# **Journal of Multidisciplinary Sciences**

www.multidisciplines.com



# Alleviation of physiological traits in lemongrass under salinity stress

Fawzy M. Salama<sup>1</sup>, Marwa M. Ragaey<sup>2\*</sup>, Sayed S. A. Abdel-Rahman<sup>3</sup>, Noha M. M. Mostafa<sup>2,4</sup>, Atef A.S. Abd El-Kader<sup>4</sup>

<sup>1</sup>Botany Department, Faculty of Science, Assiut University, Assiut 71511, Egypt

<sup>2</sup>Botany and Microbiology Department, Faculty of Science, New Valley University, El-Kharga, Egypt

<sup>3</sup>Floriculture Department, Faculty of Agriculture, Assiut University, Assiut 71511, Egypt

<sup>4</sup>Medicinal and Aromatic Plants Department, Horticulture Research Inistitute, Agricultural Research Center, Giza, Egypt

\*Corresponding author email address: marwa\_ragaey@yahoo.com (M.M.R)

Received: 28 August 2023; Accepted: 20 October 2023; Published online: 27 October 2023

**Abstract.** Lemongrass is considered one of the most economic medicinal and aromatic plants, and there is a tendency to expand the production of such important plants in newly reclaimed soils, which often suffer from salt stresses. There are natural and synthetic substances that can help plants resist stress. Therefore, this study was conducted for the purpose of using some natural substances, *Moringa oleifera* leaf extract (MLE), *Bacillus subtilis + arbuscular mycorrhizal* fungi (B+A), and synthetic substances, such as benzyl amino purine (BAP), on lemongrass plants exposed to salt stress imposed by irrigation at 0, 1000, 2000, and 4000 ppm levels. Results indicated that an increasing trend of carbohydrate content by growth stimulants was noticed as follows: Moringa leaf extract (MLE) *Bacillus subtilis + arbuscular mycorrhizal* (B+M) > benzyl amino purine (BAP). Foliar application by growth stimulants increased free proline content. Application of MLE, followed by B+M, gave the highest values of the free proline content in the two cuts for the two seasons compared to the control. It was proven that total phenol content was affected by the different growth stimulant treatments. Foliar application of the growth stimulants increased the total phenol content in the two cuts for the control. Among the growth stimulants used, foliar spraying with MLE, followed by microorganisms (B+M), shows a superior effect in decreasing the accumulation of sodium and chlorine compared to other stimulants, while improving potassium was obtained by the growth stimulants MLE, B+A, and BAP, respectively, in both seasons.

**Keywords:** Cymbopogon citratus, salt stress, Moringa oleifera leaf extract, Bacillus subtilis + arbuscular mycorrhizal fungi, and benzylaminopurine

*Cite this as:* Salama, F.M., Ragaey, M.M., Abdel-Rahman, S.S.A., Mostafa, N.M.M. & Abd El-Kader, A.A.S. (2023). Alleviation of physiological traits in lemongrass under salinity stress. J. Multidiscip. Sci. 5(2), 11-22.

# 1. Introduction

The onset of the 21st century has brought along clear evidence of the repercussions of worldwide water scarcity, escalating environmental pollution, and the salinization of soil and freshwater. Two significant challenges that jeopardize agricultural sustainability are the burgeoning population and the scarcity of arable land for crop cultivation (Shahbaz and Ashraf, 2013). Soil salinity is frequently considered one of the primary abiotic pressures that significantly impacts land on a global scale (Shrivastava and Kumar, 2015). It has an impact on the growth and development of plants and results in decreased productivity (Munns and Tester, 2008).

The medicinal and aromatic crops are of prime economic importance and occupy a prominent and vital position in different fields such as pharmaceutical industries, agro-alimentary products, perfumes, and natural cosmetic products (Grayer et al., 1996) because of the increasing and continuous demand for their products in the local and foreign markets. Lemongrass, scientifically known as *Cymbopogon citratus* (D.C.) Stapf, is mostly grown in tropical and subtropical regions of Asia, South America, and Africa for its valuable essential oil. It is widely recognized for its medicinal and fragrant properties. The oil holds

significant economic value in the pharmaceutical, fragrance manufacturing, perfumery, flavoring, cosmetics, and detergent industries. Also, its oil possesses antifungal, antibacterial, mosquito repellent, and analgesic properties (Boukhatem et al., 2014). However, salt sensitivity is directly linked to the lemongrass plant because it cannot grow well in salt-rich soil (Loake and Grant, 2007).

There are many practical attempts to mitigate the harmful effects on plants resulting from increased salinity, whether in soil or irrigation water. In this respect, cytokinin has important roles in alleviating abiotic and biotic stresses (Vanková, 2011). Moreover, application of the synthetic benzyl amino purine (BAP) significantly increases the antioxidant enzyme activities and decreases the malondialdehyde content and relative conductivity in the leaves of *Rosa hybrida* (Wang et al., 2022).

