Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1811669

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 9 - Issue 1 : 89-99 / January 2026

THE EFFECT OF ASCORBIC ACID APPLICATIONS ON BIOCHEMICAL PARAMETERS IN MEDICINAL SAGE GROWN UNDER SALT STRESS

Ezelhan ŞELEM[1]*, Lütfi NOHUTÇU[2], Rüveyde TUNÇTÜRK[2], Murat TUNÇTÜRK[2]

[1]*Van Yuzuncu Yil University, Muradiye Vocational School, Department of Landscape and Ornamental Plants Program, 65100, Van, Türkiye*
[2]*Van Yuzuncu Yil University, Faculty of Agricultural, 65100, Van, Türkiye*

Abstract: In this study, the effects of different doses of ascorbic acid (AA) applications on the physiological and biochemical responses of medicinal sage under salt stress were evaluated in a multifaceted manner. In the study, ascorbic acid was applied at different concentrations (0 ppm, 100 ppm, 200 ppm, and 300 ppm), and the plants were exposed to salt stress at different doses (0 mM, 100 mM,200 mM and 300 mM NaCl). In the study, Dualex parameters (NBI (Nitrogen Balance Index), chlorophyll, flavonoids, anthocyanins) and some biochemical components (total antioxidant activity, total flavonoids, total phenolics, and ascorbic acid content) were determined. Based on the obtained data, the total ascorbic acid content was found to be statistically insignificant, while all other parameters were found to be significant. The lowest and highest values for biochemical parameters were total antioxidant activity 68.78-176. 59 µmol TE g^{-1}, total flavonoids 6.48-13.77 mg QE 100g^{-1}, total phenolic content 174.00-237.39 mg GAE g^{-1}, and ascorbic acid 19.69-24.92 mg 100g^{-1}. In Dualex measurements, the lowest and highest values were found to be 37.50–74.70 dx for NBI, 25.83–49.40 dx for chlorophyll, 0.56–0.87 dx for flavonoids, and 0.013–0.080 dx for anthocyanins. The findings indicate that both AA doses and salt stress levels, either alone or in combination, cause significant changes in the plant's defense mechanisms and metabolic regulation.

Keywords: NBI, Anthocyanin, Antioxidant, Dualex, Phenolic

***Corresponding author:** Van Yuzuncu Yil University, Muradiye Vocational School, Department of Landscape and Ornamental Plants Program, 65100, Van, Türkiye
E mail: ezelhanselem@yyu.edu.tr (E. ŞELEM)
Ezelhan ŞELEM https://orcid.org/0000-0003-4227-5013
Lütfi NOHUTÇU https://orcid.org/0000-0003-2250-2645
Rüveyde TUNÇTÜRK https://orcid.org/0000-0002-3759-8232
Murat TUNÇTÜRK https://orcid.org/0000-0002-7995-0599

Received: October 29, 2025
Accepted: December 08, 2025
Published: January 15, 2026

Cite as: Şelem, E., Nohutçu, L., Tunçtürk, R., & Tunçtürk, M. (2026). The effect of ascorbic acid applications on biochemical parameters in medicinal sage grown under salt stress. *Black Sea Journal of Agriculture*, 9(1): 89-99.

1. Introduction

The botanical family Lamiaceae, which comprises around 230 genera and 7100 species worldwide, is of great importance for medicine, cooking, cosmetics, and the cultivation of medicinal and aromatic plants (Akacha et al., 2024). The genus *Salvia* L., one of the largest genera in the Lamiaceae family (Nepetoidae subfamily), includes over 900 species widespread in the Old and New World's regions (Jakovljević et al., 2019). The main centers of their speciation are considered to be the eastern Mediterranean, southwest Asia, South Africa, and the Americas (Maksimović et al., 2007).

Salvia officinalis L., one of the best-known species of the genus *Salvia*, thrives at altitudes of up to 1800 m and shows remarkable resistance to soils with a pH between 5 and 9 (Karalija et al., 2022). Although it can withstand temperatures as low as −10 °C at maturity, it is advisable for the young plants to be carefully mulched. Its habitat covers a wide range of landscapes, from forests to plains, demonstrating its ecological adaptability (Afzal-Rafii, 1976). *S. officinalis* L., a valued member of the genus, presents as a robust shrub that thrives in clumps and is characterized by lanceolate leaves and delicate inflorescences (Golob, 1999; Khare et al., 2020). In contemporary discourse, extensive research has shed light on the traditional uses of *S. officinalis* and at the same time brought new pharmacological findings to light. Its pharmacopeia includes antidiabetic, anticancer, antimicrobial, and anti-inflammatory areas, as well as cognitive and memory-enhancing effects. In addition, studies on the efficacy of *S. officinalis* as a natural preservative and its potential against lipid oxidation and microbial action underline its versatility and relevance in modern therapeutic contexts (Akacha et al., 2024).

The cultivation of *S. officinalis*, a species whose use and demand are increasing worldwide, has been carried out in various provinces of our country in recent years, and tons of sage are exported every year, providing foreign exchange income to the country. *S. officinalis*, which is cultivated in our country under different climatic, soil,

and geographical conditions and holds an important place in both domestic and international markets, requires an understanding of the impact of environmental factors on agricultural success when it comes to producing high-quality, high-yield crops that meet the standards demanded by global markets. In this review, the responses of plants to certain abiotic stress factors in *S. officinalis* cultivation practices were investigated. The studies revealed that different abiotic stresses trigger different responses in this species (Elmas, 2021).

