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Stimulatory effects of nicotinamide and Moringa oleifera leaf extract on growth and biochemical responses of *Vicia faba* under salt-induced stress

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Salinization is a more widespread abiotic stress that restricts plant growth worldwide. Plants possess effective defenses against harmful oxidative reactions by scavenging reactive oxygen species. Our study was designed to analyze the effects of nicotinamide, and *Moringa oleifera* leaf extract (MLE) used either alone or combined on the growth and fruit yield of faba bean (*Vicia faba*) plants exposed to salt stress situations. During plant growth, the foliar application of different concentrations of nicotinamide at 5, 10, and 20 mg/L and/or *Moringa oleifera* leaf extract dilutions (30 times) MLE (30) was applied. Plants were watered with saline water at a concentration of 100 mM NaCl. The results revealed the application of nicotinamide either separately or in combination with MLE increased growth criteria and yield components of the stressed plants, particularly by using 10 mg/L nicotinamide plus MLE (30). In addition, the contents of chlorophyll, total pigments, total carbohydrates, total protein, minerals, and nonenzymatic components (total phenol, anthocyanin, ascorbic acid flavonoids & proline contents) and antioxidant activity were increased by using these treatments.

Keywords: Faba bean, *Moringa oleifera*, Nicotinamide, Antioxidant enzymes, salinity, Yield

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INTRODUCTION

Vicia faba L. is among the most significant sources of plant-based protein worldwide. Dried faba bean seeds are abundant in carbohydrates, cellulose, vitamin C, and essential minerals, with a protein content of approximately 25% (Duc, 1997). Faba beans are rich in essential minerals such as Fe, Zn, K, Ca and Mg. They also contain significant amounts of amino acids, carbohydrates, and key nutraceutical compounds, making them valuable for human and animal diets and a significant asset in the food manufacturing industry (Koivunen et al., 2016). Water scarcity is a leading factor in reduced agricultural productivity and is often linked to other major abiotic stressors, including drought, salinity, and heat (Dawood et al., 2019).

About 22% of cultivated land worldwide is affected by salinity (FAO, 2020). Egypt is among the countries facing severe salinity challenges. For example, the use of saline irrigation water and low rainfall has led to the salinization of 33% of Egypt's arable land (El-Hendawy, 2004). Salinity affects the physicochemical properties of soil. Elevated sodium levels and increased soil pH modify the soil structure, reducing its Hydraulic permeability (Läuchli and Epstein, 1990). Salt stress affects seed germination, protein synthesis, lipid metabolism, photosynthesis, and plant growth (Parida and Das, 2005). The absorption and transport of mineral nutrients in plant cells, along with their availability in the soil, are influenced by ion toxicity, decreased water content, and osmotic imbalance in plant tissues (Hussein and Abou-Baker, 2018; Yang et al., 2020). Reduced growth leads to decreased nutrient uptake, disrupted plant hydration, and alterations in various biochemical and physiological processes (Mansour et al., 2023). Additionally, plants cultivated in saline environments showed damage to cell membranes, ion accumulation, generation of reactive oxygen species (ROS), and oxidative free radicals, along with a reduction in pigments synthesis (Masilamani et al., 2020; Budran et al., 2023; Mohammed et al., 2025; Zayed et al., 2025). Faba bean plants are adversely influenced by non-living environmental stresses such as salinity. Elevated concentrations of Na^+ and Cl^- ions in the soil negatively influence germination, maturity, reproduction, and grain yield. A small intake of vitamins is essential for plants to support their normal growth (Bassuony et al., 2008). They function as coenzyme systems and are crucial for regulating metabolism. Vitamin products have no negative or adverse consequences and are known to increase plant activity (Bronzetti et al., 2001). Nicotinamide (vitamin B3) is a vitamin that dissolves in water and is a crucial element of NADH and NADPH. These coenzymes are essential for numerous oxidation-reduction processes carried out by enzymes in living cells (Sadak et al., 2010). Moreover, the accumulation of secondary metabolites in plants and the activation of their defensive metabolic processes are triggered and regulated by the stress-related compound nicotinamide (Berglund, 1994; Berglund and Ohlsson, 1995). At minimal concentrations, nicotinamide may have the potential to affect various physiological plant traits (Bearder, 1980), as well as influence the levels of soluble sugars, total nitrogen, proline, and different minerals in faba bean plants (Abdelhamid et al., 2013).

The *Moringa oleifera* plant belongs to Moringaceae family. Its various components—roots, bark, leaves, flowers, fruits, seeds, seed oil, and gum—hold significant value due to their remarkable effects in food and medicinal applications (Moyo et al., 2011). The use of *Moringa oleifera* leaf extract (MLE) is an effective natural method to promote plant growth, especially in helping them withstand drought and other environmental stresses. This extract is rich in proteins, antioxidants (ascorbic acid, flavonoids, phenols, and carotenoids), and essential minerals (Mn, Cr, Zn, K, Ca, Fe, P, and Cu). It also contains amino acids and plant hormones (Azra Yasmeen et al., 2012). This research aimed to examine how the utilization of foliar nicotinamide and/or the *Moringa* leaf extract (MLE 30) affects both the quantity and quality of faba bean plants cultivated in saline conditions.