Moringa oleifera is renowned for its exceptional properties. Siddhuraju and Becker (2003) demonstrated the antioxidant effects of moringa leaf extracts (MLE). The plant is abundant in plant growth regulators such as cytokinin and zeatin (Foidl et al., 2001; Yang et al., 2006). Zeatin promotes cellular proliferation and elongation. It contains several enzymes, exhibits antioxidant characteristics, and safeguards plant cells against the aging effects caused by reactive oxygen species (Taiz and Zeiger, 2010; Yasmeen et al., 2013).

Grover et al. (2011) postulated that some microorganisms have the ability to induce plants to resist various stresses resulting from climate change. It can also reduce the damage to plants caused by soil salinity by activating various biochemical and physiological processes within the plant cells (Abo Nouh et al., 2021; Ali et al., 2022). Therefore, the aim of the current study was to assess the effect of foliar application of benzyl amino purine (BAP), Moringa oleifera leaf extract (MLE), and inoculation with *Bacillus subtilis* + *arbuscular mycorrhizal* fungi (B+M) on physiological responses and essential oil constituents of lemongrass under salt stress conditions.

#### 2. Materials and Methods

#### 2.1. Experimental site

The Experimental Farm of Floriculture, located at the Faculty of Agriculture, Assiut University in Assiut, Egypt, conducted a pot experiment on two occasions. The experiments took place during the 2019 and 2020 seasons. This work aimed to study the effect of application growth stimulants, including natural and synthetic substances, on the morphological traits, oil yield, and biochemical and physiological responses of lemongrass (*Cymbopogon citrates*) plants under salt stress conditions. The natural substances represented in *Moringa oleifera* leaf extract as foliar spray and *Bacillus subtilis* and *arbuscular mycorrhizal* fungi (AMF) as inoculation. While the synthetic one is represented by benzylaminopurine (BAP) as a foliar spray. Healthy uniform plants of lemongrass were procured from the Department of Medicinal and Aromatic Plants Research, Horticultural Research Institute, Agricultural Research Center, Egypt. The plants were relocated into earthenware plastic pots with a diameter and height of 40 cm, which had perforated bottoms. Each pot was filled with 20 kg of sandy loam soil, and its physical and chemical parameters were assessed using the methods outlined by Jackson (1973).

Table 1. The physical and chemical analysis of the used media at the beginning of the experiment.											
Soil type	Soluble ions meq/100 g soil							Soluble K	pН	EC	OM
	Cations				Anions			mg/100g soil		Ds m <sup>-1</sup>	%
	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na⁺	K⁺	HCO₃ <sup>-</sup>	Cl-	SO4⁼	0.20	7.80	2.16	1.22
Sandy Ioam	2.24	2.14	2.21	0.05	0.54	3.80	2.30	1			
Note. pH: Potential of Hydrogen, EC: Eectrical Conductivity, OM: Organic Matter											

At the beginning of May, two seedlings will be planted in each pot in both seasons. Two weeks after transplanting, plants were treated with BAP and *Moringa oleifera* leaf extract as foliar applications or inoculated with *Bacillus subtilis* plus mycorrhizal fungi three times (two weeks after transplanting, three weeks later, and two weeks after the first cut). The Unit of Biofertilizers at Ain Shams University in Shobra El-Kheima, Egypt supplied an active strain of *Bacillus subtilis* (108 CFU/mL) and *arbuscular mycorrhizal* fungi (*Glomus irradicans*). The soil was treated with a combination of microorganisms, specifically *B. subtilis* and 25 spores of *Glomus irradicans* each pot, at a rate of 20 mL per pot (Demir and Onogur, 1999). Additionally, uninoculated plants are included as a control group.

Benzyl amino purine (BAP) was obtained as a commercial chemical substance from El-Gomhorya Company, Egypt. It will be applied at a rate of 100 ppm, while *Moringa oleifera* leaf extract will be applied at 5% for foliar applications. Also, untreated plants are involved as a control. The plants received regular irrigation with tap water for duration of three weeks following transplantation. Subsequently, the plants were exposed to varying degrees of salt (0, 1000, 2000, and 4000 ppm NaCl).

#### 2.2. Preparing Moringa oleifera leaf extract (MLE)

The leaves of *Moringa oleifera* were collected from fully grown trees. To extract the desired components, a mixture of 100 grams of fresh leaves and 1000 milliliters of distilled water was prepared using a household mixer. This solution was subsequently refined by filtering it through a muslin towel. The extract was further purified by passing it through No. 2 Whatman filter paper (Fuglie, 1999). The resulting solution was diluted with distilled water at a concentration of 50% (volume/volume) and thereafter applied via direct spraying onto the plants. It was utilized within a span of five hours after being obtained by cutting and extraction. If the extract or the produced solution was not immediately ready for use, it was stored in the refrigerator at a temperature of 0°C and only removed when required. Each plant received an application of 25 mL of the solution.