In *S. officinalis*, a plant widely cultivated in various provinces of our country, in order to achieve agricultural success, it is necessary to determine the types, intensity, and duration of stress sources to which the plant is exposed, the type of tissue and organ exposed to stress, the physiological responses and response processes of the plant under stress conditions, and the tolerance mechanisms they create. the duration and persistence of physiological damage in the plant, and the changes occurring at the cellular and genetic levels in the plant will contribute to efforts to increase plant productivity (Yuan et al., 2013; Selmar and Kleinwächter, 2013; Sönmez, 2015).

Salinity reduces osmotic potential in plants, making it difficult for them to absorb water, nutrients, and minerals, leading to toxicity and causing disruption in the plant's physiological and biochemical metabolism (Jouyban, 2012). Studies have shown that medicinal and aromatic plants exhibit common responses to salt and drought stress (Tiryaki, 2018). The general symptoms of damage caused by salt stress in plants are similar to those of drought stress, including a decrease in plant growth rate, and in cases of prolonged exposure, aging and death (Jouyban, 2012). *Salvia officinalis* is a moderately salt-tolerant glycophytic species. Whether glycophytic or halophytic, salinity causes a decrease in plant biomass when it exceeds a certain threshold in all plant species (Tounekti et al., 2012). Studies have shown that the extent of growth inhibition in *S. officinalis* varies depending on the type and concentration of salt applied. Many researchers have reported that *S. officinalis* is negatively affected by salt stress conditions in terms of many vegetative growth characteristics such as plant height, number of branches, green and dry herb, and biomass (Hendawy and Khalid, 2005; Taarit et al., 2009; Taarit et al., 2010; Çamlıca et al., 2019; Torun, 2019; Kulak et al., 2020).

The present study aimed to determine the effects of ascorbic acid applications on *S. officinalis* plants against salt stress from physicochemical and biochemical perspectives.

2. Materials and Methods

This study was carried out on June 2021 at the Department of Field Crops, Faculty of Agriculture, Van Yuzuncu Yil University (Van YYU) in growth chamber condition. The plant material used in this study was the "Elif" variety of *Salvia officinalis* L., which is registered by the Aegean Agricultural Research Institute. Characteristics of the species include an upright trunk, grayish-green leaves, purple flowers, an angular stem, black seeds, an average height of 50 cm, a yield of 500 kg da^{-1}, and an essential oil content of 2%.

The seeds of *Salvia officinalis*, obtained from the garden of medicinal and aromatic plants of Van YYU, were planted in 500cc pots in a mixture of peat + perlite + soil (1: 1: 2) and kept at growth chamber (65% RH; 8/16 hours dark/light intervals; 25 ^{o}C).

Seeds were planted in August 2021, and ascorbic acid applications began when the plants had 5-6 leaves. Ascorbic acid doses were determined as negative control (untreated), 100 ppm, 200 ppm, and 300 ppm. Ascorbic acid was applied four times, four days apart, using the drench method (Uçar et al., 2023). Nine weeks after planting, plants were treated with negative control (untreated), 100 mM, 200 mM, and 300 mM NACl (sodium chloride). A total of five applications were made, and the study concluded at week 12.

Several physiological and biochemical parameters were measured with harvesting. Chlorophyll, flavonoid, Anthocyanin content and nitrogen balance index (NBI) were measured according to Cerovic et al. (2015) using Dualex scientific + (FORCE-A, France) instrument. Measurements were made on the leaves of the plant, with a total of 3 measurements for each plant, and their averages were taken.

Total phenolic compounds content was measured according to Obanda, Owuor (1997) method. The antioxidant activity was also based on the Antioxidant Power (FRAP) (Iron (III) antioxidant power reduction) method (Benzie and Strain 1996). FRAP reagent was prepared by modifying the FRAP method (Benzie and Strain, 1996) by mixing 250 mL of 300 mM CH3COONa buffer (pH 3.6), 25 mL of TPTZ solution (10 mM TPTZ solution in 100 mM HCl), and 25 mL of 20 mM FeCl3.6H2O. 3 mL of FRAP solution was added to 100 µL of extract and incubated for 4 min at 37°C and 200 rpm with shaking. Absorbance values at 593 nm were read and antioxidant activity values were recorded as Trolox equivalent (TE) mg^{-1}. The total flavonoids content was determinated with some modifications according to the method developed by Quettier-Deleu et al. (2000). For this purpose, 1 ml of 2% $AlCl_3$ is added onto 1 ml of plant extract and kept for 70 min at room temperature in the darkness. The total amount of flavonoid was measured at 415 nm and recorded as mg $100g^{-1}$.

The determination of ascorbic acid was determined spectrophotometrically (AOAC, 1990). 400 µL of 0.4% oxalic acid and 4.5 ml of 2,6-dichlorophenolindophenol solution were added to 100 µl of supernatant and absorbance values were determined spectrophotometrically at 520 nm. The amount of ascorbic acid in the samples was calculated in mg $100g^{-1}$ with the help of the calibration curve drawn with pure ascorbic acid.

All the recorded data were subjected to analysis of variance using the COSTAT (6.3 version) software. The average data were also compared by Duncan Multiple Range Test at P<0.05 (Genç and Soysal, 2018).

