



Studying the effect of salt stress on some chemical properties of apricot seedlings (*Prunus armeniaca* L)

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Article Info

P-ISSN: 3051-3421

E-ISSN: 3051-343X

Volume: 07

Issue: 01

January - June 2026

Received: 14-01-2026

Accepted: 12-02-2026

Published: 10-03-2026

Page No: 41-45

Abstract

The study was conducted in one of the private nurseries in the Al-Haydariya district of Najaf Governorate. The seedlings of the apricot variety were equal in size and age. The study was implemented as a factorial experiment (3×3) using a completely randomized block design (RCBD) with two factors with three replications. The first factor represents the cultivated varieties (Zaghenia, Kaisy, and Labib). The second factor represents the salinity levels of the irrigation water (regular irrigation water 1.5, 2, and 4 mS m⁻¹). The results showed that the salinity levels of the irrigation water (1.5 mS m⁻¹) gave the highest increase in the chlorophyll content of the leaves. The concentration of 4 Ms. m⁻¹ was superior to other characteristics: tannins, flavonoids, and proline. The Labib apricot variety excelled in all studied characteristics (chlorophyll content, tannins, flavonoids, and protein).

Objectives: To study the effect of salt stress on apricot seedlings and its impact on leaf chlorophyll content, as well as the concentration of secondary metabolites such as tannins and flavonoids, and to study the plant's response to external stress factors by encouraging the production of antioxidants such as proline. The main objective is to utilize stress-prone apricot trees, especially since apricot orchards in Iraq are susceptible to water stress, to serve as a source for producing secondary metabolites as natural sources for medicinal compounds.

DOI: <https://doi.org/10.54660/JADR.2026.7.1.41-45>

Keywords: Apricot, salt stress, Ethbeab, Zaghenia, Kaisy

Introduction

The apricot (*Prunus armeniaca* L.) is a medium-sized deciduous fruit tree belonging to the rose family. Its original habitat is China and Central Asia, and it grows in temperate regions. It is one of the stone fruit trees (Krichen *et al.*, 2008). Apricots are stone fruits belonging to the genus *Prunus* and Rosacea family, an economically and nutritionally important fruit in Iraq, as fruit is not only consumed fresh but also dried and is used to make jams. In the last few years apricot production has decreased, as statistics indicate Iraq's apricot production for the year 2000 reached 27000 tons and for 2010, 18926 tons, recording a 29% reduction, and for the year 2016, production recorded 13871 with a 48.6% decrease (FAO, 2016). One of these reasons is the scarcity of irrigation water.

Apricot leaves contain bioactive medicinal compounds, most notably phenols, flavonoids, terpenoids, and vitamin K, giving them antioxidant, hepatoprotective, antimicrobial, and antifungal properties. A decoction of apricot leaves is traditionally used to treat toothaches, malaria, colds, coughs, and intestinal infections, as well as arthritis. Soil salinity is one of the most critical abiotic stresses, severely impacting global plant growth and agricultural productivity. The accumulation of excess salts in soil disrupts essential metabolic processes, leading to osmotic stress, ion toxicity, and oxidative damage in plants. With the intensification of climate change, the prevalence of saline soils is projected to rise significantly, posing a substantial threat to food security and agricultural sustainability (Muhammad *et al.*, 2024)^[17].

Salinity is considered a major abiotic stress that stimulates plants to alter their metabolic pathways, leading to the accumulation of secondary metabolites (such as phenols, flavonoids, proline, and alkaloids) as a defense mechanism to regulate osmotic potential and protect cells from oxidative stress (ROS). These compounds are typically increased in plants (Flowers, 2004) ^[9]. Chlorophyll is one of the most important plant pigments in chloroplasts and has the ability to absorb visible light and convert light energy from sunlight into chemical energy used in the production of energy-rich compounds that contribute to the building of organic materials (Hopkins, 2003) ^[12].

Chlorophyll is a green pigment that consists of a tetrapyrrole ring with a central magnesium ion. It has a long hydrophobic phytol chain in its structure. It is found in some varieties of plants and algae (Aminot, 2000). Two types of chlorophyll, a and b, are present in green algae and terrestrial plants. The difference between these two chlorophylls is a methyl moiety in chlorophyll a replaced by a formyl group in chlorophyll b. The ratio of chlorophyll a to chlorophyll b in higher plants is approximately 3:1. Chlorophyll absorbs light mainly in the red (650 – 700 nm) and the blue-violet (400 – 500 nm) regions of the visible spectrum. Green light (~550 nm) is not absorbed but reflected, giving chlorophyll its characteristic color. Chlorophyll a possesses a green-blue color, and chlorophyll b possesses a green-yellow color (Arnon, 1849). Tobita and Dionisio (2000) ^[8] concluded that salt stress leads to a decrease in photosynthetic pigment content as a result of reduced cytochrome synthesis in the roots and reduced translocation to the shoot system.

