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Methanol foliar spraying improves biochemical and physiological attributes of coriander (*Coriandrum sativum*) plants under salt stress

Original paper

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Abstract: Salinity stress is one of the most significant factors limiting plant growth; therefore, using compounds such as methanol to mitigate the harmful effects of stress is of great importance. This study aimed to investigate the effect of methanol spraying on the physiological and biochemical characteristics of coriander (*Coriandrum sativum*) plants under salinity stress conditions in a greenhouse experiment. Salinity treatment was applied at two levels (control and 80 mM NaCl) and methanol was exogenously applied at four concentrations (control, 10%, 20%, and 30%). The results indicated that under salinity stress conditions, the application of methanol led to a significant increase in various traits. Superoxide dismutase enzyme activity increased by 23% with 30% methanol spraying under salinity stress. Additionally, polyphenol oxidase enzyme activity rose by 51% with 30% methanol at 80 mM salinity, while catalase activity increased by 29%. Proline content also increased by 124% under salinity stress, while malondialdehyde content decreased by 63% in these conditions. Overall, 30% methanol enhances stress tolerance in plants under salinity conditions by improving antioxidant enzyme activities, protein content, and osmoprotective mechanisms. These findings suggest the role of methanol's in mitigating the effects of salinity stress on plant physiology.

Keywords: Electrolyte leakage, enzyme, malondialdehyde, pigment, sodium chloride.

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Introduction

Coriander (*Coriandrum sativum*) is a valuable herb and aromatic plant known for its medicinal and nutritional properties. This plant is rich in vitamins, minerals, and antioxidants, particularly serving as a good source of vitamins C and K, potassium, and fiber (Gantait et al., 2022). The medicinal properties of coriander include improving digestion, reducing inflammation, and helping in blood sugar control. Additionally, coriander exhibits antibacterial and antifungal effects and may assist in detoxifying the body. Consumption of coriander may also help reduce anxiety and enhance mental health (Diederichsen et al., 2020; Gantait et al., 2022).

Salinity stress is one of the most significant environmental factors affecting plant growth and development, particularly in arid and semi-arid regions (Hosseini et al., 2023). Soil salinity can arise from groundwater extraction or irregular rainfall, leading to decreased soil fertility and disrupted plant growth (Singh, 2022). Plants under salinity stress experience reduced water uptake capacity, diminished greenness, and, in severe cases, death (Khalvandi et al., 2019; Rabiei et al., 2020; Hosseini et al., 2023). Salinity causes the accumulation of toxic ions such as sodium and chloride in plant tissue, which can disrupt metabolic activities (Hosseini et al., 2023).

On the other hand, methanol, as a simple organic compound, can stimulate and improve physiological processes in plants. Some studies have shown that foliar spraying of methanol can act as a stimulant in correcting the negative effects of salt stress (Wei et al., 2015). We hypothesize that methanol enhances reactive oxygen species (ROS) scavenging under salinity stress, helping to maintain the internal balance of the plant by affecting the activity of enzymes and antioxidant systems and preventing the harmful effects of stress factors (Tavassoli and Galavi, 2011; Dorokhov et al., 2018). One of the most important effects of methanol is to improve the nitrogen and carbon status in plants, which can lead to an increase in chlorophyll levels and an improvement in the photosynthetic capacity of the plant (Wei et al., 2015; Dorokhov et al., 2018).

Antioxidant enzymes, including peroxidase, catalase, and superoxide dismutase, play a crucial role in mitigating the damaging effects of free radicals in plant cells (Singh et al., 2022). Salinity stress induces oxidative stress, increasing the production of free radicals, which necessitates a more robust antioxidant defense system (Yilmaz et al., 2023). Investigating changes in antioxidant enzyme activity in response to salinity stress, along with the positive effect of methanol, can enhance our understanding of plant responses to these conditions.

Another important parameter in assessing the effects of salinity stress is the relative water content (RWC) of leaves (Patanè et al., 2022). Reduced water content in stressed plants often leads to an imbalance in water and nutrient uptake. This is one of the primary reasons for the reduction of photosynthetic energy and, consequently, reduced growth and yield in plants (Browne et al., 2020). Studying changes in RWC can provide a valuable tool for investigating the effects of salinity stress on plant water status (Patanè et al., 2022).

Proline, considered a non-essential amino acid, plays a signifinant role in plant resistance to environmental stresses such as salinity and drought (Yan et al., 2024). In fact, the accumulation of proline in plant tissues is recognized as a marker of stress tolerance, as this compound can help stabilize proteins and cell membranes (Shahid et al., 2022). Electrolyte leakage (EL) is another measure for assessing damage caused by salt stress in plants (Abdelaal et al., 2021). Increased EL indicates damage to the cell membrane and the irreversibility of stress-induced damage (Kovaleski and Grossman, 2021). Studying EL can provide further insights into the level of cllular damage caused by salt stress (Abdelaal et al., 2021).

Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) are other important indicators for assessing oxidative stress and its resulting damage (Goncharuk et al., 2022). Increased levels of these compounds usually occur due to increased free radical activity and environmental stresses such as salt stress (Hosseini et al., 2023). Examining the levels of these compounds can provide a more accurate assessment of the effects of methanol as a protective factor against salt stress. Total soluble sugar (TSS) also plays an important role as an energy and metabolic source in plants and can help regulate plant metabolism under stress conditions

(Hosseini et al., 2023). Changes in soluble sugar levels under stress can indicate the metabolic status of the plant and serve as a marker for assessing the effects of salinity stress (Hosseini et al., 2023).

In summary, this research uniquely investigates the relationship between salinity stress and methanol foliar application, focusing on its effects on antioxidant enzyme activity, chlorophyll content, relative water content (RWC), proline levels, electrolyte leakage (EL), malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and total soluble sugars (TSS). Unlike previous studies, which often explored individual aspects of plant responses to either salinity or methanol, this study provides a comprehensive analysis of multiple physiological and biochemical traits in coriander plants under combined stress conditions. This research not only enhances our understanding of coriander plant responses to salinity stress but also offers innovative strategies to improve plant performance under saline conditions and amid climate change challenges.