MATERIALS AND METHODS

Plant material and growth conditions

The study was conducted in a greenhouse at the Botany Department of the Faculty of Education, Ain Shams University in Egypt. Faba bean (*Vicia faba* L. cv. Nubaria, 1) seeds were acquired from the Agricultural Research Center in Giza, Egypt. According to Chapman and Pratt (1962), the chemical, physical, and nutritional characteristics of the soil were analyzed and reported in Table 1. Homogenous and healthy faba bean seeds were sterilized by using ethanol (70%) for two minutes, then treated with NaOCl (5%) for ten min, and then washed with distilled water. Faba bean seeds were seeded in pots containing 3 kg of uniformly sized loamy clay. From young, fully-grown *Moringa* leaves at the botanical garden at Ain Shams University, young leaves and branches were collected.

Preparation of *Moringa oleifera* leaf extract

MLE was prepared following the procedures outlined by Azra Yasmeen et al. (2012). The MLE extraction was done by soaking 1/10 (w/v) of the leaf powder in distilled water. After stirring the mixture with an electric stirrer for 4 hours, it was allowed to sit in a dark room for 28 h and then filtered. The obtained filtrate was centrifuged at 8000 g in 15 min. Finally, the filtrate was diluted thirty times with distilled water. Table 2 demonstrates the substance profiles of *Moringa* leaf extract, as reported by Hanafy (2017).

Table 1. Physical and chemical properties of the tested soil

Chemical variables (%)	
Clay	55.4
Silt	20.6
Sand	24.0
Soil characteristics	
pH	7.8
EC (dS m^{-1})	0.74
Organic matter%	21.5
$CaCO_3$ (%)	10.33
Total N%	0.38
Dissolved ions (meq L^{-1})	
Ca^{2+}	7.40
Mg^{2+}	3.22
K^+	1.70
Na^+	1.65
Cl^-	6.20
HCO_3^-	4.0

Table 2. Chemical components of *Moringa oleifera* leaf extract (MLE)

Chemical Components (mg g^{-1} DW)			
Calcium	6.693	Proline	25.010
Magnesium	5.443	Ascorbic acid	4.274
Potassium	26.59	Tocopherol	110
Phosphorus	5.101	Amino acids	167.6
Sodium	0.664	Iron	2.137
Soluble phenols	2.501	Copper	0.249
Phytohormones (µg g^{-1} DW)			
IAA	0.810	Zeatin	0.920
Gibberellins	0.702	Abscisic acid	0.234

Treatment and sample preparation

In this work, two distinct growing saline and non-saline were applied. Irrigation with 100 mM salt water was conducted twice a week. Three different doses of nicotinamide (5, 10, and 20 mg/L) and *Moringa* leaf extract (MLE 30) were used individually, and their combination was applied twice weekly (from 30 days after sowing, 20 ml/plant) as foliar spraying.

Botanical sampling

After 60 days of sowing, plants were gathered for each treatment and analyzed to investigate the following parameters of growth constituents (plant height, number of leaves per plant, number of branches per plant and fresh, dry weights of the plant. In addition, photosynthetic pigment elements (chlorophyll a, b, carotenoids, and total photosynthetic pigments) were determined as well as some biochemical analyses, including antioxidative compounds, such as phenols, flavonoids, anthocyanin, and osmo-protectants, including ascorbic acid and proline, oxidative damage (lipid peroxidation (MDA), hydrogen peroxide (H_2O_2), and antioxidant activity, and mineral ions content (N, P, K, and Na). At the harvest stage (after 73 days of sowing), the yield characteristics (pod number per plant, seed index (weight of 100 seeds per plant), and some biochemical constituents (total carbohydrates and total protein) were estimated. Five replicates, each consisting of ten plants, were collected to assess growth and yield parameters, while three replicates, each with five plants, were utilized for biochemical analyses.

Chemical analysis

Analysis of pigment content

The contents of chlorophyll (chlorophyll a, chlorophyll b & carotenoids) in fresh leaves of faba bean plants were measured using a spectrophotometer (VEB Carl Zeiss Germany) as described by Lichtenthaler and Buschmann (2001). The absorbance was taken at wavelengths 645, 663, and 470 nm using a spectrophotometer (VEB Carl Zeiss Germany).

Determination of secondary metabolites

Total phenolic content (TPC) was assessed using tannic acid equivalent (TAE) based on the Folins-Ciocalteu colorimetric procedure, as reported with modifications by Neugart et al. (2015), and total flavonoids (TFC) were measured by the method of Bushra Sultana et al. (2009).

Determination of non-enzymatic antioxidants

Anthocyanin level was estimated according to the method of Mancinelli (1990), ascorbic acid was measured using the method of Mukherjee and Choudhuri (1983), and proline content was determined by Bates et al. (1973).

Determination of oxidative damage

Lipid peroxidation levels were calculated by determining malondialdehyde (MDA) as described by Heath and Packer (1968), and hydrogen peroxide (H_2O_2) content was measured according to the method of Jana and Choudhuri (1981).

Assaying of minerals content

The total nitrogen in plant material was determined using the micro-Kjeldahl system in a solution of H_2SO_4 and $HClO_4$ (Yemm and Willis, 1956). The percentage of inorganic phosphorus was determined using the molybdenum blue method, as described by Paech and Tracey (1956). Total potassium (K) levels of dried leaf tissues were assessed following digestion with perchloric acid ($HClO_4$) and nitric acid (HNO_3) using the micro-Kjeldahl digestion device following Cunniff (1995).