3. Results

The data for the parameters examined in the study are presented in Tables 1 and 2. NBI is a non-destructive plant nutrition index obtained with Dualex that indicates the nitrogen nutrition level of the plant by measuring the balance between the amounts of chlorophyll and flavonoids in the leaves. In conditions where the plant is not under stress, low to moderate doses of ascorbic acid (around 100–200 ppm) usually support plant health by optimising redox homeostasis, sustaining chlorophyll biosynthesis, and promoting nitrogen metabolic balance. However, high doses (around 300 ppm) may go beyond the hormetic beneficial window, resulting in metabolic load and suppressed redox signalling. This can ultimately lead to physiological inhibition and reduced overall plant vitality. In the study conducted, the difference in NBI values between the means of ascorbic acid (AA) treatments and AAxS interaction was found to be statistically significant at the 1% level, while the difference between the means of salt stress (S) was found to be significant at the 5% level (Table 1). In AA treatments, the highest values were obtained from AA0, AA1 and AA2 treatments in the same Duncan group. In salt stress treatments, the highest value was obtained from the control (54.75 dx) group, the lowest value was obtained from S200 (48.44 dx), but it was observed that there was no statistical difference between them with S100 and S300. In the interaction, the highest value was obtained from AA2xS300 with 74.70 dx, and the lowest value was obtained from AA3xS300 with 37.50 dx (Figure 1). Considering the current findings, it is thought that high ascorbic acid applications may create an inhibitory effect or metabolic load on the antioxidant system.

In the study conducted, the difference in chlorophyll values between the means of ascorbic acid (AA) treatments and AAxS interaction was found to be statistically significant at the 1% level, while the difference between the means of salt stress (S) was found to be significant at the 5% level (Table 1). In ascorbic acid applications, the highest chlorophyll content was obtained from AA1 and AA2 applications in the same Duncan group, while the lowest value was obtained from AA0 and AA3 applications in the same Duncan group. Under salt stress, the highest value of 54.75dx was obtained from the control group, while the lowest value of 48.44dx was obtained from the S200 application. In interactions, the lowest and highest values were determined between 25.83 (AA0xS300) and 49.40 (AA2xS300) (Figure 2).

In the study conducted, the difference in flavonoid values between the means of ascorbic acid (AA) treatments and salt stress (S) was found to be statistically significant at the 1% level, while the difference between the means of AAxS interaction was found to be significant at the 5% level (Table 1). It was determined that there was an increase in ascorbic acid applications in parallel with increasing dose. Under salt stress, the highest value of 0.72dx was obtained from the S200 application, while the lowest value of 0.64dx was obtained from the control group. In interactions, the lowest and highest values were determined between 0.56 and 0.87dx (Figure 3). The results show that high doses of AA can trigger the production of flavonoids when the plant is under stress. Flavonoids are key secondary metabolites. They are involved in mitigating oxidative stress. Their increased accumulation suggests that AA promotes the activation of plant defense mechanisms. It also suggests that it promotes antioxidative response mechanisms.

Among ascorbic acid treatments for anthocyanin, the highest value was obtained from the AA2 treatment at 200 ppm, at 0.042 dx, while the lowest value was obtained from the AA1 treatment at 100 ppm, at 0.023 dx. It was determined that the amount increased in parallel with the increasing salt dose in the salt treatments, ranging from 0.017 to 0.062. This suggests that anthocyanins are a direct indicator of stress and that the accumulation of protective pigments increases under high salt conditions. In the within-group interaction, the highest value was obtained from the AA2xS300 interaction, at 0.080 dx, and it was found to be in the same Duncan group as the AA0xS300 interaction (0.077 dx). Fluctuations were observed in the intra-group interactions, as shown in Figure 4.

AA applications generally increased the total antioxidant activity of the plant. The highest antioxidant activity was observed in the AA2-S100 (176.59) application. This finding indicates that 200 ppm AA stimulates the antioxidant system at an optimal dose and makes the plant more resistant to oxidative stress conditions caused by salt (Figure 5). In salt stress applications, the highest values were obtained from the S100 (111.83) and S300 (114.33) applications in the same Duncan group, while the lowest value was obtained from the control group at 77.49. In ascorbic acid applications, the highest value was obtained from the AA100 application at 113.16. The lowest antioxidant activities were observed in combinations where 300 ppm AA was applied and low stress conditions were present. This indicates that the effectiveness of AA depends not only on its dose but also on the stress level.