Materials and Methods

Plant materials and treatments

To investigate the effect of methanol foliar spraying on the coriander medicinal plant under salinity stress, the present experiment was conducted in greenhouse conditions at the National Skills Training Center for Girls in Amol-(Tohid). A pot experiment was designed as a factorial experiment in a completely randomized design with three replications. The first factor was salinity stress, which was applied at two levels (control and 80 mM salinity) from a sodium chloride source. The second factor was methanol spraying, which included four levels (control, methanol at concentrations of 10%, 20%, and 30%).

After physical purification, the seeds were disinfected and sterilized in sodium hypochlorite solution for 1 minute, followed by several washedwith distilled water. The seeds were then soaked in distilled water for 2 hours to facilitate the dehydration process.

Soil preparation

To prepare the cultivation medium, a layer of sand was poured into each pot (20 cm deep and 10 cm wide) for drainage, followed by the addition of 5 kg of soil. The results related to the physical and chemical properties of the soil are presented in Table 1.

Nutrient supplementation

To meet the nutritional needs of the plants, specific amounts of different fertilizers were calculated for each pot, which included 0.6 g of phosphorus (P_2O_5), 0.6 g of potassium sulfate (K_2SO_4), and 0.3 g of nitrogen (from urea), based on the plants' fertilizer requirements.

Greenhouse conditions

The greenhouse conditions during the experiment were carefully maintained, with a minimum temperature of 17°C and a maximum temperature of 23°C. The light intensity was approximately 300 µmol m^{-2} s^{-1}, and the photoperiod was set to 16 hours of light and 8 hours of darkness, with humidity levels kept around 60-70% to ensure optimal growth conditions.

Experimental design

For the experimental design, a total of 30 coriander plants were used, with 10 plants assigned to each treatment group and three replicates for each treatment to ensure statistical reliability. During seed selection for the experiment, weak seeds were discarded, as they have lower germination rates and viability, exacerbated by salt stress.

Treatment application

Two weeks after planting, salinity stress was applied to the plants, and methanol foliar spraying was conducted every 7 days until the end of the growth stage.

Sampling

At the end of the growing season, samples were collected from the leaves of the coriander plants to measure various biochemical and physiological parameters. These samples were immediately placed in liquid nitrogen and transferred to a -80°C refrigerator for further analysis.

Characteristic measurements

The amount of hydrogen peroxide (H_2O_2) was determined using the method of Alexieva et al. (2001), based on the reaction of H_2O_2 with potassium iodide. Protein content was measured using the Bradford method (Bradford, 1976). The activity of catalase (CAT) and superoxide dismutase

(SOD) activity were assessed according to Keshavarz and Khodabin (2019), ascorbate peroxidase (APX) using the method of Nakano and Asada (1981), and polyphenol oxidase (PPO) enzymes using the method of Lee et al. (2021). Electrolyte leakage (EL) was measured following the protocol of Shi et al. (2006). The proline amino acid content was determined using the method of Bates et al. (1973) and malondialdehyde (MDA) levels were measured according to the method of (Zhang et al., 2008).

Statistical analysis

At the end of the experiment, data were tested for normality using the Kolmogorov-Smirnov method and subsequently analyzed using SAS 9.1 statistical software. Comparisons of means were conducted using the least significant difference (LSD) method at a statistical significance level of 5%, and then graphs were drawn using EXCEL software. Also, XLSTAT (2016) software was utilized for principal component analysis and cluster analysis, employing the varimax method for component rotation. the UPGMA (unweighted pair group method with arithmetic mean) and Euclidean distance were used to construct dendrograms, and boxplots were created to compare groups resulting from the cluster analysis.

Results

SOD activity

The analysis of variance revealed that SOD enzyme activity was significantly influenced by salinity stress, methanol application, and their interaction ($P \leq 0.01$) (Table 2). The interaction results indicated that the application of 30 % methanol under salinity stress condition resulted in the highest SOD enzyme activity (38.65 U mg^{-1} protein min^{-1}); In contrast, the lowest SOD activity was observed in the treatments of no methanol application under no salinity condition (8.12 U mg^{-1} protein min^{-1}) and the application of 10% methanol under no salinity condition (8.94 U mg^{-1} protein min^{-1}) (Figure 1).

Table 1. Physiochemical properties of soil.

Soil texture	K (mg/kg)	P (mg/kg)	Total N (%)	OM (%)	pH	EC	Clay (%)	Silt (%)	Sand (%)
Silt loam	153	7.4	0.08	0.56	7.83	1.12	17	54	29

Table 2. Mean squares of SOD, PPO, CAT, APX, protein and proline affected by salinity stress treatment and methanol foliar spraying in coriander plant.

Source of variation	DF	SOD	PPO	CAT	APX	Protein	Proline
Salt stress (A)	1	2272.05**	0.8978**	0.952**	11250**	14.85**	14248.4**
Methanol (B)	3	415.33**	0.207**	0.2033**	2444.94**	6.08**	938.58**
A × B	3	51.17**	0.0774**	0.0459**	671.39**	2.14**	186.06**
Residual	16	0.84	0.0007	0.0004	2.35	0.01	2.57
CV (%)		4.39	5.16	4.65	3.88	5.07	4.12

** indicates significance at the 1% probability level. superoxide dismutase (SOD), polyphenol oxidase (PPO), catalase (CAT), and ascorbate peroxidase (APX).