The researcher (Kandil, 2000) ^[13] concluded that there is an inverse relationship between chlorophyll content in leaves and salinity concentration; that is, the higher the salinity in the soil solution, the lower the chlorophyll concentration in the leaf, as studied on apricot seedlings.

Tannins are among the most important plant extracts derived from the wood and bark of forest trees. These substances are used in many chemical industries, in leather tanning, and in the production of pharmaceuticals, medicines, adhesives, and other products. Flavonoids are major secondary metabolites that play a critical role in stress mitigation through their antioxidant properties, which protect plants from growth inhibition and cell death by reducing oxidative stress through scavenging excess ROS [9]. Abu Shaiba (2022) ^[1] stated in his study on pomegranate trees that some secondary metabolite compounds increase under salt stress conditions, for example, tannins.

Flavonoids are the largest group of plant polyphenols and include flavonols, flavones, isoflavones, anthocyanidins,

flavanones, flavanols, and chalcones. (D'Amelia *et al.*, 2018) On the other hand, soil salinity is a major abiotic stress that stimulates plants to increase the production of secondary metabolites (such as flavonoids and terpenes) as a defense mechanism to adapt to osmotic and oxidative stress. Excess salinity leads to the accumulation of reactive oxygen species (ROS) and ion toxicity, prompting the plant to enhance protective compounds to reduce damage, such as proline. Haroun (2024) ^[11] stated in his study on pomegranate trees that some secondary metabolite compounds increase under salt stress conditions, especially tannins and flavonoids.

Proline One of the most important biochemical components affected in plants under stress conditions (saline or water stress) is the amino acid content (proline), which is closely related to the plant's resistance mechanism to stress conditions (Chutipaijit *et al.*, 2011).

Proline is considered one of the most important antioxidants, as it works to eliminate free radicals from plant tissues, regulates the osmotic environment within plant tissues, removes the toxic effects of ammonia accumulation in cells, and maintains the stability and vitality of proteins and cytoplasmic membranes, protecting them from damaging stress conditions (Meloni *et al.*, 2001). Cam contributes to the protection, activation, and stability of mitochondrial enzymes under stress conditions (Ashraf and Foolad, 2007).

Judy reported in 2020 that exposing apricot seedlings of the Zanjelly variety to drought stress conditions led to an increase in the plant's content of the amino acid Proline.

Graca (2008) ^[10] stated that apricot varieties differ in their content of secondary metabolites.

Materials and methods

The study was conducted in one of the private nurseries in the Al-Haydariya district of Najaf Governorate, where one-year-old young apricot seedlings were used. The seedlings were equal in size and age of the apricot variety, which were brought from the Horticulture and Forestry Station in Najaf. The seedlings at the station were chosen, that were identical in height and age. After that, the seedlings were marked with indicative signs, and 81 seedlings were brought, which were divided into three groups, with 27 seedlings representing a replicate, and 3 seedlings for each treatment. The total number of seedlings was 81. The planting soil was prepared from river sand and the soil was sterilized by exposing to direct sunlight for two weeks before planting. Then the soil was sterilized with boiling water twice and left to dry. The used soil in the experiment was examined to determine some physical and chemical properties,

Table 1:

Property	Parameter	Measuring Unit	Value
Soil separators	Sand	g kg ⁻¹	665
	Silt	g kg ⁻¹	190
	Clay	g kg ⁻¹	120
Soil texture	—	—	Sandy mixture
pH (1:1)	—	—	7.7
EC (1:1)	—	dS m ⁻¹	4.1
Elements	N	%	19
	P	%	17
	K	%	13

Experimental design

The study was implemented as a factorial experiment (3×3) using a completely randomized block design with two factors: the first factor represents the cultivated varieties (Zaghenia, Kaisy and Labib) and the second factor represents the salinity levels of the irrigation water (1.5, 2, 4) Ms.m⁻¹

Preparation of NaCl saline solution: Each concentration of saline solution was prepared separately by dissolving each concentration of sodium chloride salt in 1 liter of distilled water with continuous shaking until the salt was completely dissolved.