Table 1. Dualex parameters of *S. officinalis*

Ascorbic Acid (ppm)	Salt Stress (mM)	NBI (dx)	Chlorophyll (dx)	Flavonoid (dx)	Anthocyanin (dx)
	S0 (Control)	53.00±1.18^{bcd}	29.43±0.93^{ef}	0.56±0.03^{d}	0.013±0.01^{f}
Control (AA0)	S100	58.13±1.18^{bc}	35.73±1.22^{bcd}	0.66±0.01^{bcd}	0.023±0.01^{ef}
	S200	57.03±1.88^{bc}	34.47±2.17^{cde}	0.60±0.03^{cd}	0.033±0.01^{de}
	S300	47.90±4.13^{cd}	25.83±0.79^{f}	0.56±0.04^{d}	0.077±0.01^{a}
AA0 Avr.		54.02±3.24^{A}	31.37±3.11^{B}	0.60±0.03^{C}	0.037±0.02^{B}
	S0 (Control)	60.00±4.13^{b}	36.77±0.94^{bc}	0.62±0.04^{cd}	0.013±0.01^{f}
100 (AA1)	S100	62.90±3.62^{b}	38.30±1.75^{b}	0.63±0.03^{cd}	0.013±0.01^{f}
	S200	49.97±5.95^{cd}	34.80±0.26^{cd}	0.71±0.01^{bc}	0.033±0.01^{de}
	S300	47.23±2.58^{cd}	30.87±1.16^{e}	0.72±0.04^{b}	0.033±0.01^{de}
AA1 Avr.		55.03±4.83^{A}	35.18±2.15^{A}	0.67±0.03^{B}	0.023±0.01^{C}
	S0 (Control)	55.27±0.53^{bc}	27.07±1.79^{f}	0.69±0.02^{bc}	0.020±0.01^{f}
200 (AA2)	S100	45.60±1.95^{d}	30.33±1.04^{e}	0.69±0.03^{bc}	0.043±0.01^{c}
	S200	48.27±5.80^{cd}	33.57±1.85^{de}	0.70±0.04^{bc}	0.023±0.01^{f}
	S300	74.70±1.44^{a}	49.40±0.69^{a}	0.67±0.02^{bc}	0.080±0.01^{a}
AA2 Avr.		55.96±9.29^{A}	35.09±5.89^{A}	0.69±0.01^{B}	0.042±0.02^{A}
	S0 (Control)	50.73±2.36^{cd}	35.43±0.63^{cd}	0.70±0.02^{bc}	0.023±0.01^{f}
300 (AA3)	S100	44.10±2.07^{d}	33.53±1.74^{de}	0.77±0.05^{b}	0.030±0.01^{e}
	S200	38.50±1.75^{de}	33.43±0.98^{de}	0.87±0.02^{a}	0.037±0.01^{d}
	S300	37.50±2.27^{e}	27.87±1.64^{f}	0.74±0.01^{b}	0.057±0.01^{b}
AA3 Avr.		42.71±2.05^{B}	32.57±1.87^{B}	0.77±0.04^{A}	0.037±0.01^{B}
	S0 (Control)	54.75±2.68 ^{A}	32.17±3.04 ^{B}	0.64±0.03 ^{C}	0.017±0.01 ^{C}
Salt Stress Avr.	S100	52.68±6.03 ^{AB}	34.47±2.31 ^{A}	0.69±0.04 ^{AB}	0.027±0.01 ^{B}
	S200	48.44±2.11 ^{B}	34.06±2.40 ^{AB}	0.72±0.01 ^{A}	0.031±0.01 ^{B}
	S300	51.83±1.14 ^{AB}	33.49±6.73 ^{AB}	0.67±0.02 ^{BC}	0.062±0.01 ^{A}
Coefficient of Variation (%)		10.33	6.86	7.83	15.04
Ascorbic Acid (AA)		**	**	**	**
Salt Stress (S)		*	*	**	**
AA x S		**	**	*	**

Avr= average, AA= Ascorbic acid, AA0= Control, AA1= 100ppm, AA2=200 ppm, AA3= 300 ppm, S= Salt stres, S0= Control, S100= 100 mM salt, S200= 200 mM salt, S300= 300 mM salt, *P<0.05, ** P<0.01.

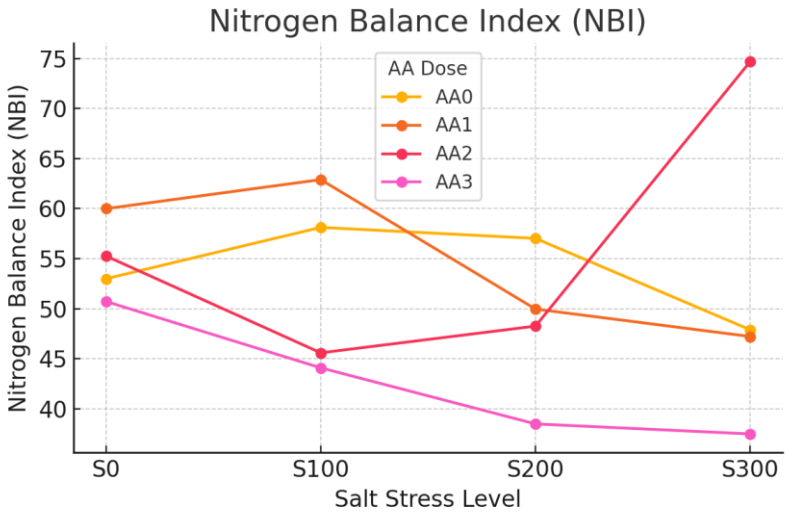

Figure 1. Effect of applied ascorbic acid and salt doses on NBI value in plants (AA: Ascorbic acid, AA0: Control, AA1: 100ppm, AA2:200 ppm, AA3: 300 ppm).

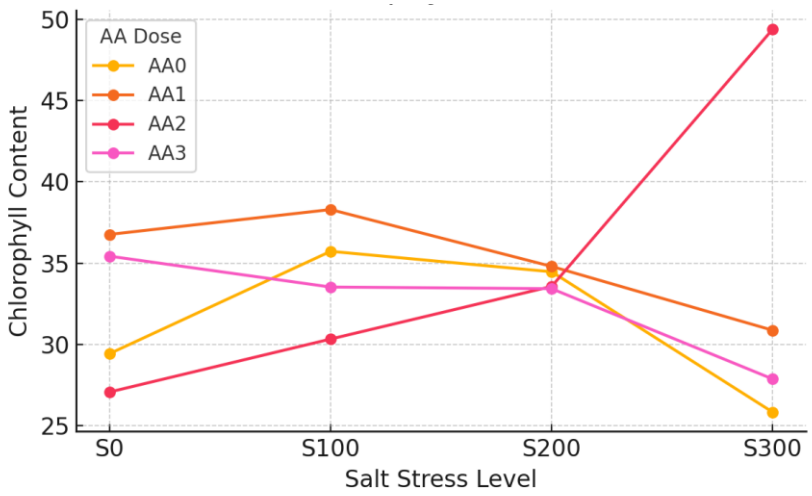

Figure 2. Effect of applied ascorbic acid and salt doses on chlorophyll value in plants (AA: Ascorbic acid, AA0: Control, AA1: 100ppm, AA2:200 ppm, AA3: 300 ppm).

