample of branches was taken in the various directions of the tree and the average branch length was computed and reported in centimeters.

2. Number of New Branches

Branches newly developed on the treated trees were counted in a uniform manner for all experimental units. The figures were reported as the average per tree number of new branches.

3. Leaf Area (cm^2)

Samples of mature, fully expanded leaves were collected from newly developed branches. Leaf area was measured using a Leaf Area Meter, and the average area of an individual leaf was calculated.

4. Leaf Dry Weight (g)

Trees were sampled by taking leaves and washing them as needed to remove dust. The samples were then dried in an electric oven at 70°C until a constant weight was achieved. They were then weighed with a very sensitive balance and the weights were given in grams.

Second: Physiological and Biochemical Characteristics

1. Leaf Chlorophyll Content

Chlorophyll content of leaves was determined by using a portable chlorophyll meter (SPAD). A number of leaves at their full expansion stage from various positions on each tree were read and the average of the readings calculated for each treatment and expressed in SPAD units.

2. Leaf Proline Content

The proline estimation in leaves was carried out using acid-ninhydrin colorimetric method of Bates et al. (1973). The amino acid was initially extracted from the plant samples, and the spectrophotometric reading of the coloured solution obtained at 520 nm was compared with a calibration curve to obtain the concentration.

3. Peroxidase Enzyme Activity (POD)

Leaf extracts were analyzed for peroxidase activity spectrophotometrically. It was followed by measuring the change in absorbance which occurs due to oxidation of the substrate in the presence of hydrogen peroxide (H_2O_2), and the activity was expressed as per the assay procedure adopted.

2.9. Statistical Analysis

Records were analyzed according to the model suitable for a factorial experiment in a randomized complete block design (RCBD) and the effects due to the first factor (salinity), the second factor (sorbitol) and their interaction were tested for significance. Each treatment was replicated three times and treatment means were separated by Duncan's multiple range test at the 0.05 probability level.

3. Results and Discussion

3.1 Effect of Salinity and Sorbitol on New Branch Length (cm)

The branch length was simply a response to the sodium chloride treatment - the higher the concentration, the shorter the length. The highest salinity (N3) showed the shortest new branches, 21.90 cm as compared to the control (N0) with longest new branches, 34.57 cm, which indicates that salt stress significantly limited the elongation of new branches. The opposite held true for the sugar alcohol sorbitol: The longest branches were produced by S2 (31.57 cm); S1 (29.47 cm) and unsprayed trees (S0, 25.40 cm) were the shortest, indicating a positive effect of the sugar alcohol on shoot elongation.

The combination N0S2 was thus the most effective in yielding the longest length (36.10 cm), followed by N0S1 (35.20 cm) and the least by N3S0 (18.20 cm); sorbitol, and especially S2, thus seem to have lessened the damage from salinity and to have maintained the growth of new branches under saline conditions.

Table1: Effect of Salinity and Sorbitol on New Branch length (cm)

Nacl	Sorbitol			Mean Nacl
	S0	S1	S2	
N0	32.40 ab	35.20 a	36.10 a	34.57 a
N2	25.60 cd	30.80 bc	33.50 ab	29.97 b
N3	18.20 f	22.40 e	25.10 d	21.90 c
Mean sorbitol	25.40 c	29.47 b	31.57 a	C.V 9.2%

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3.2 Effect of Salinity and Sorbitol on the Number of New Branches in Pomegranate Trees (branches tree⁻¹)

The results showed that increasing sodium chloride levels led to a decrease in the number of new branches. The N0 treatment recorded the highest mean of 45.03 branches tree⁻¹, whereas the N3 treatment recorded the lowest mean of 29.73 branches tree⁻¹, indicating the negative effect of salinity stress on the formation of new branches.

Regarding the effect of sorbitol, the S2 treatment recorded the highest mean of 40.97 branches tree⁻¹, followed by the S1 treatment, with a mean of 38.43 branches tree⁻¹, while the S0 treatment recorded the lowest mean of 34.17 branches tree⁻¹. This indicates the positive role of foliar sorbitol application in increasing the number of new branches.

Concerning the interaction between the two factors, the S2N0 treatment recorded the highest mean of 47.20 branches tree⁻¹, with no significant difference from the S1N0 and S0N0 treatments. In contrast, the S0N3 treatment recorded the lowest mean of 25.40 branches tree⁻¹. The results also showed an improvement in the number of branches following sorbitol application under the highest salinity level, as the mean increased from 25.40 branches tree⁻¹ in the S0N3 treatment to 33.60 branches tree⁻¹ in the S2N3 treatment. This indicates the potential contribution of sorbitol to alleviating the adverse effects of salinity stress.