Determination of total carbohydrates

The amount of total carbohydrates was calculated according to Albalasmeh et al. (2013).

Estimation of Total Protein

Total protein was described by Lowry et al. (1951).

Determination of Antioxidant Activity

Free radical scavenging activity was assessed using 2,2, -diphenyl-2-picryl-hydrazyl (DPPH) technique at an optical density of 517 nm (Turkmen et al., 2005). Antioxidant activity (%) was calculated using the following formula: A Sample (517 nm) / A Control (517 nm) × 100.

Statistical analysis

Analysis of variance (ANOVA) of complete randomized block design (RCBD) (Gomez, 1984)was used to statistically analyze the collected data using the MSTAT-C software package (Freed et al., 1991). The least significant differences test (LSD) was applied to detect significant differences between tested treatment means (Steel and Torrie, 1960).

RESULTS

Analysis of MLE extract

Table 2 demonstrates the chemical profile of *Moringa oleifera* leaf extract, as previously reported by Hanafy (2017). It was observed that *Moringa oleifera* leaf extract is abundant in specific nutrients, as well as certain antioxidants such as phenols, ascorbic acid, α-tocopherols, and flavonoids, and phytohormones like indole-acetic acid and gibberellins.

Impact of nicotinamide or MLE (30) on the growth indices of faba bean plants grown under salt stress

Table 3 indicated that irrigating faba bean plants with saline water (100 mM NaCl) caused notable decreases in their growth indices compared to the control plants. Specifically, plant height decreased from 32.6 cm to 21.4 cm, resulting in a reduction of 34.4% compared to the control group. Furthermore, the growth-related traits, specifically the number of branches and leaves per plant, fresh weight, and dry weight, were reduced from 2.25, 22.40, 37.92, and 10.72 in the control plants to 2.21, 19.73, 34.38, and 9.81 in the stressed plants. This corresponds to decreases of 1.8%, 11.9%, 9.34%, and 8.49%, respectively. In contrast, various treatments with different concentrations of nicotinamide (5, 10, and 20 mg/L), as well as MLE at 30 mg/L, enhanced the growth parameters examined under both normal and

Table 3. Impact of foliar spraying of nicotinamide and MLE (30) on growth parameters of faba beans growing under saline stress.

Treatments	Plant height (cm plant^{-1})	Number of branches plant^{-1}	Number of leaves plant^{-1}	Plant FW (g)	Plant DW (g)
0 Mm NaCl (control)	32.6 g	2.25 ef	22.40 e	37.92 g	10.72 g
100 Mm NaCl	21.4 i	2.21 f	19.73 g	34.38 h	9.81 i
100 Mm NaCl + Nicotinamide 5 mg/L	26.0 h	2.21 f	24.40 f	37.56 g	10.49 h
100 Mm NaCl + Nicotinamide10 mg/L	43.1 d	2.71 c	27.18 d	44.30 d	12.25 d
100 Mm NaCl + Nicotinamide 20 mg/L	40.6 e	2.55 d	25.00 d	42.25 e	11.50 e
100 Mm NaCl + MLE (30)	48.7 b	3.00 b	26.16 b	51.40 b	14.40 b
100 Mm NaCl + Nicotinamide 5 mg/L + MLE (30)	35.3 f	2.29 e	29.92 d	40.60 f	11.10 f
100 Mm NaCl + Nicotinamide 10 mg/L + MLE (30)	49.5 a	3.50 a	30.22 a	55.00 a	15.59 a
100 Mm NaCl + Nicotinamide 20 mg/L + MLE (30)	45.4 c	2.75 c	25.68 c	45.85 c	13.30 c
LSD at 0.05	0.43	0.06	0.28	0.45	0.10

Levels of significance are represented by at * = $P < 0.05$; ** = $P < 0.01$ and ns = non-significant ($P > 0.05$)

saline conditions. Furthermore, the data indicates that growth parameters improved with nicotinamide application , particularly at 10 mg/L. Additionally, the findings revealed that the MLE (30) treatment was more efficient than nicotinamide. Conversely, it was observed that the combination of nicotinamide at 10 mg/L and MLE (30) resulted in the most noteworthy increases in all vegetative growth parameters compared to the control group.

Changes in photosynthetic pigments

The findings in Figure 1 indicated that salinity treatment (100 mg/L) had a notable impact on the pigment content of bean plants. Chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll contents decreased in salt-treated plants by 23.6%, 46.85%, 5.5%, and 20.03%, respectively. Regarding the foliar impact of nicotinamide or MLE on the photosynthetic pigments of faba bean plants cultivated with saline irrigation, it restrained the decline in chlorophyll a, chlorophyll b, and carotenoids relative to the stressed plants. The results demonstrated a significant correlation between the rise in total pigments and the gradual increase in nicotinamide content, whereas MLE (30) treatment individually was more effective than nicotinamide treatments. The combination of nicotinamide at 10 mg/ L and MLE (30) produced the most notable increases in total photosynthetic pigments by 27.44 % compared to the control group.