Table 2. Biochemical parameters of *S. officinalis* plant

Ascorbic Acid (ppm)	Salt Stress (mM)	Total antioxidant activity (µmol TE g^{-1})	The total flavonoid content (QE 100 g^{-1})	Total phenolic compound (mg GAE g^{-1})	Total ascorbic acid content (mg $100g^{-1}$)
	S0 (Control)	73.78±2.35^{fg}	11.80±0.50^{b}	224.71±4.95^{ab}	19.96±1.23
Control (AA0)	S100	110.66±0.54^{cd}	11.31±0.48^{b}	234.36±2.06^{ab}	21.05±1.86
	S200	88.94±1.71^{ef}	8.65±0.06^{cd}	174.00±21.44^{c}	21.74±0.73
	S300	122.22±0.18^{c}	13.77±0.30^{a}	229.18±3.40^{ab}	22.37±0.47
AA0 Avr.		98.90±9.76 ^{B}	11.38±1.48 ^{A}	215.56±19.31 ^{B}	21.28±0.38
	S0 (Control)	68.78±3.43^{g}	7.85±0.16^{de}	221.86±5.36^{ab}	22.46±0.73
100 (AA1)	S100	88.78±2.17^{ef}	7.56±0.26^{e}	225.43±5.77^{ab}	22.74±1.21
	S200	118.47±4.69^{c}	13.59±0.18^{a}	233.46±1.96^{ab}	23.01±1.05
	S300	121.44±4.78^{c}	8.67±0.13^{c}	228.46±5.46^{ab}	22.83±1.21
AA1 Avr.		99.37±10.43 ^{B}	9.42±1.85 ^{B}	227.30±2.34 ^{A}	22.76±0.08
	S0 (Control)	103.63±1.35^{d}	7.85±0.39^{de}	226.68±3.81^{ab}	24.92±0.31
200 (AA2)	S100	176.59±4.87^{a}	11.80±0.29^{b}	237.39±2.58^{a}	21.37±1.31
	S200	104.25±1.89^{d}	7.96±0.03^{de}	234.00±0.21^{ab}	19.96±1.29
	S300	68.16±11.19^{d}	6.54±0.13^{f}	210.07±12.78^{b}	22.65±0.16
AA2 Avr.		113.16±10.93 ^{A}	8.53±1.57 ^{C}	227.04±8.60 ^{A}	22.22±0.77
	S0 (Control)	63.78±0.36^{g}	6.54±0.07^{f}	219.71±3.51^{b}	19.69±1.60
300 (AA3)	S100	71.28±0.36^{g}	5.52±0.05$^{g0.05}$	221.86±1.65^{b}	23.69±1.34
	S200	95.81±6.04^{de}	6.48±0.27^{f}	231.14±4.33^{ab}	21.74±2.41
	S300	145.50±4.96^{b}	10.60±0.03^{b}	234.71±0.62^{a}	24.69±0.71
AA3 Avr.		94.09±11.83 ^{B}	7.28±1.86 ^{D}	226.86±3.83 ^{A}	22.45±0.87
	S0 (Control)	77.49±12.52 ^{C}	8.51±0.44 ^{C}	223.24±2.06 ^{AB}	21.76±1.51
Salt Stress Avr.	S100	111.83±3.58 ^{A}	9.05±1.85 ^{B}	229.76±4.70 ^{A}	22.21±0.67
	S200	101.87±4.85 ^{B}	9.17±1.89 ^{B}	218.15±2.36 ^{B}	21.61±1.44
	S300	114.33±4.93 ^{A}	9.89±1.17 ^{A}	225.61±7.40 ^{AB}	23.13±0.65
Coefficient of Variation (%)		7.22	4.83	5.51	9.66
Ascorbic Acid (AA)		**	**	*	ns
Salt Stress (S)		**	**	*	ns
AA x S		**	**	**	ns

Avr= average, AA= Ascorbic acid, AA0= Control, AA1= 100ppm, AA2=200 ppm, AA3= 300 ppm, S= Salt stres, S0= Control, S100= 100 mM salt, S200= 200 mM salt, S300= 300 mM salt, *P<0.05, ** P<0.01.

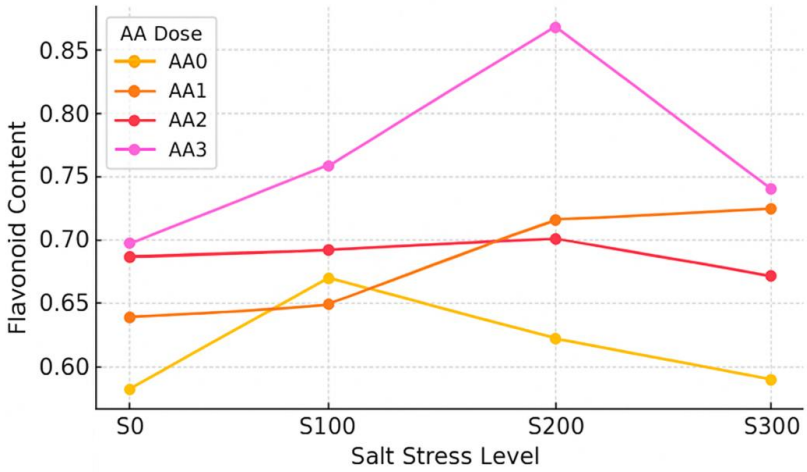

Figure 3. Effect of applied ascorbic acid and salt doses on flavonoids (dx) value in plants (AA: Ascorbic acid, AA0: Control, AA1: 100ppm, AA2:200 ppm, AA3: 300 ppm).