Table2: Effect of Salinity and Sorbitol on the Number of New Branches in Pomegranate Trees (branches tree⁻¹)

Nacl	Sorbitol			Mean Nacl
	S0	S1	S2	
N0	42.30 ab	45.60 a	47.20 a	45.03 a
N2	34.80 cd	39.50 bc	42.10 ab	38.80 b
N3	25.40 f	30.20 e	33.60 d	29.73 c
Mean sorbitol	34.17 c	38.43 b	40.97 a	C.V 9.7%

3.3 Effect of Salinity and Sorbitol on Leaf Area of Pomegranate Trees (cm²)

The results showed that increasing sodium chloride levels caused a significant reduction in leaf area. The N0 treatment recorded the highest mean of 20.10 cm², whereas the N3 treatment recorded the lowest mean of 12.47 cm², indicating the adverse effect of salinity stress on leaf expansion in pomegranate trees.

Regarding the effect of sorbitol, the S2 treatment recorded the highest mean of 18.33 cm², followed by the S1 treatment, with a mean of 16.70 cm², while the S0 treatment recorded the lowest mean of 14.37 cm². This indicates the positive role of foliar sorbitol application in improving leaf area.

Concerning the interaction between the two factors, the S2N0 treatment recorded the highest mean of 21.30 cm², with no significant difference from the S1N0 and S0N0 treatments. In contrast, the S0N3 treatment recorded the lowest mean of 10.30 cm². Furthermore, foliar application of sorbitol at the S2 concentration under the highest salinity level increased leaf area to 14.50 cm², compared with 10.30 cm² in the untreated treatment (S0N3). This indicates the potential role of sorbitol in alleviating the adverse effects of salinity stress.

Table3: Effect of Salinity and Sorbitol on Leaf Area of Pomegranate Trees (cm²)

Nacl	sorbitol			Mean Nacl
	S0	S1	S2	
N0	18.60 ab	20.40 a	21.30 a	20.10 a
N2	14.20 cd	17.10 bc	19.20 ab	16.83 b

N3	10.30 f	12.60 e	14.50 d	12.47 c
Mean sorbitol	14.37 c	16.70 b	18.33 a	C.V 9.4%

3.4 Effect of Salinity and Sorbitol on Leaf Dry Weight (g)

The results showed that increasing sodium chloride levels led to a decrease in leaf dry weight. The adverse effect of salinity stress on dry matter accumulation of leaves was observed when the lowest mean 5.87 g was obtained at N3 treatment while the highest mean 9.08 g was obtained at N0 treatment.

Regarding the effect of sorbitol, the S2 treatment recorded the highest mean of 8.39 g, followed by the S1 treatment, with a mean of 7.63 g, while the S0 treatment recorded the lowest mean of 6.68 g. This indicates the positive role of foliar sorbitol application in increasing leaf dry weight.

Concerning the interaction between the two factors, the S2N0 treatment recorded the highest mean of 9.68 g, with no significant difference from the S1N0 and S0N0 treatments, whereas the S0N2 treatment recorded the lowest mean of 4.86 g. Furthermore, foliar application of sorbitol at the S2 concentration under the highest salinity level increased leaf dry weight to 6.84 g, compared with 4.86 g in the untreated treatment (S0). This indicates the potential contribution of sorbitol to alleviating the adverse effects of salinity stress and improving dry matter accumulation.

Table 4: Effect of Salinity and Sorbitol on Leaf Dry Weight (g)

Nacl	sorbitol			Mean Nacl
	S0	S1	S2	
N0	8.42 ab	9.15 a	9.68 a	9.08 a
N2	6.75 cd	7.82 bc	8.64 ab	7.74 b
N3	4.86 f	5.92 e	6.84 d	5.87 c
Mean sorbitol	6.68 c	7.63 b	8.39 a	C.V 8.4%

3.5 Effect of Salinity and Sorbitol on Leaf Chlorophyll Content in Pomegranate Trees (SPAD Units)

The results showed that increasing sodium chloride levels led to a decrease in leaf chlorophyll content. The N0 treatment recorded the highest mean of 54.77 SPAD units, whereas the N3 treatment recorded the lowest mean of 41.97 SPAD units, indicating the adverse effect of salinity stress on chlorophyll content in pomegranate leaves.

Regarding the effect of sorbitol, the S2 treatment recorded the highest mean of 51.63 SPAD units, followed by the S1 treatment, with a mean of 48.87 SPAD units, while the S0 treatment recorded the lowest mean of 45.10 SPAD units. This indicates the positive role of foliar sorbitol application in maintaining chlorophyll content and improving the physiological status of the leaves.