#### 2.3. Experimental design

The experiment included 16 treatments, which were the combination of four salinity levels (0 "tap water," 1000, 2000, and 4000 ppm NaCl) and four synthetic and natural growth substances (control, 100 ppm BAP, 5% moringa leaf extract, and *B. subtilis* + AMF). The treatments were arranged in a split-plot in the Randomized Complete Blocks Design (RCBD) with three replicates in this experiment. The four salinity levels (0 "tap water," 1000, 2000, and 4000 ppm NaCl) represented the main plots, while the four synthetic and natural growth substances (control, 100 ppm BAP, 5% moringa leaf extract, and *B. subtilis* + AMF) represented sub-plots.

#### 2.4. Biochemical and physiological parameters

#### 2.4.1. Total carbohydrates

The proportion of total carbohydrates in dried leaves was determined colorimetrically at a wavelength of 630 nm using the anthron sulphuric acid method, following the procedure described by Hansen and Moller (1975) after each cut. The data were quantified as glucose equivalents using a calibration curve spanning from 20 to 100 parts per million (ppm). In a conical flask, 0.2 g of anthrone, 30 mL of distilled water, 8 mL of 100% ethyl alcohol, and 100 mL of concentrated H<sub>2</sub>SO<sub>4</sub> (with a density of 1.84) were combined while being continuously cooled in an ice bath. It is necessary to always prepare this reagent fresh.

#### 2.4.2. Free proline content

Bates (1973) utilized a technique involving the blending of 0.5 mg of plant material with 10 mL of a 3% aqueous sulfosalicylic acid solution to assess the amount of free proline present in dried sweet basil leaves. The resulting homogenate was then filtered through a Whatman 2 filter paper. A volume of 2 mL of the filtrate was combined with 2 mL of ninhydrin and 2 mL of glacial acetic acid in a test tube. The mixture was then heated at a temperature of 100 degrees Celsius for 1 hour. Finally, placing the test tube in an ice bath stopped the reaction. The reaction mixture was subjected to extraction using 4 mL of toluene, which was aggressively agitated with a test tube stirrer for duration of 15-20 seconds. Using a water-based solution to separate the chromophore from toluene, it was heated to room temperature and its absorbance was measured at a wavelength of 520 nm. Toluene was used as a reference solution. The proline concentration was quantified using a standard curve and expressed on a dry weight basis using the following formula: [(µg proline/mL × mL toluene)/115.5 µg/µmoles]/[(g sample)/5] = µmoles proline/g of dry weight material.

# 2.4.3. Total phenol content

The quantification of soluble phenol concentration in dry leaves was conducted after each cut using a modified Folin method, as outlined by Vasco et al. (2008). Weighed samples of finely ground dried leaves (0.1 g) were subjected to two extractions at room temperature with continuous stirring for 1 hour. The first extraction involved a mixture of methanol and water (50:50 v/v) in a 20 mL volume, followed by a second extraction with acetone and water (70:30 v/v) also in a 20 mL volume. Intermittent centrifugation was performed at 4000 rpm for 15 minutes. The liquid portions were combined in containers with a specific volume, and the volumes were adjusted to 50 mL using distilled water. Subsequently, a portion (0.5 mL) of the extract,

blank, or standard was introduced into a 25-mL flask. Then, the Folin-Ciocalteu reagent (0.5 mL) was added to the flask, and the resulting mixture was allowed to undergo a reaction for 3 minutes while being continuously stirred. Following this, a solution of sodium carbonate (75 g/L, 10 mL) was added to the flask and thoroughly mixed. The volume was thereafter adjusted to 25 mL using distilled water and allowed to stand at room temperature for 1 hour. The study measured total phenol content at 750 nm using a UV visible spectrophotometer (Optizen Pop, Mecasys, Korea), expressing results as gallic acid equivalents (GAE) using a calibration curve.

# 2.4.4. Chemical analysis of minerals

At the completion of each cutting, specimens were gathered and exposed to a 48-hour drying procedure at a temperature of 70 °C. This was conducted to determine the chemical constituents present in the dried leaf. The concentration of potassium (K) in the leaves was determined using a flame photometer, employing the procedure outlined by Jackson (1958). The Na and Cl contents were determined utilizing the AOAC method (AOAC, 1990).

# 2.5. Statistical analysis

The data from two seasons will be analyzed using Statistix 8.1 software, and the means will be compared using the least significant difference (LSD) test (Dowdy and Wearden, 1983).

# 3. Results

# 3.1. Total carbohydrate percentage

Total carbohydrate percentages gradually decreased with the increasing salinity level, from 1000 up to 4000 ppm (Figure 1). However, the higher total carbohydrate percentage of lemongrass was recorded with untreated plants, followed by those treated with the lower concentration (1000 ppm). The lowest values were achieved with the higher salinity concentration (4000 ppm). There was a significant improvement in total carbohydrate content as a result of the growth stimulants used on lemongrass. Our results proved that an increasing trend in carbohydrate contents caused by growth stimulants was noticed as follows: Moringa leaf extract (MLE) > *Bacillus subtilis* + *arbuscular mycorrhizal* (B+M) > benzylaminopurine (BAP).