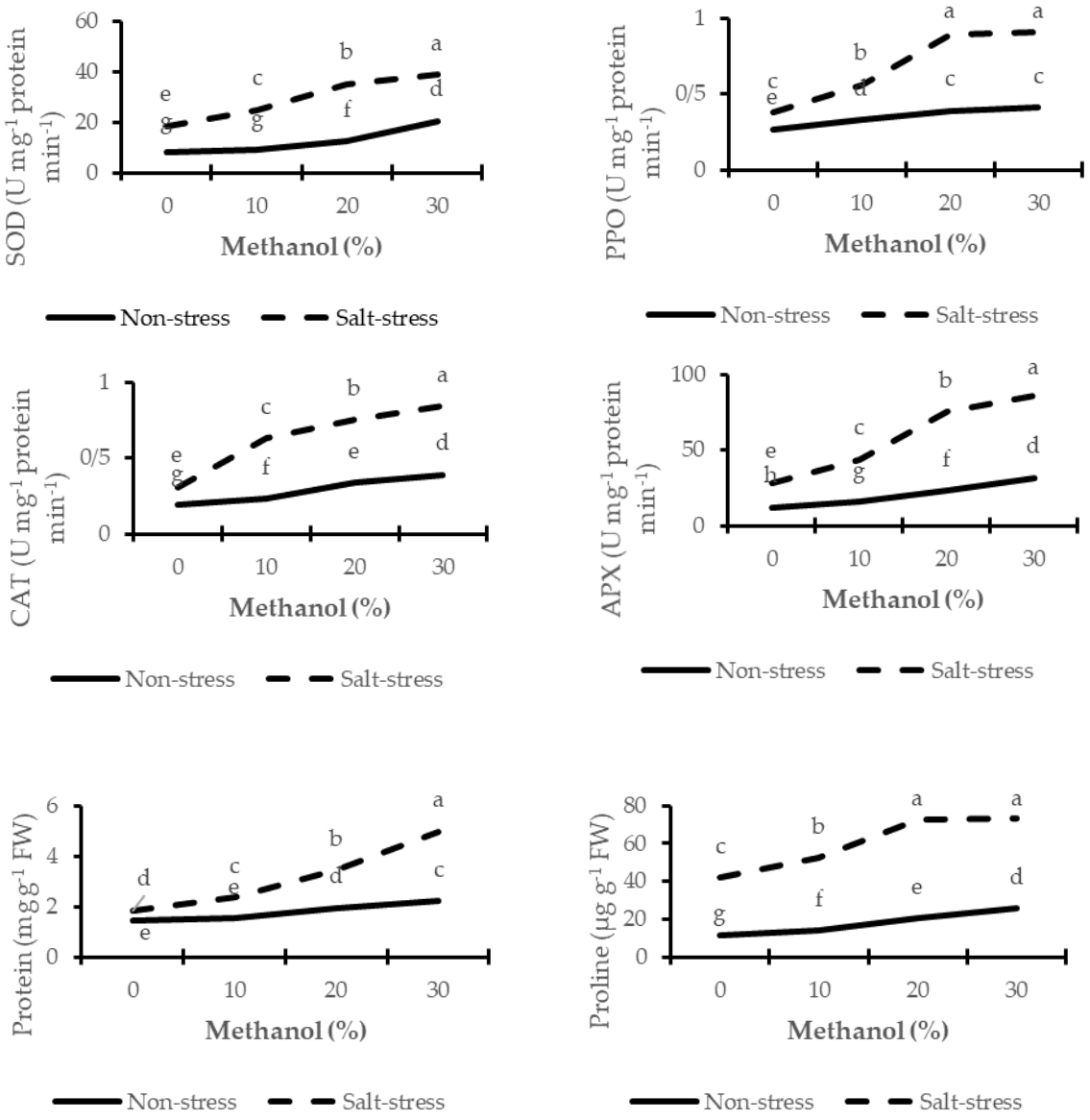

Figure 1. Results of mean comparison of superoxide dismutase (SOD), polyphenol oxidase (PPO), catalase (CAT), ascorbate peroxidase (APX), protein and proline characteristics under salinity stress (normal and 80 mM salinity stress) and methanol foliar spraying (non-spraying of methanol, 10% methanol, 20% methanol and 30% methanol) in coriander plants. The columns in the chart represent the mean ± SE. Columns with the same letters do not statistically differ from each other.

PPO activity

Data from the experiment indicated that the effects of salinity stress, methanol, and their interaction were significant for the PPO enzyme activity ($P \leq 0.01$) (Table 2). In the absence of salinity stress, the application of 20 and 30% methanol treatments improved the PPO enzyme activity by 44% and 51%, respectively compared to the control treatment without methanol (Figure 1). The highest PPO activity was recorded with 20% and 30% methanol

under 80 mM salinity conditions, measuring 0.89 and 0.91 U mg^{-1} protein min^{-1}, respectively. In contrast, the lowest PPO enzyme activity was observed in the treatment without methanol application and salinity stress (0.27 U mg^{-1} protein min^{-1}) (Figure 1).

CAT activity

Changes in CAT enzyme activity was significantly influenced by salinity stress, methanol, and their interaction ($P \leq 0.01$) (Table 2). The results indicated that the treatment using 30% methanol under 80 mM salinity conditions had the highest CAT activity (0.84 U mg^{-1} protein min^{-1}); while the lowest CAT activity was found in the control treatment without salinity stress and methanol application (0.19 U mg^{-1} protein min^{-1}) (Figure 1).

APX activity

Analysis of variance showed that APX enzyme activity was significantly affected by salinity stress, methanol, and their interaction ($P \leq 0.01$) (Table 2). Mean comparison results showed that the treatment of 30% methanol under 80 mM salinity conditions had the highest APX enzyme activity (85.69 U mg^{-1} protein min^{-1}), while the treatment without methanol application under no salinity conditionshad the lowest APX enzyme activity (12.32 U mg^{-1} protein min^{-1}) (Figure 1).

Protein content

The analysis of variance revealed that protein content was significantly affected by salinity stress, methanol application, and their interaction ($P \leq 0.01$) (Table 2). The results showed that applying methanol at concentrations of 10%, 20%, and 30% under salinity stress conditions increased protein content by 28%, 87%, and 171%, respectively, compared to the treatment without methanol application under salinity stress conditions (Figure 1). Also, based on the results obtained under non-salinity stress conditions, it was determined that there was no significant difference in protein content between the treatments without methanol application and the application of 10% methanol (Figure 1). Under non-salinity stress conditions, the application of 20 and 30% methanol improved the protein content by 32 and 51%, respectively, compared to the treatment without methanol application. The highest protein content was observed in the treatment with 30% methanol application under salinity stress conditions (4.98 mg g^{-1} FW); However, the lowest protein content was observed in the treatment without methanol application and salt stress (1.47 mg g^{-1} FW) (Figure 1).

Proline content

Analysis of variance showed that the proline content was significantly affected by salinity stress, methanol application, and their interaction ($P \leq 0.01$) (Table 2). In non-salinity stress conditions, the application of 10%, 20%, and 30% methanol increased proline levels by 25%, 73%, and 75%, respectively, compared to the treatment without methanol application (Figure 1). Also, in salinity stress conditions, the application of 10, 20, and 30% methanol treatments increased proline by 21, 78, and 124%, respectively, compared to the treatment without methanol application (Figure 1). The results showed that the treatments of 20% methanol application in salinity stress conditions (72.54 μg g^{-1} FW) and 30% methanol application in salinity stress conditions (73.25 μg g^{-1} FW) had the highest proline content; while the treatment of no methanol application in non-salinity stress conditions (11.41 μg g^{-1} FW) had the lowest proline content (Figure 1).