Changes in antioxidant compounds

Antioxidants serve as the primary defense against damage caused by free radicals. Data in Figure 2 revealed that treatment with 100 mM NaCl caused notable alterations in total phenols, flavonoids, proline, ascorbic acid, and anthocyanin contents in salt-stressed plants compared with control plants. In addition, foliar spraying of bean plants with notable increases were seen in all concentrations of nicotinamide and MLE (30), either alone or in combination with antioxidant compounds relative to control. Comparatively, the MLE (30) was more effective than the concentrations of nicotinamide under stress conditions. At a concentration of 10 mg/L of nicotinamide combined with MLE (30), induction was notably higher relative to the corresponding control.

Changes in lipid peroxidation and H_2O_2 contents

Results in Figure 3 show that the values of lipid peroxidation and H_2O_2 were notably increased in context with NaCl stress (56.2% and 30.3%, respectively) relative to control. In contrast, foliar application of nicotinamide and/or MLE caused significant decreases in MDA and H_2O_2 contents compared with salt-stressed plants. Moreover, a notable decrease in both MDA and H_2O2 contents (53.6% and 56.2%) was observed in response to the combination of nicotinamide at 10 mg/L and MLE (30) treatment compared with stressed plants. Moreover, antioxidant activity was notably decreased in stressed plants by 16.99 % compared to control (Figure 3C). While a substantial increase was detected in antioxidant activity (20.7%) in response to the combination of nicotinamide at 10 mg/ L and MLE (30) treatment as compared with control plants.

Effect of Nicotinamide or MLE on minerals content (N, P, K and Na)

This research revealed that under saline conditions, a significant reduction in N, P, and K nutrient contents was detected relative to control plants (Figure 4A, B and C, respectively). In contrast, the proportion of Na content rose in the faba bean to give a value of 100% compared with control plants (Figure 4D). All concentrations of nicotinamide or MLE (30), whether used individually or in combination, led to remarkable

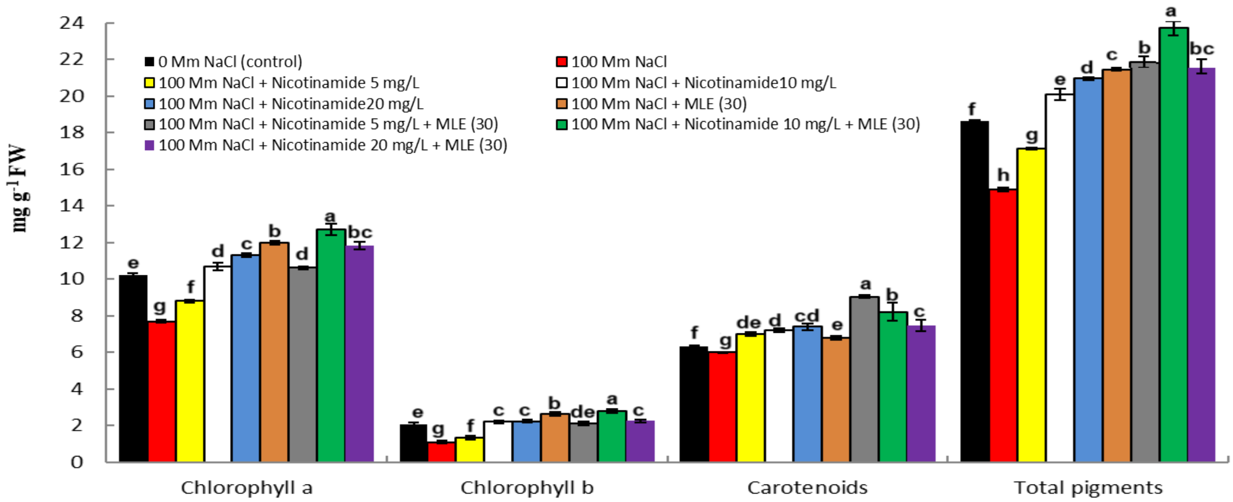

Figure 1. Impact of foliar spraying of nicotinamide and/or MLE (30) on photosynthetic pigments of fresh faba bean leaf at 70 days after sowing.

rises in N, P and K contents compared to both stressed and normal conditions. Alternatively, Na content was decreased in treated stressed plants compared with non-treated stressed plants. Furthermore, nicotinamide application at 10 mg/L in combination with MLE (30) caused remarkable rises in N, P, and K contents, accompanied by an obvious decline in Na content compared with stressed plants. Foliar administration of NAM or MLE (30), either individually or in combination, effectively mitigated the harmful effects of excessive sodium by reducing its uptake but also induced considerable rises in the absorption of vital mineral elements, including N, P, and K contents.

Impact of nicotinamide or MLE (30) on yield components of faba beans growing under salt stress

Salt stress has a deleterious effect on seed production and yield components in harvested faba beans, but there are mitigation strategies that can help alleviate these impacts. Data in Table 4 revealed that pods number, seeds number, seeds weight per plant, and 100 seeds weight of salt- stressed plants were decreased drastically relative to the control. Application of nicotinamide (10 mg/L and 20 mg/mL) and/or MLE (30) used either alone or combined caused obvious increases in faba bean plant yield under stress conditions. The combination of nicotinamide at 10 mg/L and MLE (30) caused the greatest rises in yield components relative to control

Impact of nicotinamide or MLE on certain biochemical components of the yielded seeds of faba beans cultivated under salt stress

A notable reduction in the percentage of total carbohydrates and total protein (13.59% and 14.89%, respectively) was noticed in stressed plants relative to control (Figure 5). In contrast, the foliar utilization with *Moringa* leaf extract led to accumulations of total carbohydrates that were higher than those in stressed plants. All concentrations of MLE (30) singly or in combination with nicotinamide caused crucial increases in these biochemical components of the yielded seeds of faba beans relative to control. Data clearly showed that the combination of nicotinamide at 10 mg/L and MLE (30) was found to be the most effective treatment, which resulted in considerable induction in these biochemical components.