Figure 1. Total carbohydrates percentage (%) of lemongrass as affected by moringa leaves extract (MLE), benzyl amino purine (BA) and Bacillus subtilis + arbuscular mycorrhizal (B+M) under salinity stress, as average seasons of 2019 and 2020.

# 3.2. Free proline content

Our data showed that salinity levels in the two cuts during the two seasons had an impact on free proline content (Figure 2). Clearly, the amount of free proline in the plants increased as the salinity level rose from 1000 to 4000 ppm, compared to lemongrass plants that were not salinized. The free proline content was affected by the growth stimulant treatments in the two cuts during both seasons. Application of growth stimulants to the lemongrass plant under salinity stress increased the free proline content. Application of MLE, followed by B+M, gave the highest values of the free proline content in the two cuts for the two seasons compared to the control.



Figure 2. Free proline content (mg/ g DW) of lemongrass as affected by moringa leaves extract (MLE), benzyl amino purine (BA) and Bacillus subtilis + arbuscular mycorrhizal (B+M) under salinity stress, as average seasons of 2019 and 2020.

#### 3.3. Total phenol content

The highest values of total phenol content were registered with 2000, followed by 1000 ppm NaCl, and while the least values were recorded with plants treated with the higher level (4000 ppm), as shown in **Figure 3**. In response to salinity stress, plants have developed different biochemical and physiological mechanisms to tolerate or adapt to stress. Data shows that salt stress at 2000 and 1000 ppm treatments enhanced the phenolic contents. From our results, it was noticed that total phenol contents were affected by the different growth stimulant treatments. Foliar application of the growth stimulants increased the total phenol content compared to the control. However, application of MLE resulted in the highest values of total phenol content in the two seasons compared to the control.



Figure 3. Total phenols content (mg/ GAE/g DW) of lemongrass as affected by moringa leaves extract (MLE), benzyl amino purine (BA) and *Bacillus subtilis* + *arbuscular mycorrhizal* (B+M) under salinity stress, as average seasons of 2019 and 2020.

#### 3.4. Na+, CI-, and K+ contents

Salinity levels showed significant differences in the sodium, chlorine, and potassium contents of lemongrass leaves in the first and second cuts for the two seasons, as presented in **Figures 4–6**. There is a gradual increase in the accumulation of these contents with increasing salinity concentrations up to 4000 ppm. The highest values were associated with salinity at a rate of 4000 ppm. Among the growth stimulants used, foliar spraying with moringa leaf extracts (MLE), followed by microorganisms (B+M), and shows a superior effect in decreasing the accumulation of sodium and chlorine while improving potassium contents in lemongrass. The most effective treatments for decreasing sodium and chlorine while improving potassium were MLE, B+A, and BA, respectively, in both seasons.



Figure 4. Sodium content (mg/ g DW) of lemongrass as affected by moringa leaves extract (MLE), benzyl amino purine (BA) and *Bacillus* subtilis + arbuscular mycorrhizal (B+M) under salinity stress, as average seasons of 2019 and 2020.







Figure 6. Potassium content (mg/ g DW) of lemongrass as affected by moringa leaves extract (MLE), benzyl amino purine (BA) and Bacillus subtilis + arbuscular mycorrhizal (B+M) under salinity stress, as average seasons of 2019 and 2020.

# 4. Discussion

The obtained results showed that total carbohydrates were gradually decreased with the increasing salinity level from 1000 to 4000 ppm. In addition, total carbohydrates increased with the application of different growth stimulants, especially moringa leaf extract (MLE) and *Bacillus subtilis* + *arbuscular mycorrhizal* (B+M). These results agreed with those obtained by Munns (2002)

and Mangena (2020), who reported that total carbohydrate contents decreased under high salt stress. Rahneshan et al. (2018) proved that salt stresses are critical factors that severely affect plant growth and metabolism. Also, Abdel Latef et al. (2017) pointed out that the reduction in growth, especially at severe salinity, was connected with the diminution in the total carbohydrate contents. In this regard, Farouk et al. (2001) revealed that salt stress decreased total carbohydrate content in *Mentha longifolia* shoots. Also, on *Nigella sativa*, Hajar et al. (1996) claimed that total carbohydrates decreased with the increase in NaCl salinity. The synthesis of carbohydrates was reduced by water stress due to soil salinity (Kilany et al., 2006). Moreover, abiotic stresses repress cell expansion more than cell division, resulting in diminished plant development by influencing different physiological and biochemical processes such as ion uptake, carbohydrate metabolism, and nutrient metabolism (Faroog et al., 2009).

