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Research Article

Moringa Leaf Extract Effectively Enhanced Salinity Resilience in Jatropha Shrubs by Regulating Some Biochemical and Physiological Responses

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Abstract:

Salinity is considered one of the main reasons for the decrease in growth and flowering characteristics of various ornamental species. Jatropha is a woody shrub that is widely used in modern landscaping due to the flower beauty, but its growth and development was adversely affected by salinity. To achieve the sustainable agriculture, enhancing the salt stress tolerance via eco-friendly applications is of great interest. Although reports declare that moringa leaf extract (MLE) is involved in salt-stress tolerance, little information exists about the impact of MLE on ornamental shrubs, especially jatropha under salinity conditions. This experiment was, therefore, carried out to study the impact of MLE on salinity resilience in Jatropha. Plants were exposed to 0, 30, 60 and 90 mM of NaCl, and/or foliarly sprayed with MLE at 1:30 (v:v). Salt stress markedly decreased growth and flowering parameters, total chlorophyll content and nitrogen, phosphorus, and potassium contents in the leaf tissues; however, MLE treatment alleviated these impacts. Moreover, MLE application enhanced the accumulation of proline resulting in the mitigation of salinity damage. The current results revealed the possible benefits of the application of MLE and its mechanisms that can improve the growth and flowering of jatropha shrubs under salinity.

1. Introduction

Salinity is considered one of the main reasons for the reductions in growth and flowering characteristics of various ornamental species (Díaz-López et al., 2012; Ashour and Mahmoud, 2017; Muhammad et al., 2024) due to ionic imbalance and osmotic stress (Azeem et al., 2023). Additionally, high concentrations of salt result in lipid peroxidation observed by accumulation of malondialdehyde (Attia et al., 2020) leading to membrane injury, and hence, increased ion leakage (Elkarmout et al., 2022). The induction of oxidative stress is caused by overproduction of reactive oxygen species (ROS) in salt stressed-plants (Bernstein et al., 2010), that are involved in the impairment of cell growth processes. Basically, plants detoxify ROS through enhancing the non-enzymatic and enzymatic antioxidant system which play a vital role in ROS-scavenging. Thus, proline, glycinebetaine, ascorbate, phenolics, reduced glutathione and tocopherol, as non-enzymatic antioxidants (Mansour and Salama, 2019; Elkarmout et al., 2022) have been improved in several species under salt stress which play crucial roles in ROS scavenging in plants exposed to salinity.

To enhance salinity tolerance in plant, many efforts have been made worldwide. In this context, application of gibberellic acid (Ali et al., 2014), polysaccharides (Liu et al., 2019), β-aminobutyric acid (Ali and Hassan, 2019), nitric oxide (Nabi et al., 2019), and salicylic acid (Ghasemi et al., 2020). To achieve the sustainable agriculture and to protect the environment, enhancing the plant growth and development and improving the salt stress tolerance via eco-friendly applications is of great interest (Ali et al., 2022; Moussa et al., 2024; Fadil et al., 2025; Mazrou et al., 2025). There are several biostimulants that able to ameliorate the salt stress in plants. Among them, moringa leaf extract (MLE) is a good applicant due to its content of vitamins, phenols, hormones such as cytokines and gibberellic acid, essential elements, sugars, sterols, and some antioxidants that make it beneficial for plant growth and flowering (Ali et al., 2018; Muhammad et al., 2023) and alleviating the adverse effects of salinity in several plants (Hassanein, et al., 2019; Hassan et al., 2021; Arif et al., 2022).

Jatropha (*Jatropha curcas* L.) is a versatile woody shrub that belongs to the Euphorbiaceae family. Due to the beauty of the flowers, they are used in landscaping in addition to using as sturdy hedges (Neuwinger, 1996). Moreover, seed oil has been used as an alternative to fossil fuels to help preserve the environment (Abdelgadir et al., 2010) and also has been used in some cosmetics and medicinal formulations (Asase et al., 2005; Abdelgadir and Staden, 2013). Given the importance of jatropha, it is necessary to study the impact of salinity on its growth and flowering traits. Despite the published reports on jatropha, little information is available about the effect of salinity on its growth. Furthermore, studies on foliar application of MLE on jatropha are limited. Therefore, this experiment was conducted to investigate the efficacy of MLE on amelioration the negative effects of salinity in jatropha.