DISCUSSION

The chemical composition of *Moringa oleifera* leaf extract is shown in Table 2. MLE (30) has been identified as a rich source of various nutrients, antioxidants including ascorbic acid, α- tocopherol, flavonoids, phenols, and phytohormones like gibberellins and indole acidic acid. Additionally, nicotinamide (NAM) is a compound associated with stress that promotes and controls the buildup of secondary metabolites and/or the activation of defense strategies (Berglund, 1994). Abiotic stress, like extreme temperatures, salinity, and drought may seriously hinder the productivity of various crops, leading to substantial annual crop losses. As a result, various techniques have been suggested to enhance plant performance in saline environments. Among these is the use of vitamins or cost-effective natural extracts from various plant parts, such as *Moringa oleifera* leaf extract. This novel biostimulant is known to contain high levels of antioxidants, minerals, proteins, amino acids, vitamins, and plant hormones, making it particularly advantageous for plant growth (Arif et al., 2023).

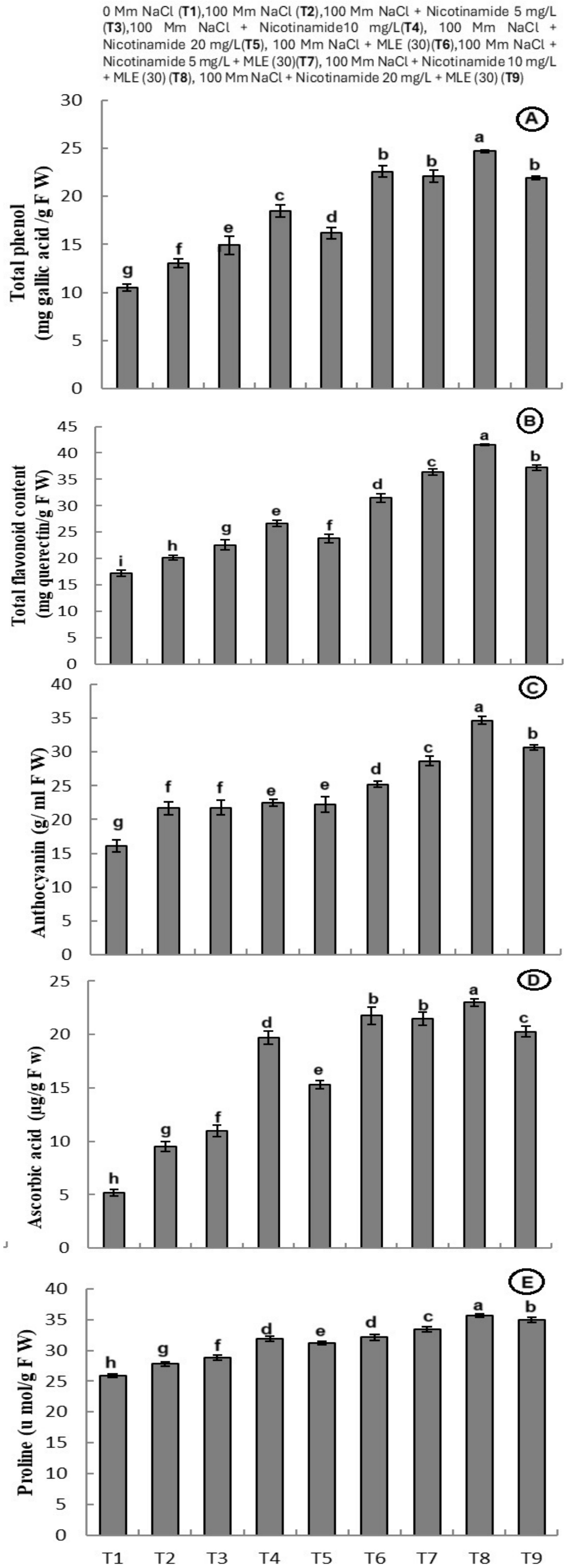

Figure 2. Impact of nicotinamide (NAM) and/or MLE (30) on antioxidative compounds and osmo-protectants content of faba beans under salt stress conditions.