These results were in agreement with those of Desoky et al. (2019), who revealed that spraying MLE was more effective in increasing total carbohydrates in Sudan grass than other treatments under different levels of soil salinity. Application of moringa leaf extract led to an increase in total carbohydrates in sweet basil (Hassanein et al., 2019). However, the use of MLE gave a great chance for the translocation of nutrients assimilated into a healthy cell to be utilized in different metabolic processes, such as carbohydrate synthesis under salinity conditions (Semida et al., 2014). Plant growth-promoting microorganisms have direct and indirect means of mitigating salinity damage to plants (Hashem et al., 2016), such as by avoiding high salt levels inside the cytoplasm. This is achieved through modification in the cell wall construction by the formation of specific membrane proteins, exopolysaccharides, and lipids. Other adaptations for plant survival under salt stress include the development of proteins and enzymes that are capable of performing metabolic functions (Kunte, 2006). Studies concluded that salt-tolerant plant growth-promoting rhizobacteria (ST-PGPR) produce various types of phytohormones, such as gibberellins, auxins, and cytokinins (Dodd et al., 2010), that regulate plant defense systems and increase carbohydrate contents (Hashem et al., 2016).

Our results pointed out that free proline content increased with increasing salinity stress, from 1000 to 4000 ppm. Also, application of growth stimulants increased free proline content, especially with foliar application of MLE, followed by inoculation with B+M, compared to the untreated plants. The same results were recorded by Mukarram et al. (2022), who reported that proline, a key osmolyte, was quantified in lemongrass to assess osmoprotection during salt stress, and the highest improvement was brought by the higher level of NaCl application (240 mM). Also, a positive correlation between proline content and stress intensity was found in several plant species (Pavlovic et al., 2019). However, the upregulation of osmolyte (proline) during salt stress is a common defense mechanism in several plant species to render enhanced protection against stress-induced osmotic damage (Foyer, 2018; Noctor et al., 2018). Our findings of increased proline content with the increase in salt stress may have been due to the fact that many cellular enzymes are affected by salinity stress, such as enzymes involved in the metabolism of nitrogen and the synthesis of amino acids such as proline. These were in agreement with Nathawat et al. (2005) and Siddiqui et al. (2008). Also, Garcia et al. (2019) reported that the contents of nutrients, metabolites, and proteins are disrupted by salt stress. Additionally, compatible solutes play a diverse role in plant physiology to alleviate salt stress effects under harsh conditions. These compatible solutes include proline, hydroxyproline, sugars, and sugar alcohols (Zulfigar et al., 2020).

The obtained results were in accordance with Hassan et al. (2021), who found that free proline increased compared to the control in response to salinity in damask rose. They added that application of MLE increased proline contents in plants grown under stress. Enhancing antioxidant defense systems in response to MLE application and increasing scavenged reactive oxygen species (ROS) and thus improved membrane stability, which in turn increased tolerance to high salinity, as has also been shown in several studies (Hassan and Fetouh, 2019). On the other hand, application of B+A produced higher proline in lemongrass compared to the control. PGPB (plant growth-promoting bacteria) and endophytic fungi can produce antioxidant enzymes, which scavenge excess reactive oxygen species generated in plants under saline stress (Afridi et al., 2019); therefore, they minimize salt effects on plant growth (Ghaffari et al., 2016). Moreover, proline is thought to function as an osmoprotectant for proteins (Bohnert and Jenson, 1996). Accumulation of proline provides an environment compatible with the macromolecular structure and helps plants adapt to the negative consequences of salinity (Jaleel et al., 2007). In the present study, the different growth stimulants, especially MLE, increased proline accumulation in the leaves of lemongrass under salt stress.

Results showed that the higher contents of total phenols were due to using salinity at a rate of 2000 ppm, followed by 1000 ppm, while the lowest one was recorded at 4000 ppm. Using the growth stimulants resulted in an increase in total phenol content, especially with MLE spraying. Salinity-induced disturbances in metabolic processes lead to an increase in the synthesis of phenolic compounds, according to Keutgen and Pawelzik (2009) and Dawood and El-Awadi (2015). But when plants are under a lot of salt, reactive oxygen species (ROS) build up and the net carbon gain changes. This can have a big impact on the biosynthesis of carbon-based secondary compounds, especially polyphenols (Rady et al., 2013). However, phenolic compound production in plants under

salinity is dependent on the salt sensitivity of the plant (Kim et al., 2008). The soluble phenolic compounds are secondary metabolites in plants and could be increased as powerful antioxidants in plant tissues under salinity conditions. This judgment agrees with Abou El-Leel et al. (2018).

The results of Rehman et al. (2022) revealed that applying *Cymbopogon flexuosus* to salicylic acid produced the maximum phenolic compounds compared to the control. However, phenolic compounds are significant food metabolites that can help to prevent diseases, including neurological and cardiovascular diseases as well as cancer (Goyal et al., 2014). The effect of MLE, BA, and B+M treatments on lemongrass resulted in different degrees of improvement in phenolic compounds, with improvements being particularly noticeable with MLE. The obtained results were in line with Zrige et al. (2022). Creating secondary metabolites, such as phenolic compounds, is one way plants deal with salt stresses (Agati et al., 2007). These compounds help protect plants from ROS. The phenolic compounds are synthesized when photosynthetic metabolisms or aerobic respiration are disrupted by environmental stresses (Bettaieb et al., 2011). Thus, the considerable increment in total phenol of lemongrass applied by growth stimulants might be a result of improved plant resistance mechanisms to adapt to adverse salinity conditions (Alu'datt, 2017). Also, the enhancement of total phenols in lemongrass with the application of MLE may be due to an increment in K+ content, which plays an important role in activating most enzymes in pathways of biosynthesis (Pavarini et al., 2012). Moreover, the application of stimulants might improve the production of antioxidant enzymes and plant defensive metabolites (Cappellari et al., 2020).