2. Materials and Methods

2.1. Plant Material

A pot experiment was conducted to study the efficacy of MLE on alleviation the negative impacts of salinity in jatropha (*Jatropha curcas* L.) at the experimental farm of the Faculty of Agriculture, Tanta University, Egypt, during the 2024 and 2025 summer seasons. One-year seedlings were transplanted in pots (30×20 cm) filled with clay loamy soil (Clay, 37.6%; Silt, 39.9%; Sand, 22.5%). One month later, the seedlings were cut to a 40 cm and were irrigated with tap water until the treatment beginning.

2.2. Treatments and experimental layout

Seedlings were exposed to salinity at 30, 60 and 90 mM NaCl after two weeks from cuttings. To prevent shock, irrigation was started with 30 mM and increased by 30 mM in each subsequent irrigation until the desired salinity level (90 mM) was reached. Irrigation with saline solution was performed every 3 days at a rate of 250 mL per pot and the treatments were continued for 108 days. The pots were washed once with tap water every two irrigations with salt to ensure salt homogeneity (Hassan et al., 2021). At the same time, the control plants were irrigated with tap water only for the same period. Moringa leaf extract (MLE) was prepared at a 1:30 volume-to-volume ratio as described by (Ali et al., 2018). Spraying with MLE was performed once a month in the morning one day after salt treatment until the end of the experiment. Control plants were sprayed with distilled water. Seven treatments with three replicates each were arranged in a completely randomized design.

2.3. Growth and flowering characters

Every 30 days the height of the plants was measured (cm) from the highest point of the plant to the soil surface. By the end of treatments, main branches/plant, fresh and dry weight of shoots (g) were measured. During the experimental period the number of flowers /plant was recorded.

2.4. Chlorophyll determination

Fresh leaf samples (0.1 g) were used for chlorophyll extraction using methanol following the protocol described by Dere et al. (1998). Chlorophyll content was measured using a UV-VIS spectrophotometer, and the results were expressed in milligrams per gram of fresh weight using the formulae established by Lichtenthaler (1987).

2.5. Proline determination

The free proline content was measured following the method outlined by Bates et al. (1973). The proline concentration was determined using a standard curve and expressed as μmol g^{-1} fresh weight (FW).

2.6. Mineral content

Acid digestion of dried leaf samples was performed using a mixture of perchloric acid and sulfuric acid in a 1:5 volume ratio, as outlined by the A.O.A.C. (1995). Nitrogen (N) content was quantified using the micro-Kjeldahl method, as described by Nelson and Sommers (1973). Phosphorus (P) content was measured spectrophotometrically (Pharmacia, LKB-Novaspec II) by assessing the formation of a blue color complex, following the procedure of Jackson (1978). Potassium (K) and Sodium (Na) concentrations were determined using flame emission photometry (Corning, Tewksbury, MA, USA).