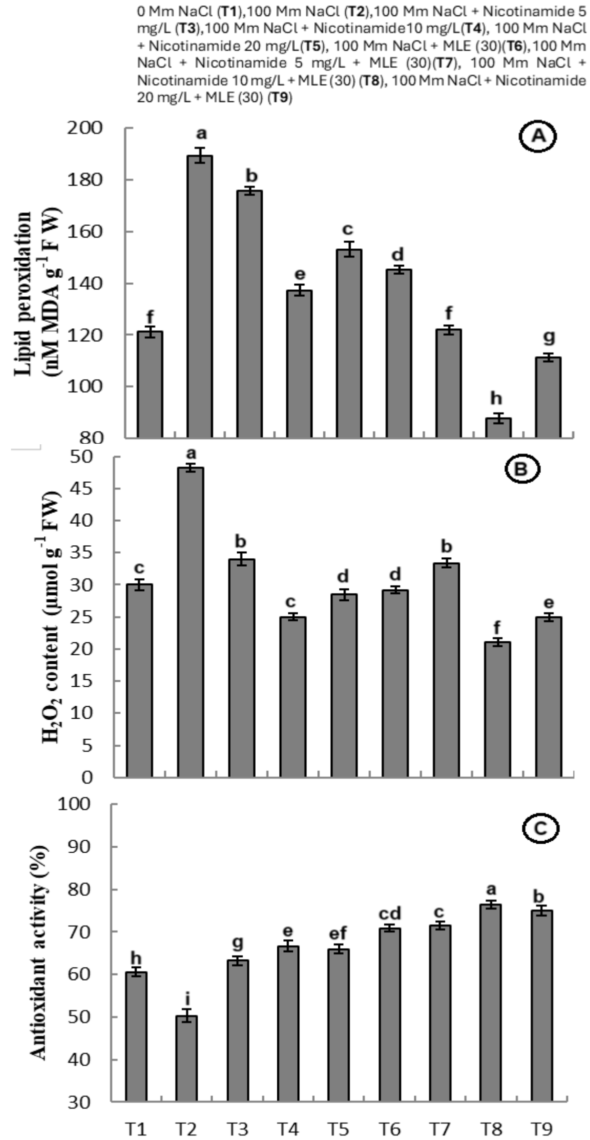

Figure 3. Effect of nicotinamide and/ or MLE (30) on lipid Peroxidation, H_2O_2 and antioxidant activity of faba bean plants grown under salt stress

Our obtained results indicated that salt stress led to a decrease in all growth variables in faba bean plants (Table 3). These findings align with those of Abbas and Akladious (2013), who found that salt stress at 100 mM inhibited the growth in cowpea seedlings, as well as with the results from Akladious and Mohamed (2018), which showed similar effects in bean plants. The inhibition of growth in response to abiotic stresses can result from several factors, including reduced cell elongation, decreased cell division, hormonal imbalances within the plant, and diminished activity of the apical meristem. Additionally, a decline in water absorption and the activity of root metabolic processes can contribute to this reduction in growth (Abou-Leila et al., 2012).

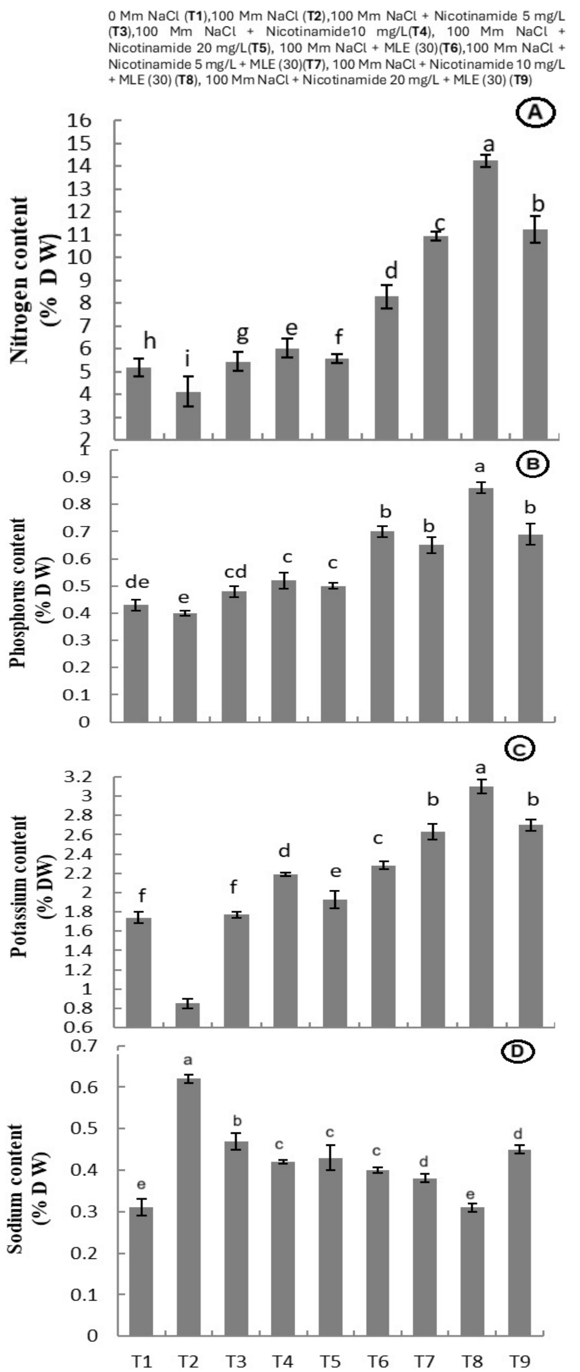

Figure 4. Effect of nicotinamide and/ or MLE (30) on mineral ions content of faba bean plants grown under salt stress

The decline in *Phaseolus vulgaris* productivity in saline environments can be attributed to the osmotic effects of salinity stress. This stress results in an elevated level of growth inhibitors, such as abscisic acid, while reducing the concentration of gibberellins and indole-3-acetic acid. Additionally, it disrupts the balance of water in plants impacted by salinity (Rady and Mohamed, 2015).