MDA content

Variance analysis results indicated that the MDA content was significantly affected by salinity stress, methanol, and their interaction ($P \leq 0.01$) (Table 3). Based on the results, it was observed that the MDA content decreased with the application of different methanol treatments in salt stress and non-salt stress conditions. A comparison of the mean data in non-salt stress conditions showed that the application of 30% methanol had the lowest MDA content (13.52 nmol g^{-1} FW); while the highest MDA content was related to the treatment of no methanol application (31.25 nmol g^{-1} FW) (Figure 2). Based on the results obtained in salt stress conditions, it was determined that the treatment of no methanol application had the highest MDA content (67.54 nmol g^{-1} FW); in contrast, the treatment of 30% methanol application had the lowest MDA content (24.82 nmol g^{-1} FW).

Table 3. Mean squares of MDA, EL, H_2O_2, TSS, RWC, and TChl affected by salinity stress treatment and methanol foliar spraying in coriander plant.

Source of variation	DF	MDA	EL	H_2O_2	TSS	RWC	TChl
Salt stress (A)	1	7598.97**	8516.43**	8.1**	3111.81**	1722.25**	36.63**
Methanol (B)	3	1346.25**	643.22**	2.11**	1996.2**	257.16**	23.15**
A × B	3	353.04**	1433.39**	0.58**	681.45**	57.63**	1.62**
Residual	16	1.34	3.11	0.06	6.79	6.92	0.24
CV (%)		3.23	4.56	10.7	4.87	3.64	7.25

** indicates significance at the 1% probability level. Malondialdehyde (MDA), electrolyte leakage (EL), total soluble sugar (TSS), relative water content of leaf (RWC), and total chlorophyll content (TChl).

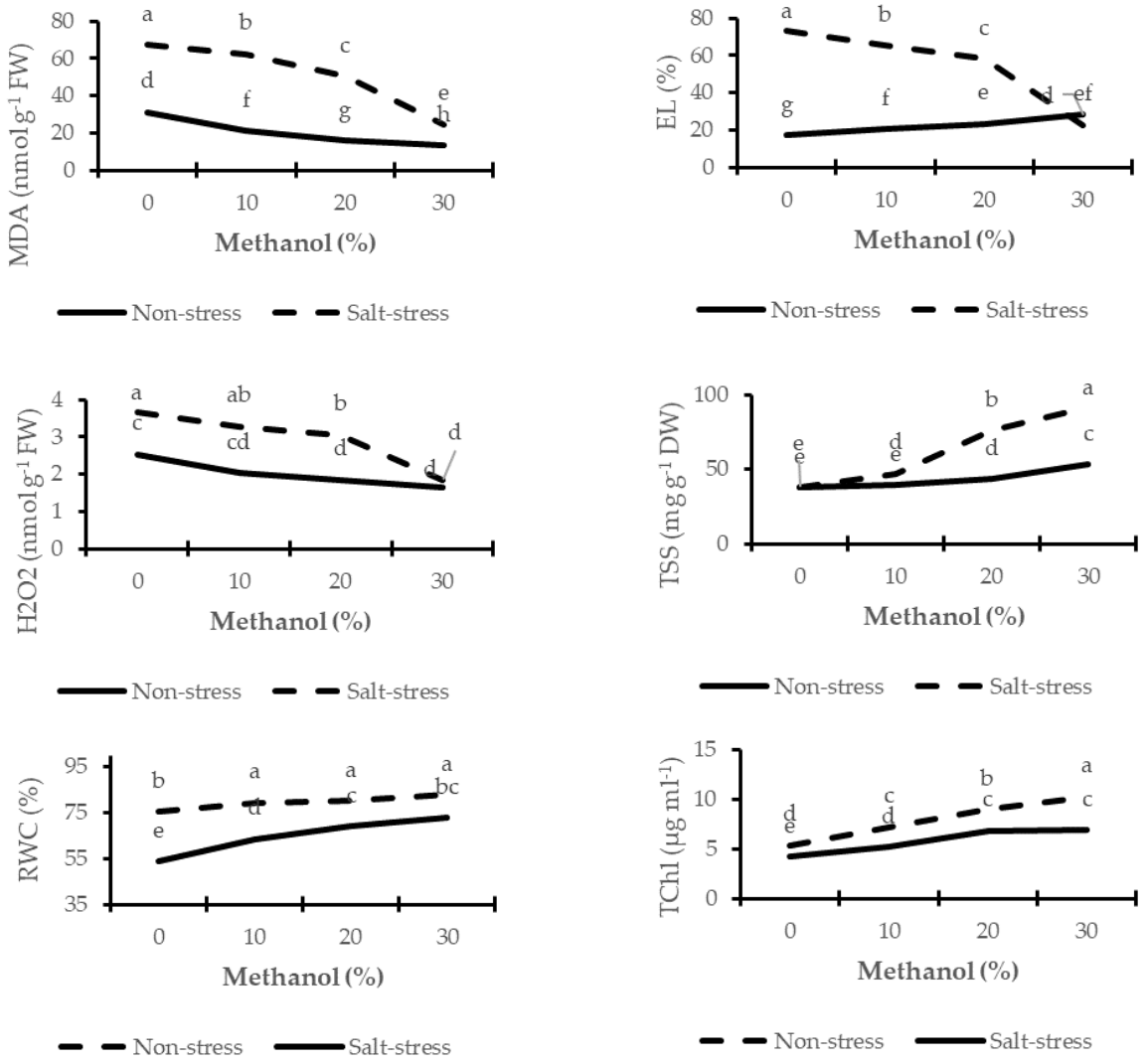

Figure 2. Results of comparison of mean related to malondialdehyde (MDA), electrolyte leakage (EL), H_2O_2, total soluble sugar (TSS), relative water content of leaf (RWC) and total chlorophyll content (TChl) characteristics under salinity stress (normal and 80 mM salinity stress) and methanol foliar spraying (non-spraying of methanol, 10% methanol, 20% methanol and 30% methanol) in coriander plants. The columns in the chart represent the mean±SE. Columns with the same letters are not statistically significantly different from each other.