2.7. Statistical analysis

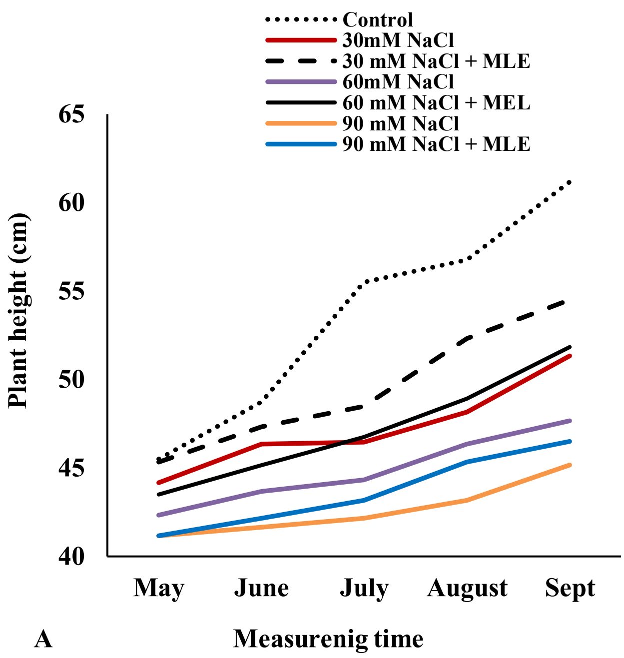
Data of each season were statistically analyzed, and the analysis of variance (ANOVA) was performed using Costat v x86 (cohort6\clapboarded). The LSD test was employed to separate means at a significance level of 0.05 (Snedecor and Cochran, 1980).

3. Results

3.1. Growth characters

Data presented in Figure (1) clearly show that plant height was gradually increased with advance of growth from May to September in treated or non-treated plants in in the two experimental seasons. However, the increment level in plant height was higher in control plants. The lowest increase was observed in salt stressed plants in both seasons. Otherwise, application of MLE enhanced the height in stressed plants in both seasons. For instance, at September of the first season the plant height was 61.17 cm in control plants, while it reduced to 47.67 cm when 60 mM NaCl was applied but it enhanced to 51.83 cm after MLE treatment.

Furthermore, increasing salinity level led to a gradual decrease in the number of branches per plant and shoot fresh and dry weights, reaching the maximum reductions when plants were exposed to 90 mM NaCl in both seasons (Table 1). For example, by applying this treatment, the branch number was decreased by 60% and 57.6% relative to the control in both seasons, respectively. However, the application of MLE effectively mitigated the negative effects of salinity on the growth characters under salt stress conditions in both seasons. For instance, the number of branches decreased only by 27.97 % and 26.99% when plants were treated with 90 NaCl + MLE salinity compared to the control. Additionally, MLE treatment improved the fresh and dry weights of shoots under salt stress compared to plants exposed to salt-stressed plants and ameliorated the reductions observed due to salinity.