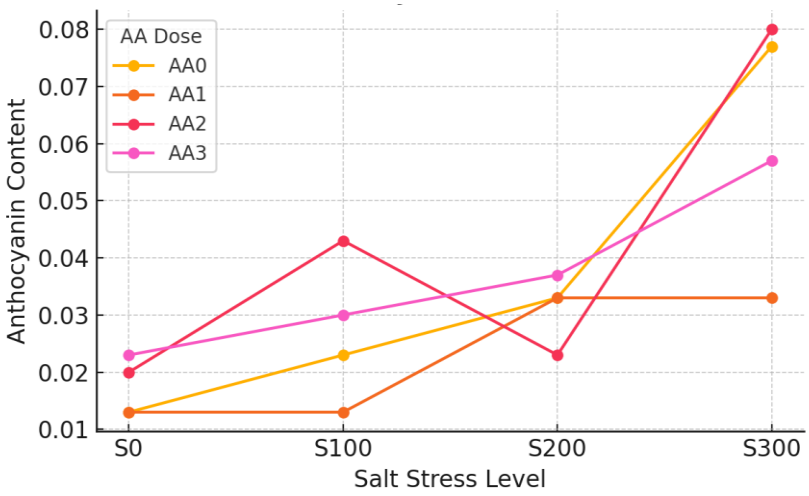

Figure 4. Effect of applied ascorbic acid and salt doses on anthocyanin value in plants (AA: Ascorbic acid, AA0: Control, AA1: 100ppm, AA2:200 ppm, AA3: 300 ppm).

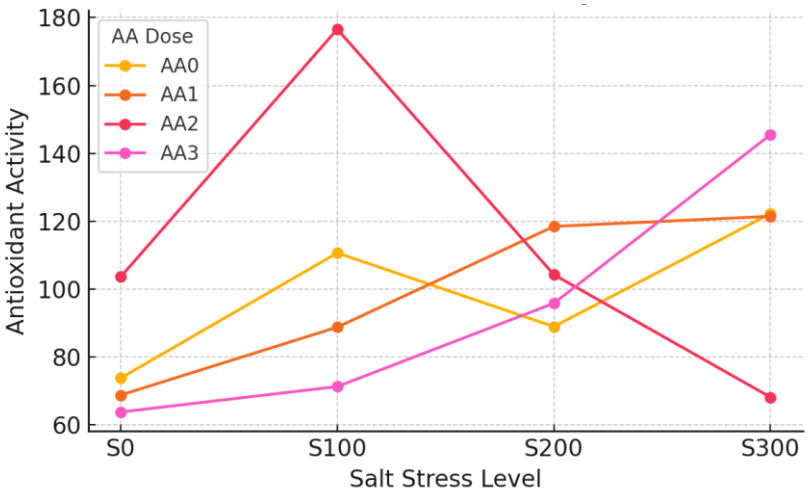

Figure 5. Effect of applied ascorbic acid and salt doses on total antioxidant activity in plants (AA: Ascorbic acid, AA0: Control, AA1: 100ppm, AA2:200 ppm, AA3: 300 ppm).

The total flavonoid content was also significantly affected by both AA and salt stress. Notably, flavonoid levels generally showed a decreasing trend as the AA dose increased. The highest flavonoid levels were observed in the control groups and low-dose AA applications. This suggests that high-dose AA applications may suppress

certain secondary metabolic pathways or that flavonoids may be consumed outside the defense system. In salt stress, an increase in total flavonoid content was observed in parallel with increasing salt doses. The results obtained from within-group interactions are shown in Figure 6, with the highest values obtained from the AA0x S300 (13.77) and AA1xS200 (13.59) interactions within the same Duncan group.

Total phenolic compound levels were found to be quite high in all groups and increased particularly in the 300 ppm AA and high salt stress treatments. The highest value in intra-group interactions was obtained in the AA2xS200 (237.39) and AA3xS300 (234.71) combinations within the same Duncan group (Figure 7). This increase indicates that phenolic compounds provide an important response to stress signals in the plant's defense system and that high doses of AA may enhance these defense responses.

When the total ascorbic acid content in plant tissues was evaluated, no statistically significant difference was observed between the treatments. Considering the results obtained, ascorbic acid applications showed changes in the range of 21.28-22.76, salt stress applications in the range of 21.61-23.13, and group-internal interaction in the range of 19.69-24.92. This result suggests that externally applied AA may not directly reflect plant content or that internal (endogenous) regulatory mechanisms limit the accumulation of this compound.

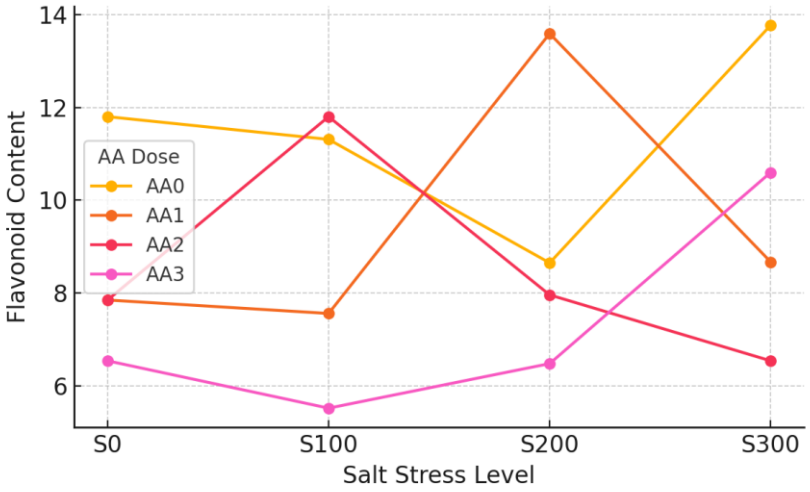

Figure 6. Effect of applied ascorbic acid and salt doses on total flavonoid content value in plants (AA: Ascorbic acid, AA0: Control, AA1: 100ppm, AA2:200 ppm, AA3: 300 ppm).