Electrolyte leakage (EL)

The changes in the content of EL were affected by the treatments used; so the effect of salinity stress, methanol, and their interaction was significant for the EL content ($P \leq 0.01$) (Table 3). Based on the results obtained from comparing the mean data, it was observed that the highest content of EL was related to the treatment of not using methanol under salinity stress conditions (73.48%); while the lowest amount of EL was observed in the treatment of not using methanol and no salinity stress (17.21%) (Figure 2).

H_2O_2 content

Based on the results obtained from the analysis of variance of the data, it was determined that the effect of salinity stress, methanol, and their interaction was significant for the H_2O_2 content ($P \leq 0.01$) (Table 3). The highest content of H_2O_2 was observed in the treatment of no methanol application under salinity stress conditions (3.65 nmol g^{-1} FW), which was not statistically significantly different from the treatment of 10% methanol application under salinity stress conditions (3.28 nmol g^{-1} FW) (Figure 2). On the other hand, the lowest amount of H_2O_2 was observed in the treatments of 20% methanol application under non-salinity stress conditions (1.85 nmol g^{-1} FW), 30% methanol application under non-salinity stress conditions (1.65 nmol g^{-1} FW) and 30% methanol application under salinity stress conditions (1.85 nmol g^{-1} FW), which were not statistically significantly different from the treatment of 10% methanol application under non-salinity stress conditions (2.03 nmol g^{-1} FW) (Figure 2).

Total soluble sugars (TSS)

Analysis of variance showed that TSS was significantly affected by salinity stress, methanol and their interaction ($P \leq 0.01$) (Table 3). Based on the results of mean comparison, it was determined that the treatment of 30% methanol application under salinity stress conditions (91.84 mg g^{-1} DW) had the highest TSS content; while the treatments of no methanol application under non-salinity stress conditions (37.74 mg g^{-1} DW), 10% methanol application under non-salinity stress conditions (39.45 mg g^{-1} DW) and no methanol application under salinity stress conditions (38.25 mg g^{-1} DW) had the lowest TSS content (Figure 2).

Relative water content of leaf (RWC)

Based on the results obtained from the present experiment, it was observed that RWC was significantly affected by salinity stress, methanol, and their interaction ($P \leq 0.01$) (Table 3). Under non-salinity conditions, methanol application at 10%, 20%, and 30% increased RWC to 79.35%, 80.21%, and 82.84%, respectively, while the lowest RWC (53.71%) was observed in the no-methanol treatment under salinity stress (Figure 2).

Total chlorophyll content

Based on the results of variance analysis, it was determined that the effects of salinity stress, methanol, and their interaction were significant for the total chlorophyll content ($P \leq 0.01$) (Table 2). The use of methanol under stress and non-salinity stress conditions increased the total chlorophyll content. Based on the results, it was determined that the treatments of applying 30% methanol under non-salinity stress conditions (10.23 µg ml^{-1}) and not applying methanol under salinity stress conditions (4.23 µg ml^{-1}) had the highest and lowest total chlorophyll content, respectively (Figure 2).

Principal component analysis (PCA)

The PCA results indicated that the first and second components had eigenvalue higher than one and were identified as influential components (Figure 3). The relative variance in the first and second components was 57.90 and 36.61%, respectively, and accounted for 94.52% of the total variance (Figure 3). The biplot from the first and second components showed that the treatments of 80 mM salinity stress with no methanol and 80 mM salinity stress with 10% methanol treatments were grouped together, showing strong correlation with the H_2O_2, MDA and EL traits (Figure 3). On the other hand, the 80 mM salinity stress with 20% methanol and 80 mM salinity stress with 30% methanol treatments were separated into an independent group and showed a strong correlation with the proline, SOD, TSS, PPO, APX, CAT and protein traits (Figure 3). The biplot evaluation obtained from the first and second components showed that the no stress with non-spraying of methanol, no stress with 10% methanol, no stress with 20% methanol and no