Concerning the interaction between the two factors, the S2N0 treatment recorded the highest mean of 56.80 SPAD units, with no significant difference from the S1N0 and S0N0 treatments, whereas the S0N3 treatment recorded the lowest mean of 37.60 SPAD units. Furthermore, foliar application of sorbitol at the S2 concentration under the highest salinity level increased chlorophyll content to 46.00 SPAD units, compared with 37.60 SPAD units in the untreated treatment (S0N3). This indicates the potential contribution of sorbitol to alleviating the adverse effects of salinity stress and maintaining leaf chlorophyll content.

Table 5: Effect of Salinity and Sorbitol on Leaf Chlorophyll Content in Pomegranate Trees (SPAD Units)

Nacl	sorbitol			Mean Nacl
	S0	S1	S2	
N0	52.40 ab	55.10 a	56.80 a	54.77 a
N2	45.30 cd	49.20 bc	52.10 ab	48.87 b
N3	37.60 f	42.30 e	46.00 d	41.97 c
Mean sorbitol	45.10 c	48.87 b	51.63 a	C.V 10.9%

3.6 Effect of Salinity and Sorbitol on Proline Content in Pomegranate Leaves (mg g^{-1} Fresh Weight)

The concentration of proline in the leaves increased with an increase in the level of sodium chloride. The N3 produced the highest salinity (3.74 mg.g-1 fresh weight), followed by N2 (2.46 mg.g-1 fresh weight) and the least value was the control (N0) (1.67 mg.g-1 fresh weight) and it was obvious that the amino acid is accumulated in the tissue due to salinity stress.

The opposite effect was found with sorbitol. The least amount of proline was found in trees sprayed at S2 (2.26±0.15 mg g-1 of fresh weight), the intermediate level in trees sprayed at S1 (2.60±0.15 mg g-1 FW) and the highest level in unsprayed trees (S0) (3.01±0.15 mg g-1 FW). Thus, the foliar spray seems to have helped relieve proline buildup that would occur under stress.

The maximum is 4.36 mg.g-1 fresh weight obtained by S0N3 combination and minimum is 1.54 mg.g-1 obtained by S2N0 combination. Under the highest salinity condition, application of sorbitol at S2 reduced proline to 3.15 mg g-1 compared to the unsprayed S0N3 which accumulated proline to 4.36 mg g-1 indicating that sorbitol can help in suppressing proline accumulation under saline condition.

Table 6: Effect of Salinity and Sorbitol on Proline Content in Pomegranate Leaves (mg g^{-1} Fresh Weight)

Nacl	sorbitol			Mean Nacl
	S0	S1	S2	
N0	1.82 f	1.65 f	1.54 f	1.67 c
N2	2.85 d	2.42 e	2.10 e	2.46 b
N3	4.36 a	3.72 b	3.15 c	3.74 a
Mean sorbitol	3.01 a	2.60 b	2.26 c	C.V 1.9%

3.7 Effect of Salinity and Sorbitol on Peroxidase (POD) Activity in Pomegranate Leaves (Enzyme Activity Units g^{-1} Fresh Weight)

The results indicated that increasing sodium chloride (NaCl) levels led to an increase in peroxidase (POD) activity. The highest salinity treatment (N3) recorded the highest mean value of 4.23 enzyme activity units g^{-1} fresh weight, compared with the control treatment (N0), which recorded 2.35 enzyme activity units g^{-1} fresh weight.

In contrast, foliar application of sorbitol reduced enzyme activity. The concentration of 4,000 mg L^{-1} (S2) recorded the lowest mean value of 2.94 enzyme activity units g^{-1} fresh weight, compared with the non-sprayed treatment (S0), which recorded 3.41 enzyme activity units g^{-1} fresh weight.

Regarding the interaction between the two factors, the N3S0 treatment recorded the highest value of 4.85 enzyme activity units g^{-1} fresh weight, whereas the N0S0 treatment recorded the lowest value of 2.15 enzyme activity units g^{-1} fresh weight. This may indicate that foliar application of sorbitol contributed to alleviating oxidative stress and reducing the need for increased activity of the antioxidant enzyme.