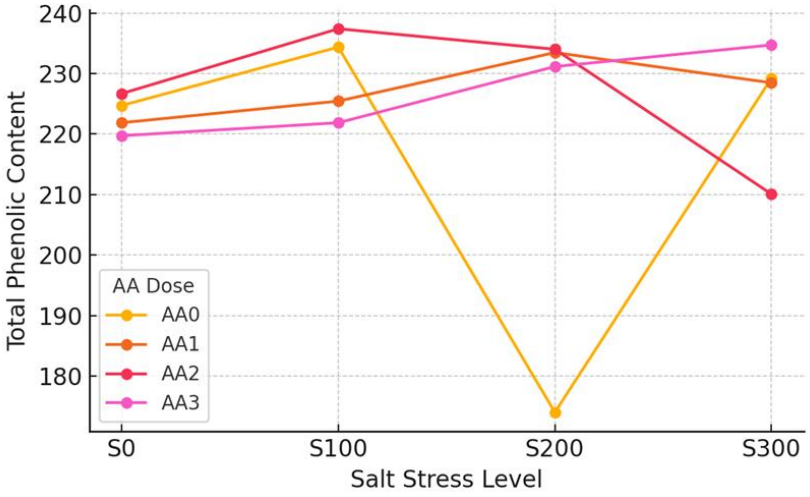

Figure 7. Effect of applied ascorbic acid and salt doses on total phenolic content in plants (AA: Ascorbic acid, AA0: Control, AA1: 100ppm, AA2:200 ppm, AA3: 300 ppm).

4. Discussion

The degree of cellular oxidative damage in plants exposed to abiotic stress such as salt stress is controlled by the plants' capacity to produce antioxidant substances (Aziz et al., 2013). The response of *S. officinalis* to abiotic stress factors involves the regulation of secondary metabolite levels, including essential oils and phenolic compounds. Phenolic compounds exhibit antioxidant activity by inactivating free radicals and/or preventing the decomposition of hydroperoxides into free radicals (Valifard et al., 2014).

It has been reported that moderate salinity in *S. officinalis*

supports antioxidant protection by increasing the accumulation of carotene and α-tocopherol in plant leaves, but at higher salinity levels, it affects the photosynthetic process by disrupting photosynthetic enzymes, chlorophylls, and carotenoids (Tounekti et al., 2011; Tounekti et al., 2012).

Altay (2015) investigated the total phenolic content, total flavonoid content, and antioxidant activity parameters of the *Salvia fruticosa* plant. According to the study, the total phenolic content was found to be 175.2±5.07 μg mg^{-1}, and the total flavonoid content was 108.9±2.61 μg mg^{-1}. The flavonoid content of *Salvia macrochlamys*, *Salvia kronenburgeii*, and *Salvia huberi* extracts was calculated as 34.2, 30.1, and 21.2 mg g^{-1}, respectively (Akıcı, 2018). The total phenolic content of methanol extracts prepared from the aerial parts of *Salvia verticillata* subsp. *amasica* was found to be 275.76±2.14 mg g^{-1}, and the flavonoid content was 15.05±0.83 mg g^{-1}. In *Salvia adenophylla*, the total phenolic content in methanol extracts was determined to be 92.12±1.78 mg g^{-1} and the flavonoid content was 25.32±2.50 mg g^{-1} (Hatipoğlu, 2010). Alimpic et al. (2017) conducted a biological activity study on *Salvia amplexicaulis* Lam. and found that the phenolic content of this plant was 99.1±0.74 mg g^{-1} in methanol extracts and the flavonoid content was 42.7±1.54 mg g^{-1} in methanol extracts. The studies conducted support this study, and when compared, it can be said that it has sufficient phenolic and flavonoid content.

In *S. officinalis* plants exposed to salt stress, the total phenolic content was lowest in the control group (34.58 mg GAE g^{-1} DW) and highest in the 75 mM salt treatment (40.66 mg GAE g^{-1} DW) (Taarit et al., 2012). Moshari-Nasirkandi et al., (2024) reported TPC values for the 102 *Salvia* plants, ranging from 12.67 to 62.46 mg GAE g^{-1} DW, for *S. officinalis* ranged from 2.02 to 184 mg GAE g^{-1} DW. Alizadeh and Shaabani (2012) reported a TPC content of 25.13 mg GAE g^{-1} DW for Iranian *S. officinalis*. Similar ranges of phenolic amounts were obtained for some *Salvia* species such as *S. lanceolata*, *S. dolomitica*, and *S. garepensis* (54.2, 53.0, and 45.6 mg GAE g^{-1} DW, respectively) (Kamatou et al., 2010).

Salt stress causes a decrease in AA (ascorbic acid) biosynthesis. AA catabolism increases compared to its synthesis and renewal during salinity (Song et al., 2005; Amor et al., 2006). In some plants that adapt to salt stress, an increase in ascorbate content has been observed. Ascorbic acid, along with other antioxidants, helps minimize oxidative damage and stabilize membranes by detoxifying H_2O_2 and other AOTs (Shao et al., 2007). The IAA (Indol Asetic Acid) content in plant tissues increases due to ascorbic acid application, which promotes cell division and expansion, thereby improving plant growth (Hassanein et al., 2009).

Torlak (2019) conducted a study in which he applied salt + ascorbic acid to corn plants and found that, similar to our study, it did not cause significant changes in ascorbic acid content. This is because total ascorbic acid content is tightly regulated in plants, so exogenous AA is rapidly recycled through the ascorbate–glutathione cycle, maintaining overall levels while still enhancing antioxidant activity.