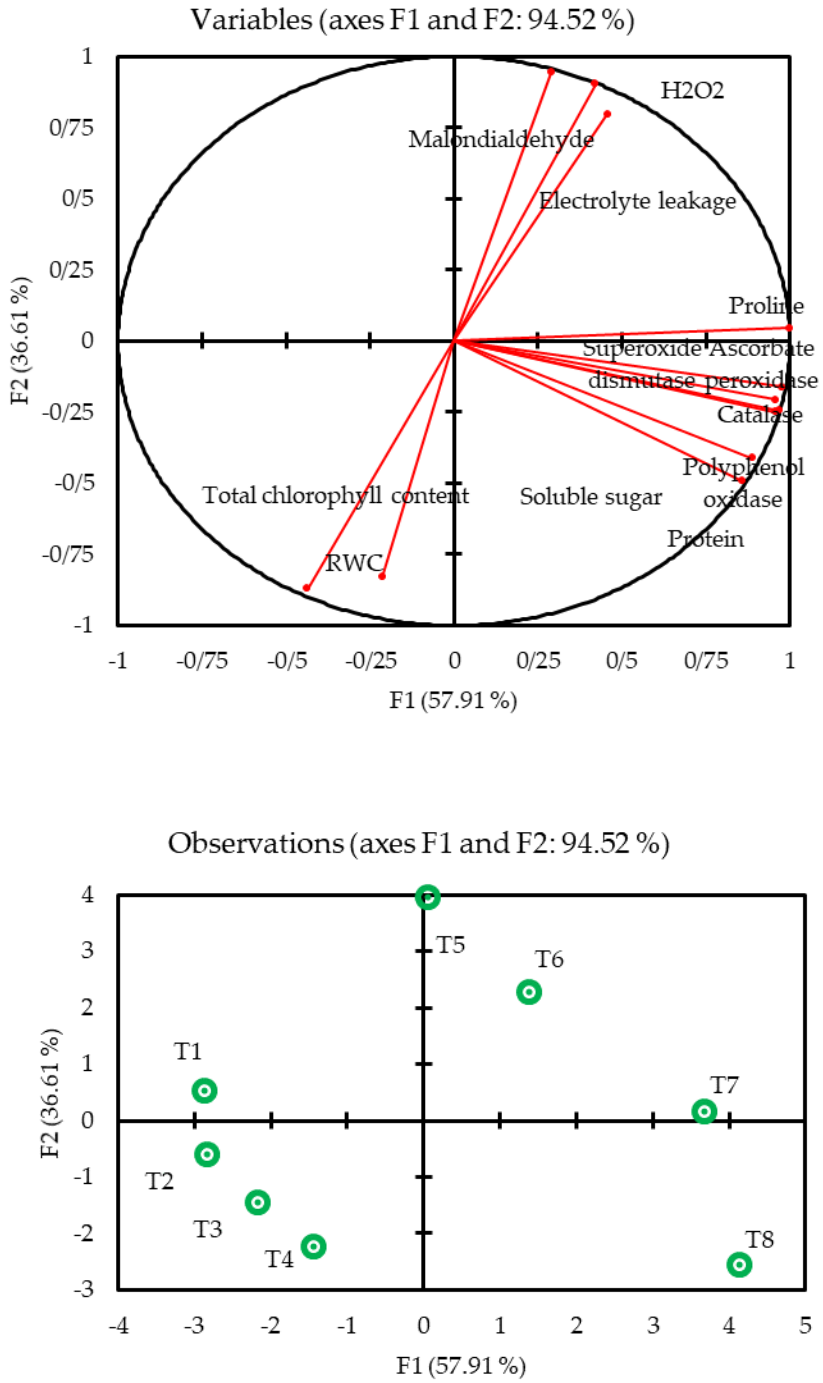

Figure 3. Biplot of the first and second components based on superoxide dismutase (SOD), polyphenol oxidase (PPO), catalase (CAT), ascorbate peroxidase (APX), protein, proline, malondialdehyde (MDA), electrolyte leakage (EL), H_2O_2, total soluble sugar (TSS), relative water content of leaf (RWC) and total chlorophyll content (TChl) traits in coriander plants under salt stress and methanol foliar spray treatments. T1: no stress with non-spraying of methanol, T2: no stress with 10% methanol, T3: no stress with 20% methanol, T4: no stress with 30% methanol, T5: 80 mM salinity stress with non-spraying of methanol, T6: 80 mM salinity stress with 10% methanol, T7: 80 mM salinity stress with 20% methanol and T8: 80 mM salinity stress with 30% methanol.

stress with 30% methanol treatments were placed in the same group and showed a strong correlation with the total chlorophyll content and RWC traits (Figure 3).

Cluster analysis

The dendrogram resulting from cluster analysis showed that the eight treatments were divided into three distinct groups; The first group included treatments without stress and non-spraying of methanol, treatments with 10%, 20%, and 30% methanol (Figure 4). The second group comprised the treatments of 80 mM salinity stress with no methanol and 80 mM salinity stress with 10% methanol. The third group included the treatments of 80 mM salinity stress with 20% and 30% methanol (Figure 4). Comparison of the groups derived from cluster analysis showed that the RWC and total chlorophyll content were significantly higher in the first group compared to the other groups (Figure 5). On the other hand, the second group was superior in terms of MDA, EL and H_2O_2 compared to the first and third groups. Box plots showed that in the third group, the SOD, PPO, CAT, APX, protein, proline and TSS traits were superior compared to the first and second groups (Figure 5)

Discussion

Salt stress significantly influences the physiological and biochemical activities of plants, and effective management strategies are crucial to mitigate its detrimental effects. In the present experiment, the exogenous application of methanol demonstrated multiple positive effects on the studied characteristics of coriander plants.

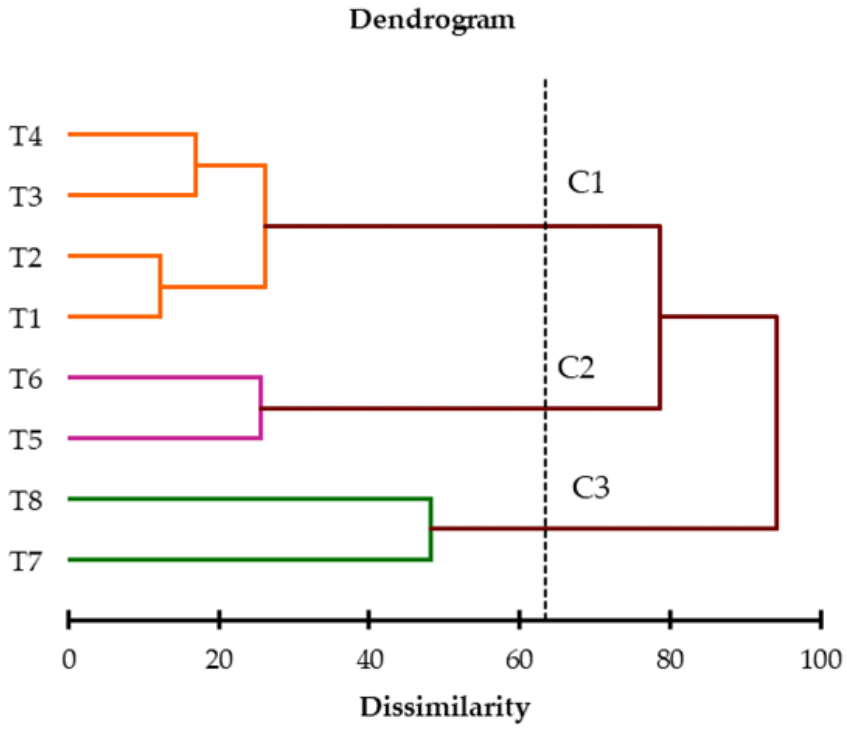

Figure 4. Dendrogram resulting from cluster analysis based on superoxide dismutase (SOD), polyphenol oxidase (PPO), catalase (CAT), ascorbate peroxidase (APX), protein, proline, malondialdehyde (MDA), electrolyte leakage (EL), H_2O_2, total soluble sugar (TSS), relative water content of leaf (RWC) and total chlorophyll content (TChl) traits in coriander plants under salt stress and methanol foliar spray treatments. T1: no stress with non-spraying of methanol, T2: no stress with 10% methanol, T3: no stress with 20% methanol, T4: no stress with 30% methanol, T5: 80 mM salinity stress with non-spraying of methanol, T6: 80 mM salinity stress with 10% methanol, T7: 80 mM salinity stress with 20% methanol and T8: 80 mM salinity stress with 30% methanol.