The following chemical properties were measured in leaves.

1. The s total chlorophyll content of leaves (mg.g⁻¹ soft weight). were measured asmentioned by

(Ranganna, 1977)^[19]

2. Tannin content in apricot leaves% Dry weight were measured asmentioned by (Al-Ghanimi *et al*, 2007)^[3]
3. Flavonoids content in apricot leaves (mg /g Dry weight) were measured asmentioned by (Budrat and Shotipruk,2008)^[6]
4. Leaf Proline content (RWC): were measured asmentioned by (Bates *et al.*, 1973)^[5]

Statistical Analysis: At the end of the study, the results were recorded, then the variance was analyzed and the factors and their interactions were tested using a computer and the ready-made statistical program Genstat. The differences between the means were compared according to the least significant difference (LSD) test at a probability level of 0.05.

The results

Table2: Effect of irrigation water salinity and variety and their interaction on total chlorophyll content of leaves (mg.g-1 soft weight)

Salinity Level (dS m ⁻¹)	Treatment	Zaghenia	Kaisy	Labib	Mean (Saline Solution Effect Rate)
1.5	Regular irrigation water	0.442	0.580	0.584	0.535
2.0	—	0.364	0.360	0.324	0.349
4.0	—	0.317	0.330	0.286	0.311
Variety Effect Mean	—	0.374	0.423	0.398	—

The results in Table 2 showed that there are significant differences between the concentrations of levels of salinity of irrigation water where 1.5 Ms.m⁻¹ concentration was significantly superior to the other concentrations gave the 0.535 mg.g-1 soft weight. While the comparison treatment 4 Ms.m⁻¹ gave the lowest average of total chlorophyll content of leaves gave the 0.311 mg.g-1 soft weight. It is clear from the results of the table2 that there are significant differences between Variety, The Labib variety gave the highest chlorophyll content. was significantly superior to the other

variety and gave the highest chlorophyll content of 0.398 mg.g-1 soft weight. The interactions of the table2 also showed significant differences between the experimental factors, as the interaction consisting of 1.5 Ms.m-1 and The Labib variety was superior and gave the highest rate of chlorophyll content, reaching 0.584 mg.g-1 soft weight. While the interaction treatment consisting of 4 Ms.m-1 and Labib variety was recorded as the minimum average of of chlorophyll content was 0.286 mg.g-1 soft weight.

Table 3: Effect of irrigation water salinity and variety and their interaction on tannin content in apricot leaves%

Salinity Level (dS m ⁻¹)	Treatment	Zaghenia	Kaisy	Labib	Mean (Saline Solution Effect Rate)
1.5	Regular irrigation water	11.17	11.90	12.81	11.96
2.0	—	11.22	12.40	12.78	12.13
4.0	—	12.56	13.90	14.11	13.52
Variety Effect Mean	—	11.56	12.73	13.23	—

The results in Table 3 showed that there are significant differences between the concentrations of levels of salinity of irrigation water where 4 Ms.m-1 concentration was significantly superior to the other concentrations gave the 13.52% Dry weight. While the comparison treatment 1.5 Ms.m-1 gave the lowest average of tannin content of leaves gave the 11.96% Dry weight.

It is clear from the results of the table3 that there are significant differences between Variety. The Labib variety gave the highest tannin content. was significantly superior to

the other variety and gave the highest tannin content of 13.23% Dry weight.

The interactions of the table3 also showed significant differences between the experimental factors, as the interaction consisting of 4 Ms.m-1 and The Labib variety was superior and gave the highest rate of tannin content, reaching 14.11% Dry weight. While the interaction treatment consisting of 1.5 Ms.m-1 and Zaghenia variety was recorded as the minimum average of of tannin content was 11.17% Dry weight.

Table 4: Effect of irrigation water salinity and variety and their interaction of Flavonoids content in apricot leaves(mg / g)

Salinity Level (dS m ⁻¹)	Treatment	Zaghenia	Kaisy	Labib	Mean (Saline Solution Effect Rate)
1.5	Regular irrigation water	1.57	1.87	1.90	1.78
2.0	—	1.45	1.97	2.11	1.84
4.0	—	2.56	2.90	2.99	2.81
Variety Effect Mean	—	1.86	2.24	2.33	—

The results in Table 4 showed that there are significant differences between the concentrations of levels of salinity of irrigation water where 4 Ms.m-1 concentration was significantly superior to the other concentrations gave the 2.81 mg / g Dry weight. While the comparison treatment 1.5 Ms.m-1. M-1 gave the lowest average of Flavonoids content of leaves gave the 1.78 mg / g Dry weight.