Salinity levels showed a gradual increase in the accumulation of Na+, Cl-, and K+ contents with increasing salinity concentrations up to 4000 ppm. Application of moringa leaf extract (MLE), followed by microorganisms (B+M), resulted in a decreasing accumulation of sodium and chlorine while increasing potassium content in lemongrass. Our results were consistent with those of Ghassemi-Golezani et al. (2022). Under salinity conditions, which cause reductions in plant growth because of specific ion toxicities such as Na+ and Cl- and ionic imbalance acting on the biophysical and/or metabolic components of the plant growth (Grattan and Grieve, 1999). However, Malagoli et al. (2008) discovered that Na+ is a toxic ion that roots' cells quickly absorb. Thus, disruption in the Na+ exclusion process at the root and subsequently its translocation to the shoots under salinity stress led to an increase of this ion in the roots and leaves of the plant (Ghassemi-Golezani et al., 2022). Na+ and K+ concentrations are important ions associated with the salinity tolerance of plants (Azadi et al., 2011). High concentrations of Na+ in lemongrass under salt stress can also interfere with the accumulation of other ions such as Cl- and K+ (Ji et al., 2019). However, plant tolerance to osmotic stresses and Na+ toxicity determine the overall plant responses to salt stress (Munns and Tester, 2008). In this regard, Shabala and Cuin (2008) reported that under salinity conditions, Na+ ions enter apoplastic lumens and, with the substitution of Ca<sup>2+</sup> ions in the cell membrane, depolarize the membrane and interfere with selective absorption of the essential minerals.

One of the most important strategies for ion balance in the cytoplasm is reducing Na+ uptake, limiting its accumulation in plant tissues, and increasing the K+/Na+ ratio (Munns and Tester, 2008). However, alleviating salt toxicity by growth stimulants, especially MLE, may be related to reducing the Na+ in the cytosol (Ghassemi-Golezani and Farhangi-Abriz, 2018). Also, Ahanger (2018) reclaimed that foliar spraying with cytokinins regulates the fundamental cellular pathways and preserves them from Na+ toxicity with an improvement in K+ uptake. On the other side, alleviation of water stress by different microorganisms such as mycorrhiza and bacteria, which promote plant growth, has been reported earlier by Chakraborty et al. (2013). Gurmani et al. (2011) proved that the used plant growth promoters (ABA and BA) lowered Na+ accumulation, while K+ concentration was increased under saline field conditions in rice plants. Iqbal and Ashraf (2006) revealed that BA was effective in reducing Na+ levels in wheat plants under salt stress. Na+ affects the uptake of the K+ ion due to the chemical similarities between the two ions in plants (Rodríguez-Navarro, 2000).

#### 5. Conclusions

It is clear from the study that treating lemongrass plants with some growth stimulants such as moringa leaf extract (MLE), microorganisms (B+M), and benzyl amino purine (BAP) could improve the biochemical and physiological parameters as well as the chemical composition of the volatile oil under salt stress conditions. Foliar application of MLE at 5%, followed by B+M, was the most effective treatment for inducing the biochemical and physiological characteristics under low salinity conditions, especially with lower salinity levels (1000 and 2000 ppm NaCl). From here, it can be recommended when planting lemongrass, especially in salt-affected soils, to apply these treatments under study to obtain higher vegetative growth and productivity.

Conflicts of interest. The authors mentioned that none of them have a conflict of interest when it comes to this article.