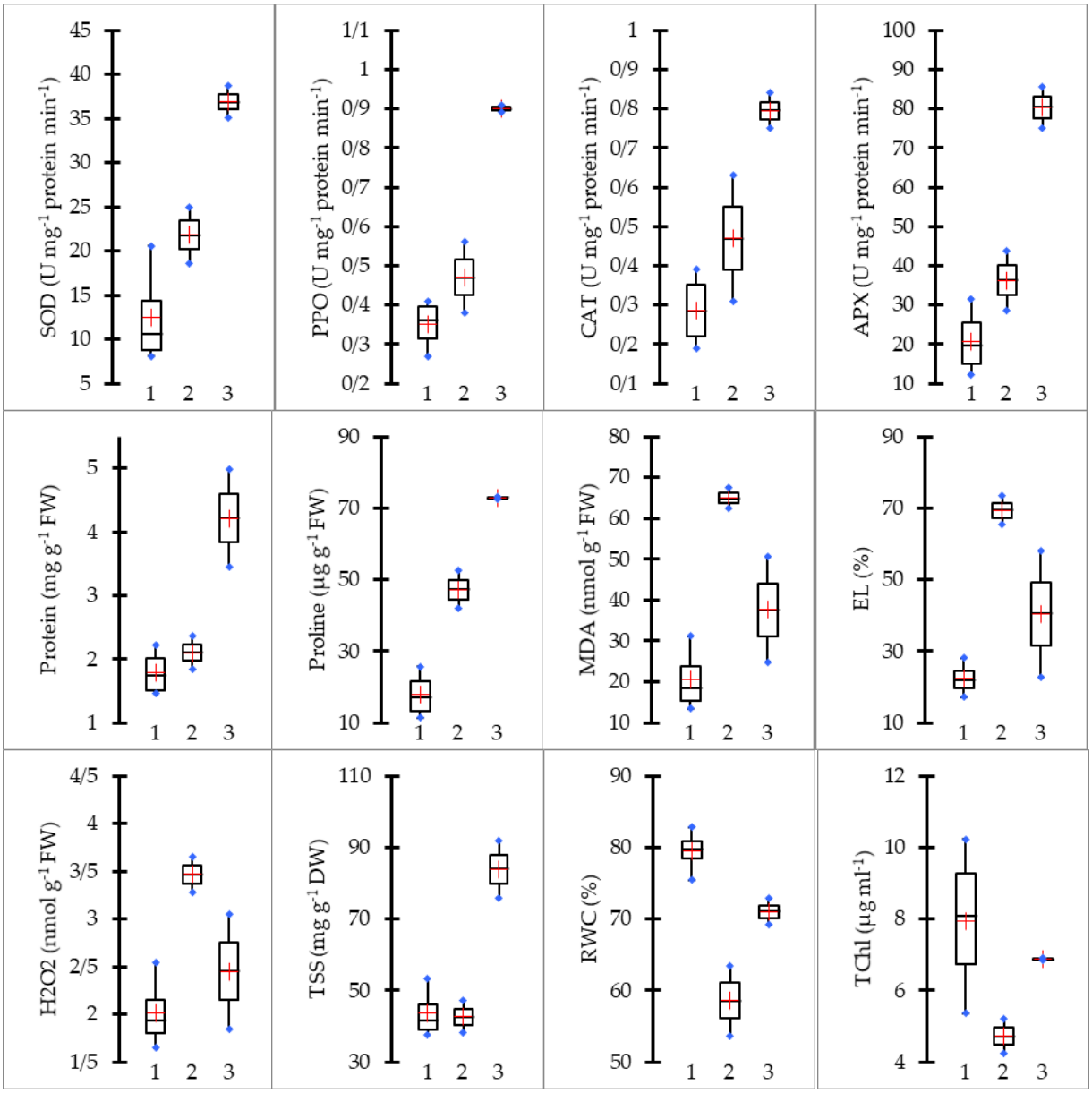

Figure 5. Box plot of comparison between groups resulting from cluster analysis in superoxide dismutase (SOD), polyphenol oxidase (PPO), catalase (CAT), ascorbate peroxidase (APX), protein, proline, malondialdehyde (MDA), electrolyte leakage (EL), H_2O_2, total soluble sugar (TSS), relative water content of leaf (RWC) and total chlorophyll content (TChl) traits in coriander plants under salt stress and methanol foliar spray treatments.

Our findings indicated that salt stress increased the activity of antioxidant enzymes, including SOD, PPO, CAT, and APX. These results align with those of (Hosseini et al., 2023), who reported elevated antioxidant enzyme activity in various mint species subjected to salt stress. Similar increases in antioxidant enzyme activity have also been documented in coriander (Rabiei et al., 2020), peppermint (Khalvandi et al., 2019), basil (Yilmaz et al., 2023), and sage (Bayat et al., 2022) under saline conditions, further corroborating our findings.

Salinity stress induces the production of ROS, which can damage cellular components and lead to oxidative stress (Hosseini et al., 2023). In response, plants enhance the production of antioxidant enzymes to mitigate this damage (Yilmaz et al., 2023). These enzymes are crucial for protecting cell membranes and organelles by neutralizing ROS (Rabiei et al., 2020). Additionally, salinity stress

activates specific signaling pathways, stimulating the production of antioxidant enzymes (Azeem et al., 2023). Under saline conditions, plants require more energy, and antioxidant enzymes play a vital role in maintaining healthy metabolism (Yilmaz et al., 2023). An increase in antioxidant enzyme activity indicates an active plant response to environmental stresses (Hosseini et al., 2023), allowing for better tolerance to saline conditions and improved resistance to various stressors. Moreover, antioxidant enzymes help regulate the levels of essential nutrients and metabolites in response to salinity. By reducing oxidative stress, these enzymes can prevent DNA damage and genetic destruction (Hosseini et al., 2021; Kesawat et al., 2023; Yilmaz et al., 2023). Enhanced antioxidant enzyme activity can also lead to improved crop quality under salt stress conditions (Khalvandi et al., 2019).

Furthermore, our experiment demonstrated that methanol application positively influenced the activity of antioxidant enzymes. Methanol can serve as a valuable carbon source for the synthesis of secondary metabolites, which support the production of antioxidant enzymes (Dorokhov et al., 2015; Wei et al., 2015; El Moukhtari et al., 2020). By activating signaling pathways and increasing oxidative stress levels, methanol stimulates the production of antioxidant enzymes (Dorokhov et al., 2015; Wei et al., 2015; Dorokhov et al., 2018). Moreover, methanol can help regulate plant osmotic pressure, creating better conditions for antioxidant enzyme activity (Tavassoli and Galavi, 2011; Dorokhov et al., 2018). This compound also acts as a stimulator of plant responses to environmental stresses, including salinity and drought (Dorokhov et al., 2018). In our results, methanol application also increased chlorophyll content under salt stress conditions, likely providing the necessary carbon for photosynthesis and improving carbon fixation (Wei et al., 2015) .