It is clear from the results of the table3 that there are significant differences between Variety, The Labib variety

gave the highest Flavonoids content. was significantly superior to the other variety and gave the highest Flavonoids content of 2.33 mg / g Dry weight.

The interactions of the table4 also showed significant differences between the experimental factors, as the interaction consisting of 4 Ms.m-1 and The Labib variety was superior and gave the highest rate of Flavonoids content, reaching 2.99 mg / g Dry weight.

Table 5: Effect of irrigation water salinity and variety and their interaction of prolein content in apricot leaves(mg/g)

Salinity Level (dS m ⁻¹)	Treatment	Zaghena	Kaisy	Labib	Mean (Saline Solution Effect Rate)
1.5	Regular irrigation water	0.970	0.878	0.870	0.906
2.0	—	1.047	0.867	0.996	0.970
4.0	—	1.043	0.983	1.006	1.011
Variety Effect Mean	—	1.020	0.909	0.957	—

The results in Table 5 showed that there are significant differences between the concentrations of levels of salinity of irrigation water where 4 Ms.m-1 concentration was significantly superior to the other concentrations gave the 1.011 mg / g Dry weight. While the comparison treatment 1.5 Ms.m-1 gave the lowest average of prolein content of leaves gave the 0.906 mg/g Dry weight.

It is clear from the results of the table3 that there are significant differences between Variety, The Labib variety gave the highest protein content. was significantly superior to the other variety and gave the highest Fla prolein content of 0.957 mg / g Dry weight.

The interactions of the table2 also showed significant differences between the experimental factors, as the interaction consisting of 4 Ms.m-1 and The Labib variety was superior and gave the highest rate of prolein content, reaching 1.006 mg / g Dry weight.

Discussion

The decrease in chlorophyll content in leaves is due to an increase in free radicals (Rs) such as H₂O₂, which cause the oxidation of lipids and proteins associated with chlorophyll. This leads to the breakdown and destruction of the chlorophyll molecule, resulting in the activation of the chlorophyllase enzyme responsible for separating phytol from the chlorophyll molecule and its subsequent breakdown. This, in turn, leads to the unavailability and absorption of magnesium due to ionic competition with sodium. This leads to easy damage to chloroplast membranes due to high salt levels. Furthermore, these results are consistent with those obtained by (Kandil, 2000)^[13] on apricot plants, where he demonstrated that salinity damages chlorophyll.

Increased tannin and flavonoid concentrations in plants are a defensive response to salt stress, with the concentration of these phenolic compounds increasing as a direct response to environmental challenges. This finding is consistent with (Abu Shaiba', 2022)^[11] study on pomegranate trees. This result is also consistent with what researcher (Haroun, 2024)^[11] concluded in his study on pomegranate trees.

The accumulation of proline is due to a deficiency in its oxidation on the one hand and an increase in protein breakdown and its conversion into amino acids, including riboflavin (Molinari *et al.*, 2007). The accumulation of proline is due to the disruption in the breakdown of amino acids related to protein breakdown and aging resulting from salinity. Another reason for proline accumulation in plants is a deficiency in the activity of both proline dehydrogenase and

proline oxidase. (Khedr *et al.*, 2003)^[14]. This result is consistent with what Judy reported in 2020 when studying apricot plants.

The differences in the content of secondary metabolites among apricot varieties may be due to genetic factors specific to the variety itself. These results are consistent with what (Graca, 2008)^[10] reported in his study on apricot plants.

Conclusion

Salinity is a major problem facing the agricultural sector in Iraq and worldwide. Apricots are among the most important fruit crops in Iraq and globally, as their fruit has high nutritional value and their leaves are rich in medicinally active compounds. Therefore, the salinity problem can be viewed positively, as we have concluded that irrigating apricot varieties with different salinity levels stimulated the seedlings to produce medicinally active secondary metabolites. Therefore, we recommend studying the effect of salt stress on other apricot varieties and other plants rich in medicinally active compounds.

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How to Cite This Article

Ethbeab IJ, Hmiz D. Studying the effect of salt stress on some chemical properties of apricot seedlings (*Prunus armeniaca* L). *J Agric Digit Res.* 2026;7(1):41–45. doi:10.54660/JADR.2026.7.1.41-45

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