A study reported that the L-ascorbic acid content in *S. officinalis* increased with plant development depending on harvest time, reaching a maximum level of approximately 17.6 mg·100 g^{-1} FW (fresh weight) prior to the flowering stage (Baranauskiene et al., 2011). In a broader study involving intra-family and interspecific comparisons within the Lamiaceae, ascorbic acid content in different *Salvia* species was reported to range from approximately 36 (*Salvia nemorosa*) to 50 (*Salvia multicaulis*) mg AA·100 g^{-1} DW (dry weight) (Moshari-Nasirkandi et al., 2024).

It is certain that drought-affected plants have significantly higher chlorophyll concentrations per cm^2. However, it should not be forgotten that drought-affected plants consistently have smaller leaf areas. Since chlorophyll production does not slow down while leaf expansion slows down during ongoing drought, it is expected that chlorophyll concentrations will be higher on a per-area basis in drought-affected plants (Fletcher, 2021). It was found that chlorophyll values decreased compared to the control group in 4 soybean varieties under 5 different salt applications (Özçınar et al., 2022). Additionally, in bean plants exposed to salt stress, it was determined that chlorophyll content decreased in the plant after a certain dose (Çirka et al., 2022).

Flavonols have been reported to prevent excessive damage to plants by triggering a cooling mechanism in response to stress caused by salt and drought (Agati et al., 2012). In a study conducted on black cumin, it was found that the plant increases its flavonol content as a protective mechanism against stress (Aghajanzadeh-Gheshlaghi et al., 2021). Oral et al. (2024) found that the highest flavonol content in soybean plants was 0.66 dx in T4, and the lowest was 0.38 dx in T0, as a result of salt dose applications (control, 50, 100, 150, 200 mM).

Anthocyanins, a member of the flavonoid family, contribute to increased resistance to environmental stress factors in plants (Chalker-Scott 1999). In *Hyssopus officinalis* L., phenol and anthocyanin levels were found to increase in response to salt stress (Jahantigh et al., 2016). It is thought that nitrogen allocation to leaves under drought conditions increases as plants create a nitrogen source before the decrease in soluble substance transport in the phloem (Sevanto, 2014). Oral et al. (2024) reported that the highest NBI value of 46.2 mg g-1 was obtained from control applications, while the lowest value of 33.7 mg g^{-1} was obtained from the 200 mM dose in soybean plants following salt applications. In a similar study, it was reported that the NBI value in fenugreek plants decreased to a range of 54.5–59.85 mg g^{-1} under drought stress (Yolci et al., 2022). The study findings indicate that AA supports NBI and, therefore, nitrogen metabolism in plants, particularly under stress. However, this effect occurs within a critical dose range. Higher doses may inhibit nitrogen metabolism due to the risk of

creating an imbalance in the antioxidant system and metabolic overload. Therefore, AA applications should be considered as a biochemical regulator with high potential in the context of plant nutrition and stress physiology, but requiring dose optimization.

Oral et al. (2021) found that plants grown under limited irrigation stress in soybeans (*Glycina max* L.) had a nitrogen balance index of 70.64–82.90 (dualex value), flavonol 0.375–0.398 (dualex value), and anthocyanin 0.016–0.045 (dualex value) and reported that the ratios decreased due to stress. Zhang et al. (2024) determined chlorophyll levels of 31.43–26.47 ug cm^{-2} and flavonoid levels of 0.043–0.048 ug cm^2 in *Gossypium hirsutum* plants under control and 150 mmol·L^{-1} NaCl stress, respectively.

Buchaillot et al. (2025) applied salt stress at 2, 5, and 10 dS m^{-1} levels in Moringa and Pomegranate plants. In Moringa plants, at 2.5 and 10 dS m^{-1} doses, the chlorophyll content was 18.48, 25.96, and 22.86 µg cm^{-2}, respectively, and the flavonoid content was 1.98, 1.99, and 1.96 relat. Units, anthocyanins were 0.08, 0.06, and 0.08 relat. Units, NBI was determined to be 9.48, 13.01, and 11.49, while in pomegranate plants, chlorophyll content was 9.16, 16.86, and 13.83 µg cm^{-2}, flavonoid content was 2.04, 2.02, and 1.93 relat. Units, anthocyanins were determined to be 0.22, 0.13, and 0.20 relat. Units, and NBI was determined to be 4.44, 8.57, and 6.56. In line with the results of the relevant literature, it was observed that the damage caused by salt stress in sage was reduced by ascorbic acid applications.

5. Conclusion

The data obtained clearly demonstrate that ascorbic acid application provides protective effects against salt stress in medicinal sage plants. In particular, it has been determined that the 200 ppm dose provides the optimal effect in terms of many parameters, increasing the plant's stress tolerance by stimulating physiological and biochemical defense systems. Increases in NBI and chlorophyll content demonstrate improved plant health through strengthened photosynthetic performance and balanced nitrogen metabolism, and the accumulation of flavonoids and anthocyanins reflects adaptive activation of stress-responsive defense mechanisms; however, the reductions observed at high ascorbic acid doses indicate potential disruption of redox homeostasis and metabolic burden-related physiological inhibition, suggesting a dose threshold beyond which plant health may be adversely affected.

Additionally, the presence of significant interactions between salt stress levels and AA applications indicates that plant responses to stress conditions are not dependent on a single factor but rather involve an interactive and multifaceted regulatory mechanism. Therefore, AA applications may be considered a potential area of application in agricultural biotechnology and stress physiology.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	E.Ş.	L.N.	R.T.	M.T.
C	25	25	25	25
D	25	25	25	25
S	50	50		
DCP			50	50
DAI	25	25	25	25
L	25	25	25	25
W	25	25	25	25
CR	25	25	25	25
SR	25	25	25	25
PM	25	25	25	25
FA	25	25	25	25

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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