Additionally, proline content increased in response to both salt stress and methanol application. Proline, a non-protein amino acid that accumulates in plants under environmental stresses, particularly salt stress, helps mitigate salinity's negative effects (Yan et al., 2024). By enhancing water balance in cells, proline increases the relative water content of leaves and reduces transpiration caused by salt (El Moukhtari et al., 2020). Furthermore, proline contributes to improved photosynthesis by supplying energy for metabolic processes under stress conditions (Yan et al., 2024). Under salt stress, proline accumulation can reduce oxidative damage and protect cell membranes (Shahid et al., 2022). This amino acid also boosts the activity of antioxidant enzymes, which are crucial for reducing oxidative stress, aligning with our experimental results (Singh et al., 2022). Additionally, proline is linked to the accumulation of carbohydrates in cells, serving as an energy source and providing protection against stress (Mohammadi Alagoz et al., 2023). In summary, proline not only mitigates the adverse effects of salinity but also promotes growth and development under salt stress by improving photosynthetic conditions and regulating metabolism (Hnilickova et al., 2021).

Lastly, in our experiment, the application of methanol under salt stress conditions resulted in a reduction of MDA content. It appears that methanol improved antioxidant enzyme activity and proline synthesis, reduced ROS levels, and helped prevent membrane damage, which in turn led to a decrease in MDA content (Wei et al., 2015; Hnilickova et al., 2021; Hosseini et al., 2023).

Conclusion

The results of our experiment investigating the effects of methanol foliar spraying on the physiological and biochemical traits of coriander plants under salt stress showed that methanol positively influences various characteristics of this plant. Firstly, we observed a significant increase in the chlorophyll content of the leaves in methanol-treated plants. This enhancement in chlorophyll levels likely improves the photosynthesis process, indicating greater efficiency in light absorption by the plant. On the other hand, the measured amounts of proline in methanol-treated plants also increased. This is related to the enhancement of the plant's ability to cope with oxidative stress and maintain water balance within the cells. In addition, the levels of antioxidant enzymes in plants that received methanol were significantly higher than those in control plants. This increase in the levels of antioxidant enzymes indicates a better ability of the plant to cope with damage caused by salt and oxidative stress. Furthermore, the relative water

content of leaves in methanol treatment was improved, indicating the positive effect of methanol in maintaining plant water balance under salt stress. Finally, the results showed that methanol had a positive effect on reducing the level of MDA, an indicator of oxidative damage. This improvement in the level of MDA can help reduce the damage caused by salt stress and maintain the plant's cellular structure. In summary, our findings indicate that methanol foliar spraying can serve as an effective strategy to improve salt stress resistance in coriander plants, leading to increased chlorophyll content, enhanced antioxidant enzyme function, improved proline and sugar accumulation, and better water balance, while also reducing the level of MDA. To maximize these benefits and ensure a cost-effective approach, we recommend that a concentration of 20% methanol be employed for foliar spraying under saline soil conditions. This specific concentration may provide the best balance between cost and the enhancement of physiological traits, thereby optimizing the agronomic management of coriander plants facing environmental stress. By implementing this recommendation, growers can effectively improve the resilience of their crops to salt stress, leading to better yield and quality

Supplementary Materials

No supplementary material is available for this article.

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Author Contributions

Conceptualization, M.M. and Y.N.; methodology, M.M.; software, E.H.; validation, M.M., Y.N. and M.G.; formal analysis, M.M.; investigation, M.G.; resources, E.H.; data curation, M.M.; writing—original draft preparation, M.M.; writing—review and editing, Y.N.; visualization, M.G.; supervision, M.M.; project administration, E.H.; funding acquisition, Y.N. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest Statement

The authors declare no conflict of interest.

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مقاله پژوهشی

بررسی تاثیر محلول پاشی متانول بر بهبود خصوصیات بیوشیمیایی و فیزیولوژیکی گیاه گشنیز (*Coriandrum sativum*) تحت تنش شوری

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چکیده: تنش شوری یکی از عوامل مهم محدودکننده رشد گیاهان است بنابراین، استفاده از ترکیباتی مانند متانول برای کاهش اثرات مضر تنش اهمیت زیادی دارد. این تحقیق با هدف بررسی تأثیر اسپری متانول بر ویژگی‌های فیزیولوژیکی و بیوشیمیایی گیاه گشنیز در شرایط تنش شوری در گلخانه انجام شد.. تنش شوری در دو سطح (شاهد و ۸۰ میلی‌مولار از منبع کلرید سدیم) و اسپری متانول در چهار سطح (شاهد، محلول‌پاشی متانول در غلظت‌های ۱۰٪، ۲۰٪ و ۳۰٪) اعمال شد. نتایج نشان داد که در شرایط تنش شوری، استفاده از متانول منجر به افزایش معنی‌داری در ویژگی‌های مختلف شد. فعالیت آنزیم سوپراکسید دیسموتاز با ۳۰٪ متانول در شرایط تنش شوری ۲۳٪ افزایش داشت. همچنین، فعالیت آنزیم پلی‌فنول اکسیداز با ۳۰٪ متانول در شرایط شوری ۸۰ میلی‌مولار ۵۱٪ افزایش و فعالیت کاتالاز ۲۹٪ افزایش یافت. محتوای پرولین نیز در شرایط تنش شوری ۱۲۴٪ افزایش و محتوای مالوندی‌آلدهید ۶۳٪ کاهش یافت. به طور کلی، اسپری ۳۰٪ متانول تحمل به تنش را در گیاه تحت شرایط شوری افزایش می‌دهد و فعالیت آنزیم‌های آنتی‌اکسیدانی، محتوای پروتئین و محافظت اوسموتیکی را بهبود می‌بخشد. این یافته‌ها نقش متانول را در کاهش اثرات تنش شوری بر فیزیولوژی گیاه نشان می‌دهند.

کلمات کلیدی: آنزیم، رنگدانه، کلرید سدیم، مالون‌دی‌آلدهید، نشت الکترولیت.

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