Table 7: Effect of Salinity and Sorbitol on Peroxidase (POD) Activity in Pomegranate Leaves (Enzyme Activity Units g^{-1} Fresh Weight)

Nacl	sorbitol			Mean Nacl
	S0	S1	S2	
N0	2.15 f	2.38 ef	2.52 e	2.35 c
N2	3.24 d	2.91 de	2.68 e	2.94 b
N3	4.85 a	4.21 b	3.62 c	4.23 a
Mean sorbitol	3.41 a	3.17 b	2.94 c	C.V 1.9%

First: Vegetative Growth Traits

The longer the sodium chloride concentration of the irrigation water, the lower the new branch length, the number of new branches, leaf area and leaf dry weight. Two mechanisms probably lie behind this. The first is osmotic, that is, the more concentrated the soil solution becomes, the more difficult it is for the roots to absorb water. The second is ionic: sodium and chloride accumulate in the tissues and disturb the mineral nutrient balance, thus reducing the amount of the necessary nutrient that the tree can utilize. In this manner, less dry matter was deposited, less leaves and branches were produced and cell division and cell elongation were suppressed.

Similarly Liu et al., (2020) conducted a salt stress experiment in 18 pomegranate cultivars. Dry weight, branch length, number of branches and leaf area were all decreased and the cultivars varied in their salt tolerance. The authors attributed the lack of growth to poor root system and uptake of water and nutrients.

Similarly, Calzone et al. (2020) reported a good agreement of the present data. As the concentration of sodium chloride increased in the various levels, there were ionic balance and physiological changes in the plants of both cultivars; the two cultivars adopted different salt coping strategies. Such writers pointed to the distribution of sodium and chloride in the various parts of the plant as a consideration in plant growth and biomass maintenance.

Liu et al. (2018) also found salinity impeded growth of pomegranates cuttings, especially under high salinity and that chlorophyll decreased and antioxidant enzymes activity varied.

In the present experiment the vegetative growth was improved with increasing concentration of the spray for sorbitol. One might suggest that this could be due to sorbitol maintaining the osmotic balance and water status of the cells, thus permitting the continued elongation of branches, leaf production and accumulation of dry matter. This, however, is an interpretation of the results which have been achieved here. The studies in pomegranate cited above did not include sorbitol, but other external treatments.

Regarding the interaction between salinity and sorbitol, the improvement observed in the sorbitol-sprayed treatments under salinity stress may be attributed to the possible contribution of sorbitol to alleviating the physiological effects of salinity and maintaining the functional efficiency of plant tissues. This general trend is consistent with the findings of Rashedy et al. (2022), who found that the combined application of proline or calcium with humic acid improved the nutritional status and osmotic regulation of pomegranate trees under saline conditions and reduced sodium and chloride accumulation.

Second: Leaf Chlorophyll Content

The results showed a decrease in chlorophyll content with increasing salinity levels, whereas foliar application of sorbitol improved this trait. The reduction in chlorophyll may be attributed to the effects of salinity on the integrity of chloroplasts and cell membranes, in addition to disturbances in the uptake of mineral nutrients essential for pigment formation, consequently reducing photosynthetic efficiency.

These findings are consistent with those of Dichala et al. (2022), who investigated the responses of three pomegranate cultivars, namely Wonderful, Ermioni, and Grenada, to different salinity levels. Their results indicated a decrease in the levels of chlorophyll and carotenoid pigments under increasing levels of salt stress, varying among cultivars.

The same trend was observed by Khayyat et al. (2014) who found that chlorophyll content of pomegranate leaves decreased with increasing salinity in irrigation water and also the photochemical efficiency of photosystem II decreased.

Dichala et al., (2021) using different cultivars of pomegranate reported that an increase in concentration of certain salts reduced the chlorophyll content in leaves and the degree of reduction varied with both the type of salt and its concentration as well as the cultivar.

Liu et al. (2020) suggested that salinity reduces the growth, photosynthesis and mineral nutrition in pomegranate cultivars, which provide a physiological explanation for the chlorophyll reduction observed in the present trial.

The beneficial effect after the sorbitol sprays can be attributed to the maintenance of water status of the cells and to the reduced indirect salinity stress on the photosynthetic apparatus. Under these conditions, chlorophyll production may be continued and the amount of chlorophyll in the leaves might be better maintained.

Third: Leaf Proline Content

The content of proline in leaves has increased with the increase in salinity and decreased with the increase in sorbitol content. This increase likely reflects the use of proline as a compatible osmolyte, and its accumulation by cells in response to salinity. It helps to resist oxidative damage, protect proteins and membranes by regulating osmotic potential, and is part of the plant's defense mechanisms against the stress.

Khayyat et al. (2014) obtained results in line with these. In their investigation of two pomegranate cultivars, the more saline the irrigation water, the higher the level of proline in the leaves, and the lower the level of chlorophyll. Dichala et al. (2022) also reported an increase in proline content in some pomegranate cultivars exposed to salinity, with responses varying according to cultivar, salt type, and concentration.