# ORCID

Marwa M. Ragaey: https://orcid.org/0000-0001-5563-9803

# References

- Abou El-Leel, O.F., El-Shayeb, N.S.A. & El-Azzony, E.A.A. (2018). Effect of proline on growth and the active ingredients of Moringa oleifera Lam. plant under salt stress. New Egyptian Journal of Microbiology, 51, 56-85.
- Afridi, M.S., Amna, Sumaira, Mahmood, T., Salam, A., Mukhtar, T., Mehmood, S., Ali, J., Khatoon, Z. et. al. (2019). Induction of tolerance to salinity in wheat genotypes by plant growth promoting endophytes: Involvement of ACC deaminase and antioxidant enzymes. Plant Physiology and Biochemistry, 139, 569-577.
- Ahanger, M.A. (2018). Potential of exogenously sourced kinetin in protecting *Solanum lycopersicum* from NaCl-induced oxidative stress through up-regulation of the antioxidant system, ascorbate glutathione cycle and glyoxalase system. PLoS ONE 13, 1-21.
- AOAC (1990).Official methods of analysis.15th ed. Association of Official Analytical Chemist, Washington D.C.
- Abdel Latef, A.A., Abu Alhmad, M.F. & Hammad S.A. (2017). Foliar application of fresh moringa leaf extract overcomes salt stress in fenugreek (*Trigonellafoenum-graecum*) plants. Egyptian Journal of Botany, 57(1), 157-179.
- Abo Nouh, F.A., Abu-Elsaoud, A.M. & Abdel-Azeem, A.M. (2021). The role of endophytic fungi in combating abiotic stress on tomato. Microbial Biosystems, 6(1), 1037:35-48.
- Agati, G., Matteini, P., Goti, A. & Tattini, M. (2007). Chloroplast-located flavonoids can scavenge singlet oxygen. New Phytologist, 174, 77-89.
- Ali, R., Gul, H., Hamayun, M., Rauf, Iqbal, M.A., Hussain, A. & Lee, I. (2022). Endophytic fungi controls the physicochemical status of maize crop under salt stress. Polish Journal of Environmental Studies, 31(1), 561-573.
- Alu'datt, M.H. (2017). A review of phenolic compounds in oil-bearing plants: Distribution, identification and occurrence of phenolic compounds. Food Chemistry, 218, 99–106.
- Azadi, A., Hervan, E.M., Mohammadi, S.A., Moradi, F., Nakhoda, B. Vahabzade, M. & Mardi, M. (2011). Screening of recombinant inbred lines for salinity tolerance in bread wheat (*Triticum aestivum* L.). African Journal of Biotechnology, 10, 12875-12881.
- Bates, L.S. (1973). Rapid determination of free proline for water stress studied. Plant and Soil, 39, 205-207.
- Bettaieb, I., Hamrouni-Sellami, I., Bourgou, S., Limam, F. & Marzouk, B. (2011). Drought effects on polyphenol composition and antioxidant activities in aerial parts of *Salvia officinalis* L. Acta Physiologiae Plantarum, 33, 1103-1111.
- Bohnert, H.J. & Jenson, R.G. (1996). Strategies for engineering water-stress tolerance in plants. Trends in Biotechnology, 14, 8997.
- Boukhatem, M.N., Ferhat, M.A., Kameli, A., Saidi, F. & Kebir, H.T. (2014). Lemon grass (*Cymbopogon citratus*) essential oil as a potent anti-inflammatory and antifungal drugs. Libyan Journal of Medicine, 9, 25431.
- Cappellari, L.D.R., Santoro, M.V., Schmidt, A., Gershenzon, J. & Banchio, E. (2020). Improving phenolic total content and monoterpene in Mentha × piperita by using salicylic acid or methyl jasmonate combined with Rhizobacteria inoculation. International Journal of Molecular Sciences, 21, 50.
- Chakraborty, U., Chakraborty, B.N., Chakraborty, A.P. & Dey, P.L. (2013). Water stress amelioration and plant growth promotion in wheat plants by osmotic stress tolerant bacteria. World Journal of Microbiology and Biotechnology, 29, 789-803.
- Dawood, M.G. & El-Awadi, M.E. (2015). Alleviation of salinity stress on *Vicia faba* L. plants via seed priming with melatonin. Acta Biologica Colombiana, 20(2), 223-235.
- Demir, S. & Onogur, E. (1999). *Glomus intraradices* Schenck & Smith: a hopeful vesicular-arbuscular mycorrhizal (VAM) fungus determined in soils of Turkiye. Journal of Turkish Phytopathology, 28, 33-34.
- Desoky, E.M., Merwad, A.M. & Ibrahim, S.A. (2019). Humus materials and moringa (*Moringa oleifera* Lam.) leaf extract modulate the harmful effect of soil salinity stress in sudan grass (*Sorghum vulgare* L.). Egyptian Journal of Agronomy, 41(1), 29-45.
- Dodd, I.C., Zinovkina, N.Y., Safronova, V.I. & Belimov, A.A. (2010). Rhizobacterial mediation of plant hormone status. Annals of Applied Biology, 157, 361- 379.