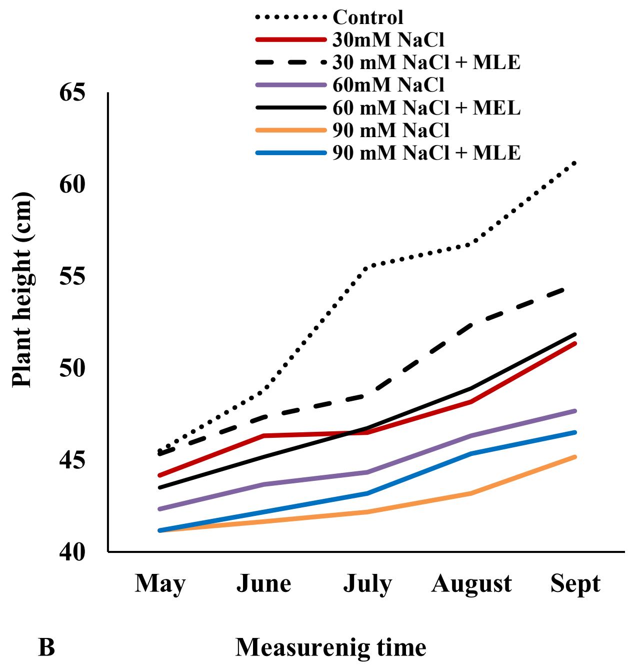


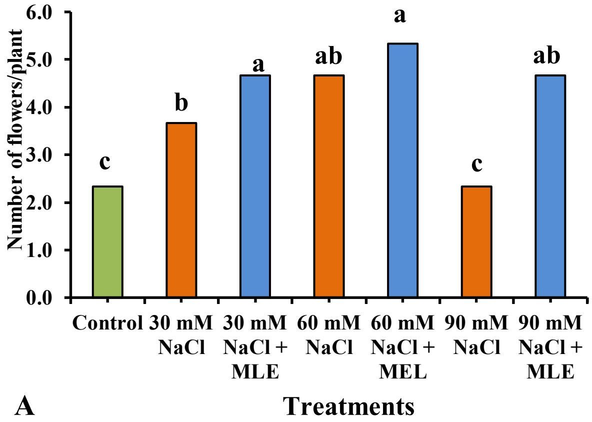
Figure 1. Impact of moringa leaf extract (MLE) on plant height of jatropha shrubs grown under salinity during the investigated period at 2024 (A) and 2025 (B) growing seasons.

Table 1. Impact of moringa leaf extract (MLE) on growth traits of jatropha shrubs grown under salinity. Means at each column having different letters are significantly different at $p \leq 0.05$.

Treatments	The first season (2024)			The second season (2025)		
	No. of branches/ plant	Shoot FW g/plant	Shoot DW g/plant	No. of branches/ plant	Shoot DW g/plant	Shoot DW g/plant
Control	8.33 a	99.33 a	95.83 a	8.67 a	33.52 a	31.16 a
30 mM NaCl	6.66 b	70.67 c	71.83 b	7.33 bc	22.68 b	21.28 b
30 mM NaCl + MLE	8.67 a	75.5 b	71.67 b	8.35 ab	23.20 b	21.69 b
60 mM NaCl	5.31 c	57.33 e	56.17 d	5.64 d	17.49 d	16.17 d
60 mM NaCl + MLE	6.33 bc	64.67 d	62.33 c	7.67 ab	19.47 c	17.75 c
90 mM NaCl	3.35 d	32.67 g	32.33 f	3.65 e	11.59 f	10.50 f
90 mM NaCl + MLE	6.00 bc	47.33 f	44.17 e	6.33 cd	12.74 e	11.52 e
L.S.D (5%)	**1.14**	**2.47**	**1.15**	**1.21**	**2.83**	**0.94**

3.2. Flower number

The results showed that low and moderate salinity levels resulted in a significant increase in flower number compared to the control in both seasons (Fig. 2). The highest number of flowers per plant was achieved by the treatment of 60 mM NaCl in the first season and 30 mM NaCl in the second one which increased the flower number by 100 and 133 % over the control, respectively. Importantly, foliar spraying with MLE on plants exposed to salt stress led to a clear improvement in the number of flowers per plant in the 2023 and 2024 seasons compared to stressed or non-stressed plants. Jatropha plants exposed to 60 mM NaCl in the first season or 30 mM NaCl in the second one recorded the highest number of flowers per plant.



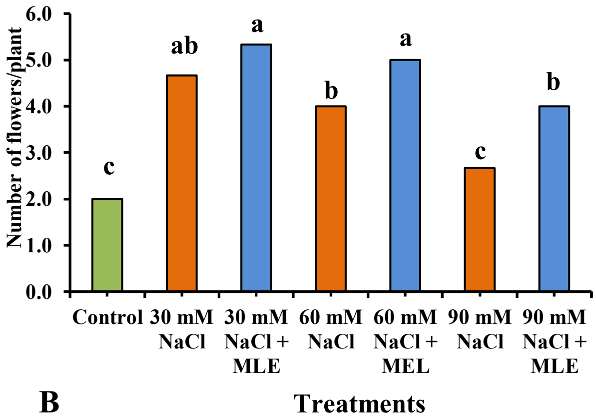


Figure 2. Impact of moringa leaf extract (MLE) on number of flowers per plant of jatropha shrubs grown under salinity during 2024 (A) and 2025 (B) growing seasons. Columns having different letters are significantly different at $p \leq 0.05$.