Similarly, Calzone et al. (2020) explained that pomegranate cultivars differ in their mechanisms of response to salinity stress, and that the accumulation of osmolytes, including proline and sugars, represents one of the physiological pathways through which plants respond to salinity.

The decreased proline level associated with sorbitol may be attributed to the osmotic potential and water status of the cells, which are now more stable and the tissue no longer needs to produce as much proline as one of the stress response compounds. The findings of Rashedy et al. (2022) showing that mechanisms of osmotic regulation and accumulation of soluble compounds were affected by application of proline or calcium + humic acid, respectively, in pomegranate leaves exposed to saline conditions support this idea.

However, a high proline value does not necessarily equate to greater tolerance. It can just be marking the intensity of the stress experience(s). This was true as reported by Singh et al. (2023), who observed that in some genotype of pomegranate the proline accumulation was more closely correlated with the sodium uptake by the tissue rather than osmotic adjustment.

Fourth: Peroxidase Enzyme (POD) Activity

As salinity increased, peroxidation activity increased and as the sorbitol concentration increased, activity decreased. The increased activity likely represents a response to oxidative stress induced by salt, which increases generation of reactive oxygen species in particular hydrogen peroxide. Peroxidase is useful in helping to decompose these compounds, thus preserving the cell's membranes, proteins and other vital cell components.

Similar results were found by Liu et al. (2018) in cuttings of the Tunisian cultivar of pomegranate. The activities of the antioxidant enzymes including POD rose gradually with salinity reaching a maximum at a certain level and then decreased thereafter, demonstrating that salinity stress response of enzymes is salinity level- and duration-dependent.

Similarly, the activity of those antioxidant enzymes (POD, CAT, SOD, APX) in salinity stress and drought stressed pomegranate trees remained higher, and the oxidative stress markers also increased in these plants, highlighting their role in the plant's physiological defense mechanism (Zarbakhsh et al. 2024).

In a similar context, the application of foliar salicylic acid (SA) was effective in mitigating the salt stress effects on physiological and biochemical traits such as antioxidant enzyme activity of pomegranate tree plants (Khalil et al., 2022).

The decrease in POD activity with the increase in sorbitol concentration might suggest a role of sorbitol in alleviating the oxidative stress, thus reducing the need for the enzymatic defense system to be activated to the same extent. However, a decrease in enzyme activity alone is not enough to establish that oxidative damage was decreased, it would be necessary to have some additional evidence, in this case the MDA and H_2O_2.

Fifth: Interaction Between Salinity and Sorbitol

The results showed that the interaction between salinity levels and sorbitol concentrations resulted in different responses among the treatments. The S0T2 treatment recorded the highest values for most vegetative growth traits and chlorophyll content, whereas the S2T0 treatment recorded the lowest values. Furthermore, the application of sorbitol under saline conditions improved several of the studied traits compared with treatments exposed to the same salinity levels without sorbitol application.

This may possibly be attributed to the combined effects of osmotic stress and the plant's defense mechanisms. Sorbitol may help maintain cellular water status and stabilize physiological processes, thereby supporting continued vegetative growth and maintaining leaf efficiency in photosynthesis. It may also contribute to limiting some of the imbalances resulting from salt accumulation, which may improve plant performance under stress conditions.

This finding is generally consistent with the results of Rashedy et al. (2022), who found that the combined application of proline or calcium with humic acid improved several nutritional and osmotic traits in pomegranate trees exposed to salinity, compared with the use of some treatments individually.

Khalil et al. (2022) also reported that foliar application of salicylic acid under salt stress resulted in significant improvement in various morphological and physiological traits in pomegranate Wonderful, indicating the potential of exogenously applied SAs in reducing the salt stress-induced damage.

The salinity level and the combined effect of Nano-NPK fertilization and Proline sprays were tested on seedlings of the same cultivar as done by Abdulkadhim and Mortada (2025). All of these combinations influenced branch number, branch length and chlorophyll content and the authors highlighted that treatment combinations are important in assessing seedling performance under saline conditions.

4. Conclusions and Recommendations

The increase in salinity caused reduction in vegetative growth and leaf chlorophyll content and increase in proline content and peroxidase (POD) activity in the pomegranate trees, which is indicative of the stress that these trees were subjected to. Vegetative growth and chlorophyll increased while proline and POD activity dropped with sprays of sorbitol; the effects were most pronounced with the highest rate. On this evidence, foliar sorbitol can be recommended as a supplementary measure for reducing the harm done by salt stress. Additional testing is warranted with other sorbitol concentrations, with different intervals between applications, and for the impact on fruit yield and quality under other environmental conditions.

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