The impact of nicotinamide (NAM) on the height of plants in excess salt conditions can be attributed to its ability to enhance cell elongation and division. Additionally, NAM protects photosynthetic pigments, boosts the photosynthesis rate, and promotes mineral uptake in plants experiencing salt stress (Abdelhamid et al., 2013; El-Bassiouny et al., 2014). In a similar study, researchers investigated the foliar application of nicotinamide on *Chenopodium quinoa* resulted in a rise in the branch lengths (Abdallah et al., 2016). Nicotinamide may serve as a growth stimulant, helping to mitigate the negative impact of salt on metabolic processes related to growth. Multiple studies have emphasized the role of MLE (micronutrients, lignin, and enzymes) in enhancing the productivity of many crops when combined with nicotinamide (Rady et al., 2013; Rehman et al., 2014; Yasmeen et al., 2012). In this regard, Zulfiqar et al. (2020) noted that MLE serves as an effective bioenhancer, providing vital elements, flavonols, phenols, sugars, vitamins, and amino acids that promote the growth of plants. Additionally, it is rich in antioxidants, which enhance activity and reduce ROS, helping to alleviate numerous biotic and abiotic stresses while also boosting plant yield. MLE mitigated the destructive outcomes of salt by preserving the characteristics of plant productivity (Howladar, 2014) and reduced the harmful consequences of salt by enhancing shoot and root length, relative water content, leaf area, biomass, and membrane integrity (Rady and Mohamed, 2015).

Salt stress in plants may cause a decrease in the levels of photosynthetic pigments in many plant species (Abbas and Akladious, 2013). The decline in chlorophyll levels in salinity-stressed plants may result from elevated chlorophyllase activity (Abbas and Akladious, 2013). Desingh and Kanagaraj (2007) suggested that salinity stress could impact the biochemical processes of photosynthesis by disrupting the structure of chloroplasts which, in turn may result in reduced activity of the photosystems. Application of nicotinamide or MLE notably enhanced the levels of these pigments compared with salt-stressed plants, and the dual application of nicotinamide + MLE further increased their concentrations under salt stress (Figure 1). The highest number of photosynthetic leaves obtained by using nicotinamide and/ or MLE treatments indicates a delay in senescence and helps maintain higher concentrations of chlorophyll (Table 3). In contrast, vitamins may help safeguard chloroplasts from oxidative stress (Munné-Bosch et al., 2001).

Nicotinamide vitamins seem to create a sink that facilitates the movement of various nutrients essential for enhancing photosynthesis. Additionally, nicotinamide plays a crucial role in activating enzymes that regulate the carbon reduction process in photosynthesis (Abdelhamid et al., 2013). The presence of essential minerals such as Mn, K, Zn, Ca, Cu, P, and Fe in MLE prevents the early onset of leaf senescence and keeps a greater leaf area for photosynthesis (Rady et al., 2013). Using MLE extraction on snap beans and common beans resulted in the synthesis of a higher concentration of cytokinins (Zulfiqar et al., 2020), which improved chloroplast plasticity, promoted chlorophyll biogenesis, and inhibited chlorophyll disintegration (Fletcher et al., 1982). The results obtained in Figure 2 show that salt stress increased the level of non-enzymatic antioxidants, owing to their ability to safeguard plants from oxidative stress. These results are in harmony with those obtained by Asami et al. (2003) and Khan and Ashraf (2008).

Our investigation showed that the salinity tolerance of bean plants was enhanced by increasing non-enzymatic antioxidants (i.e., total phenols, flavonoid, anthocyanin, AsA, and proline) by the addition of minerals, IAA, AsA, Gas, and cytokinins containing MLE (Table 2) separately or together with NAM. Nicotinamide at 10 mg/L + MLE treatment was the most effective one. According to El-Bassiouny et al. (2014), NAM promotes and regulates the protective metabolic pathways in plants. This enhancement might be ascribed to the function of phenolics in modulating plant metabolic processes, thereby influencing overall plant growth (Berglund and Ohlsson, 1995; Lewis and Yamamoto, 1990). MLE contributed to reducing the negative consequences of salt exposure by enhancing the antioxidant defensive mechanism. This improvement included increases in flavonoids, anthocyanins, ascorbic acid, and proline levels, which collectively helped decrease ROS and leakage of ions (Howladar, 2014). Phenolic compounds serve as substrates for various antioxidant enzymes, helping to reduce stress-related injuries (Khattab, 2007). Additionally, these compounds safeguard cells against oxidative damage and enhance membrane durability (Semida et al., 2014). The accumulation of proline is a frequent modification observed in plants subjected to water and salt stress and is often linked to stress resistance mechanisms. In this context, Munns (2002) highlighted proline's role in stabilizing macromolecule and organelle structures by preventing the denaturation of proteins and membranes brought on by excessive salt concentrations. Ascorbic acid is a key substrate in the Halliwell-Asada cycle, functioning as a non-enzymatic antioxidant. It directly reduces ROS during various stress conditions and plays a crucial role in regulating hydrogen peroxide (H_2O_2) levels (Del Rio et al., 2006). This condition is obvious in the buildup of AsA in our study, which was detected with both MLE and NAM treatments. The highest concentration was recorded with the combined foliar application of NAM + MLE. The increased levels of ascorbic acid (AsA) and proline in the faba bean enhanced its antioxidant system, allowing it to better tolerate salt stress. Consequently, treatment with NAM in combination with MLE mitigated the detrimental outcomes of salinity on growth and metabolic processes by reducing the accumulation of ROS and boosting the bean's antioxidant defense system, ultimately enhancing its resistance to salt stress (Hassanein, 1999).