3.3. Chlorophyll content

A significant decrease in total chlorophyll was observed under salt stress conditions (30, 60, and 90 mM NaCl) compared to the control group in both seasons. However, MLE application enhanced total chlorophyll in salt stressed plants in both seasons (Table 2). Particularly, salinity levels of 30, 60 and 90 mM NaCl reduced the chlorophyll content by 16.54, 33.83 and 48.87 %, respectively compared to the control in the first season, while after MLE treatment chlorophyll values were enhanced and the reduction was only 8.27, 19.54 and 39.09%, respectively.

3.4. Proline content

Data in Table (2) show that the proline content was gradually increased with increasing salinity level compared to the control in both seasons more so with higher levels of 60 or 90 mM. Additionally, plants exposed to salinity and sprayed with MLE recorded higher proline content compared to only salt-stressed plants in both seasons. The highest proline content was recorded by plants exposed to 90 mM NaCl + MLE foliar spray and resulted in 171.24 and 189% higher proline compared to plants under the same salinity level and not sprayed with MLE in both seasons, respectively.

Table 2. Impact of moringa leaf extract (MLE) on chlorophyll and proline contents of jatropha leaves grown under salinity. Means at each column having different letters are significantly different at $p \leq 0.05$.

Treatments	The first season (2024)		The second season (2025)	
	Total chlorophyll (mg g^{-1} FW)	Proline (µmol g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Proline (µmol g^{-1} FW)
Control	1.33 a	1.89 f	1.35 a	1.73 ef
30 mM NaCl	1.11 c	1.99 ef	1.07 c	1.58 f
30 mM NaCl + MLE	1.22 b	4.23 c	1.12 b	4.30 c
60 mM NaCl	0.88 e	2.08 ef	0.90 e	1.96 de
60 mM NaCl + MLE	1.07 d	5.50 b	1.01 d	5.60 b
90 mM NaCl	0.68 g	2.31 d	0.71 g	2.21 d
90 mM NaCl + MLE	0.81 f	6.26 a	0.83 f	6.39 a
L.S.D (5%)	0.029	0.109	0.030	0.331

3.5. Nutrient Elements

It is evident from data in Table 3 that N, P and K contents in Jatropha curcas leaves were significantly reduced with increasing salinity levels in both seasons compared to the control treatment. The contents of N, P and K elements in Jatropha leaves were reduced by 36.23, 41.65 and 24.897% in the first season and by 33.58, 44.69 and 25.33 % in the second one, respectively relative to the control when the treatment of 90 mM NaCl was applied. Conversely, sodium (Na) content showed a significant and gradual increase in response to increasing salinity level. The highest sodium accumulation was observed by 90 mM NaCl, with its content increasing by 2.71-fold and 2.73-fold in the first and second seasons, respectively, compared to the unstressed plants. However, application of MLE under salinity stress significantly enhanced N, P and K contents, compared to stressed plants without MLE. Furthermore, MLE effectively reduced Na content in salt-stressed plants compared to those solely exposed to salinity. Specifically, Na content was reduced by 46.4 and 45.74% in both seasons, respectively when 90 mM NaCl + MLE treatment was applied compared to plants exposed to the same salinity level and not treated with MLE.

Table 3. Impact of moringa leaf extract (MLE) on N, P and K contents (mg g^{-1} DW) of jatropha shrubs grown under salinity. Means at each column having different letters are significantly different at $p \leq 0.05$.

Treatments	The first season (2024)				The second season (2025)			
	N	P	K	Na	N	P	K	Na
Control	20.9 a	3.77 a	21.93 a	1.67 g	20.9 a	3.77 a	21.63 a	1.67 g
30 mM NaCl	17.83 d	3.10 b	19.23 c	2.16 d	17.83 d	3.10 b	18.93 c	2.19 d
30 mM NaCl + MLE	19.80 b	3.60 a	21.27 b	1.76 f	19.80 b	3.60 a	20.80 b	1.79 f
60 mM NaCl	15.60 e	2.73 c	17.67 d	3.74 b	15.60 e	2.73 c	17.17 d	3.77 b
60 mM NaCl + MLE	18.90 c	3.23 b	19.30 c	1.94 e	18.90 c	3.23 b	18.97 c	1.97 e
90 mM NaCl	13.33 f	2.20 d	16.47 f	4.53 a	13.33 f	2.20 d	16.13 e	4.57 a
90 mM NaCl + MLE	17.30 d	2.87 c	17.23 e	2.43 c	17.30 d	2.87 c	16.93 d	2.48 c
L.S.D (5%)	0.672	0.213	0.296	0.040	5.917	0.115	0.356	0.021

4. Discussion

Data of this investigation revealed that salt stress significantly reduced the vegetative growth characteristics of *Jatropha curcas* L. This decrease may be due to the fact that salt stress decreases water uptake, which in turn decreases relative water content (Hassan et al., 2017), decreases photosynthesis (Borsani et al., 2003), inhibits cell division (Zhao et al., 2020) or accumulates high levels of Na and Cl ions to the toxicity point (Sahi et al., 2006). These results agreed with Ashour and Mahmoud (2017), who reported that plant height, number of branches per plant, fresh and dry weights of the stem and leaves of Jatropha integerrima were significantly decreased under salinity. Our results are also consistent with the previous results reported by Badawy et al. (2023) *in Jatropha curcas*, Elkarmout et al. (2022) in Moringa, Abdel-Aziz et al. (2020) in *Doranta erecta*.

On the contrary, MLE treatment alleviated the negative impacts of salinity on the growth of Jatropha curcas L. plants. This result could be explained via the fact that MLE contains essential nutrients and a wide range of nonenzymatic antioxidants, including proline, ascorbic acid, carotenoids and phenolic compounds. Furthermore, MLE is also considered a natural source of phytohormones, such as gibberellins, cytokinins (especially zeatins), and indole-3-acetic acid, as well as vitamins, amino acids, soluble sugars (Ibrahim et al., 2023; Farhat et al., 2023). These nutrients and bioactive compounds altogether enhance the salinity tolerance in plants. In agreement with these results, Hassan et al. (2021) in roses and Abdel-Rahman and Abdel-Kader (2020) in fennel found that MLE enhanced the growth parameters under saline conditions. In the same direction, Bahgat et al. (2023) found that foliar application of MLE has a positive effect on growth of *Hibiscus sabdariffa* L. cultivated under salt stress conditions.

Increasing number of flowers in this study under lower salinity levels is consistent with a previous study on chamomile, which reported an increase in the number of flowers under salt stress (Hendawy et al., 2019). In contrast, Ashour and Mahmoud (2017) found a decrease in the number of flowers on each plant with an increase in the concentration of salt in the irrigation water compared to the plants irrigated with tap water in *Jatropha integerrima* plants. Therefore, it is suggested that the impact of salinity on flower number is also species dependent. Similarly, Badawy et al. (2023) found that increasing salinity led to a significant decrease in the number of flowers in *Jatropha curcas*. However, spraying with MLE led to a gradual and noticeable increase in number of flowers. This result may be ascribed to the enrichment of MLE with essential nutrients and phytohormones, such as gibberellins, cytokinins (especially zeatins), and indole-3-acetic acid, as well as vitamins and amino which altogether enhance the flowering traits of Jatropha. The effective role of MLE in reducing the harmful effect of salinity and therefore improving the plant flowering has been early reported (Sarhan et al., 2022; Arif et al., 2022; Sunusi et al., 2025).