In the current study, the accumulation of H_2O_2 and MDA content in the shoots of faba bean under salt stress is observed, as these are commonly used indicators of salt-triggered membrane oxidative injury (Figure 3). These results are in accordance with Mohammadkhani et al. (2016), who found that MDA concentration rose in leaves and roots of grape after salt stress. In addition, Ayala-Astoraga and Alcarza-Melendez (2010) observed that peroxidative damage to lipids enhanced with 150 and 200 mM of NaCl in *Paulownia imperialis*. Whereas foliar application of nicotinamide and/or MLE caused significant decreases in MDA and H_2O_2 contents compared with salt-stressed plants. The same treatment caused the maximum induction in the antioxidant activity of the stressed plants relative to the control (Figure 3). Our findings indicate that H_2O_2 might serve a supportive role in the salt stress signaling network by activating defense mechanisms during the initial stages of salinity stress. It was proposed that the decrease in MDA content resulted from enhanced activity of antioxidative enzymes, which lowered H_2O_2 levels and minimized membrane damage (Shalata et al., 2001). The existence of phenols and active metabolites in MLE is believed to stop excessive membrane leaking and enhance its stability, thus safeguarding the functionality of biological membranes against the oxidative effects of harmful free radicals (Howladar, 2014).

Salt stress negatively affects the absorption of vital mineral nutrients in faba bean plants. Elevated levels of sodium and chlorine ions can harm plants, disrupting the mobility of K^+ within them (Iqbal et al., 2015). Our findings of decreased absorption of essential mineral ions under salt stress align with the results reported by Yasmeen et al. (2013) for wheat and Iqbal et al. (2015) for *Brassica juncea*. Maintaining ionic homeostasis during salt stress is essential for protecting plants from the accumulation of toxic ions. Enhanced potassium (K^+) uptake positively influences growth performance by actively participating in several critical metabolic processes, such as osmoregulation, enzyme activity, and the differential permeability of sodium (Ahanger et al., 2015; Ahmad et al., 2014). Additionally, it was reported that nicotinamide increased the concentrations of ions in the shoots of stressed faba bean plants, and this effect may be attributed to its role in enhancing osmotolerance and/or managing diverse results including, the intake of nutrients from the soil (Abdelhamid et al., 2013). The application of MLE on *Pelargonium graveolens* resulted in enhanced levels of potassium, nitrogen, phosphorus and magnesium, which subsequently led to improvements in plant height, volatile oil content, and overall yield characteristics (Ali et al., 2018).

From the data in Table 4, it was observed the seed yield per faba bean was notably decreased under saline conditions. These results align with those reported by Ragab et al. (2008) and Sadak et al. (2010) on different crops. The significant decrease in seed yield recorded in faba beans under salt stress may be partly attributed to a notable reduction in leaf chlorophyll content and could also result from the detrimental impact of salt stress on growth and the disturbance of mineral uptake (Taffouo et al., 2009). The increase in plant productivity is probably referred to the role of nicotinamide and/or MLE in enhancing nutrient absorption from the soil, increasing the photosynthetic rate, enhancing protein synthesis, increasing plant growth, and consequently, seed yield productivity. In this regard, Abdelhamid et al. (2013) recorded a rise in dry matter yield in bean plants treated with nicotinamide under saline irrigation conditions at 50 mM and 100 mM NaCl. Sadak (2016) and Dawood et al. (2019) indicated that NAM had a beneficial impact on growth parameters, amounts of IAA, photosynthetic pigments, and seed yield of faba seeds. The use of MLE on pepper enhanced plant height, fruit quantity, foliage area, as well as fruit length and diameter, resulting in better growth and yield traits (Hala et al., 2017). In *Triticum aestivum*, Yasmeen et al. (2012) reported that the application of MLE enhanced various growth and yield parameters, including grain yield, and harvest index. The increase in seed yield per plant because of nicotinamide or MLE addition can be primarily attributed to their role in promoting the synthesis of protein and retarding deterioration (Sahu et al., 1993). Our results (Figure 5) show a notable reduction in totals of carbohydrates and protein in stressed plants compared to the control. These decreases may also result from the effect of salinity on enzyme activities, leading to a drop in the totals of carbohydrates and protein in the resulting seeds (Sadak et al., 2010). The accumulation of total carbohydrates in faba bean plants treated with MLE may be due to the high total pigments content (Figure 1). Stress-induced accumulation of carbohydrates can act as osmolytes to preserve cell turgor and may protect proteins and membranes from harm (Kaplan and Guy, 2004).

CONCLUSION

Salinity stress negatively impacted the growth, biochemical composition, antioxidant levels, and yield quality of faba bean plants compared to control conditions. However, the external application of Nicotinamide (Vitamin B3) and/or *Moringa oleifera* leaf extract effectively mitigated the adverse effects of salinity. These treatments led to notable improvements in biochemical and physiological parameters. The most effective approach was the foliar application of nicotinamide (10 mg/L) combined with MLE (1:30 v/v, extract: water), which enhanced the salt tolerance of the plants. This combination not only preserved growth and productivity under saline conditions but also holds considerable potential for practical agricultural applications.

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CONFLICT OF INTEREST

The authors indicated no potential conflicts of interest

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