The chlorophyll content of *Jatropha curcas* L. leaves was significantly reduced as a result of salt stress. This reduction under saline conditions may be ascribed to a decreased uptake of essential minerals, mainly magnesium, which is vital for chlorophyll biosynthesis (Sheng et al., 2008). These findings are consistent with previous studies conducted on Jatropha curcas L. under salt stress (Badawy et al., 2023). Similarly, reductions in photosynthetic pigments under salinity were observed also in *Schefflera arboricola* (Shahin and Ahmed, 2023) and *Dianthus caryophyllus* (Muhammad et al., 2023). Contrary, MLE foliar application enhanced photosynthetic pigments in salt-stressed Jatropha leaves compared to salt-stressed plants not treated with MLE. This could be explained via the fact that MLE contains many essential elements such as magnesium, nitrogen, iron, manganese and zinc, in addition to being rich in cytokines, which work altogether to delay leaf aging and maintain high chlorophyll (Batool et al., 2020; Farhat et al., 2023; Ibrahim et al., 2023). The current results agreed with the previous reports of Hassan et al. (2021) in roses; Sardar et al. (2021) in stevia and Al-Taisan et al. (2022) in mint who reported that the use of MLE improved the salt stress-induced damage to the photosynthetic pigments compared to plants not treated with moringa leaf extract.

The increase in proline content in Jatropha leaves in current study due to salinity may be observed as a plant adaptation to intracellular stress (Ashour and Mahmoud, 2017). Actually, proline functions as an osmoprotectant, contributing to osmotic adjustment within cells and playing a vital role in safe guarding photosynthetic efficiency under salinity conditions (Silva-Ortega et al., 2007). Several reports have documented the enhanced accumulation of proline as a direct response to salinity-induced stress, such as Hassan et al. (2021) on damask rose, Bandurska et al. (2022) on *Chrysanthemum grandiflorum*, Shahin and Ahmed (2023) on *Schefflera arboricola* Endl. cv. Gold Capella, and Muhammad et al. (2024) on Petunia.

In the same direction, the proline content of leaves was further increased as a result of MLE application in salt stressed plants. It is apparent that proline is a direct ROS scavenger and plays a pivotal role in preserving the subcellular structures (Kamiab et al., 2014) which support our results. Improving proline content due to MLE treatment and hence enhancing the salt stress tolerance has been previously reported (Hassan et al., 2021). In the same context, Al-Taisan et al. (2022) on Mentha and Abdou et al. (2024) on marjoram reported similar trend.

The accumulation of NaCl has been found to disturb the homeostasis of essential cations such as K and Ca and hence a decrease in K in leaves was observed which in agreement with current results (Sarhan et al., 2022; Shahin and Ahmed, 2023). It is well known that salt stress accumulates high Na ions, which causes a competition with N, P and K ions, which reduces the proportion of these elements (Marschner, 1995; Turan et al., 2007). Therefore, salt stress increased the accumulation of leaf Na and Cl ions, thereby decreasing the plant growth and the ion uptake by roots and hence ion accumulation in the shoots (Caia et al., 2014) which support our findings. These results are consistent with the results of Hassan et al. (2017) in rosemary and Elkarmout et al. (2022) in moringa. However, MLE application ameliorated the harmful effects of salinity and contributed to maintaining ionic balance. This was reflected in increased essential nutrients concentrations, such as N, P and K, while decreasing the accumulation of Na ion under salinity conditions. This result may be due to the content of MLE from essential elements and phytohormones which can indirectly or directly increase the absorbance of essential elements under saline conditions (Abdel-Rahman and Abdel-Kader, 2020; Bahgat et al., 2023). These results suggest that alteration of nutrients uptake due to MLE applications may be a mechanism for salt stress alleviation in Jatropha. These findings are consistent with the reports of Sardar et al. (2021) in stevia and Abdou et al. (2024) in marjoram.

5. Conclusions

In conclusion, this study reports that MLE foliar application could play a pivotal role in restrictive the oxidative damage caused by salt stress and activating the antioxidant system in Jatropha. MLE treatment enhanced growth and flowering traits, photosynthetic pigments and proline contents. MLE also suppressed the disturbance of ion homeostasis in Jatropha leaves. These results suggest that MLE application might be a significant treatment for enhancing salt stress tolerance in Jatropha and possibly other ornamental shrubs.

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Conflicts of Interest: The authors declare no conflict of interest.

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