- Dowdy, S. & Wearden, S. (1983). Statistics for Research (Wiley Series in Probability and Statistics). Wonder Boock, Frederick, USA.
- Farooq, M., Wahid, A., Kobayashi, N., fujita, D. & Basra, S.M.A. (2009). Plant drought stress: Effects, mechanisms and management. Agronomy for Sustainable Development, 29,185-212.
- Farouk, M.G., faisal, M.M.A. & Ibrahim, K.A. (2001). Response of wild mint (*Mentha longifolia* L. Huds. var. longifolia) plants to salinity stress. Egyptian Journal of Applied Science, 16(4), 39-52.
- Foidl, N., Makkar, H.P.S., Becker, K., Foild, N. & Km, S. (2001). The potential of *Moringa oleifera* for agricultural and industrial uses. What Development Potential Moringa Production? 20, 1-20.
- Foyer, C.H. (2018). Reactive oxygen species, oxidative signaling and the regulation of photosynthesis. Environmental and Experimental Botany, 154, 134-142.
- Fuglie, L.J. (1999). The Miracle Tree: Moringa oleifera: Natural Nutrition for the Tropics. Church World Service, Dakar.
- Garcia, C.L., Dattamudi, S., Chanda, S. & Jayachandran, K. (2019). Effect of salinity stress and microbial inoculations on Glomalin production and plant growth parameters of snap bean (*Phaseolus vulgaris*). Agronomy, 9, 545.
- Ghaffari, M.R., Ghabooli, M., Khatabi, B., Hajirezaei, M.R., Schweizer, P. & Salekdeh, G.H. (2016). Metabolic and transcriptional response of central metabolism affected by root endophytic fungus *Piriformospora indica* under salinity in barley. Plant Molecular Biology, 90, 699-717.
- Ghassemi-Golezani, K. & Farhangi-Abriz, S. (2018). Foliar sprays of salicylic acid and jasmonic acid stimulate H+-ATPase activity of tonoplast, nutrient uptake and salt tolerance of soybean. Ecotoxicology and Environmental Safety, 166, 18-25.
- Ghassemi-Golezani, K., Nikpour-Rashidabad, N. & Samea-Andabjadid, S. (2022). Application of growth promoting hormones alters the composition and antioxidant potential of dill essential oil under salt stress. Scientific Reports, 12, 14349.
- Goyal, A., Sharma, V. & Upadhyay, N. (2014). Flax and flaxseed oil: An ancient medicine & modern functional food. Journal of Food Science and Technology, 51, 1633-1653.
- Grattan, S.R. & Grieve, C.M. (1999). Salinity mineral nutrient relations in horticultural crops. Scientia Horticulturae, 78, 127-157.
- Grayer, R.J., Kite, G.C., Goldstone, F.J., Bryan, S.E., Paton, A. & Putievsky, E. (1996). Infraspecific taxonomy and essential oil chemotypes in sweet basil, *Ocimum basilicum*. Phytochemistry. 43, 1033-1039.
- Grover, M., Ali, S.K.Z., Sandhya, V., Rasul, A. & Venkateswarlu, B. (2011). Role of microorganisms in adaptation of agriculture crops to abiotic stresses. World Journal of Microbiology and Biotechnology, 27, 1231-1240.
- Gurmani, A.R., Bano, A., Khan, S.U., Din, J. & Zhang, J. L. (2011). Alleviation of salt stress by seed treatment with abscisic acid (ABA), 6-benzylaminopurine (BA) and chlormequat chloride (CCC) optimizes ion and organic matter accumulation and increases yield of rice (*Oryza sativa* L.). Australian Journal of Crop Science, 5(10), 1278-1285.
- Hajar, A.S., Zidan, M.A. & Al-Zahrani, H.S. (1996). Effect of salinity stress on germination, growth and some physiological activity of Black cumin (*Nigella sativa*) Arab Gulf. Journal of Scientific Research, 14(2), 445-454.
- Hansen, J. & Moller, I. (1975). Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. Analytical Biochemistry, 68(1), 87-94.
- Hashem, A., Abd\_Allah, E. F., Alqarawi, A. A., Al-Huqail, A. A., Wirth, S. & Egamberdieva, D. (2016). The interaction between arbuscular mycorrhizal fungi and endophytic bacteria enhances plant growth of *Acacia gerrardii* under salts tress. Frontiers in Microbiology, 7, 1089.
- Hassan, F.A.S. & Fetouh, M.I. (2019). Does moringa leaf extract have preservative effect improving the longevity and postharvest quality of gladiolus cut spikes? Scientia Horticulturae, 250, 287-293.
- Hassan, F., Al-Yasi, H., Ali, E., Alamer, K., Hessini, K., Attia, H. & El-Shazly, S. (2021). Mitigation of salt-stress effects by moringa leaf extract or salicylic acid through motivating antioxidant machinery in damask rose. Canadian Journal of Plant Science, 101, 157-165.
- Hassanein, R.A., Abdelkader, A.F. & Faramawy, H.M. (2019). Moringa leaf extracts as biostimulants-inducing salinity tolerance in the sweet basil plant. Egyptian Journal of Botany, 59(2), 303-318.
- Iqbal, M. & Ashraf, M. (2006). Wheat seed priming in relation to salt tolerance: growth, yield and levels of free salicylic acid and polyamines. Annales Botanici Fennici, 43, 250-259.
- Jackson, M.L. (1958). Soil Chemical Analysis, Prentice-Hall, London.
- Jackson, M.L. (1973). Soil Chemical Analysis. Prentice-Hall, Inc. Englewood Cliffs, N.J. New Delhi, India.

- Jaleel, C.A., Gopi, R., Sankar, B., Manivannan, P., Kishorekumar, A., Sridharan, R. & Panneerselvam, R. (2007). Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. South African Journal of Botany, 73, 190-195.
- Ji, Z., Camberato, J.J., Zhang, C. & Jiang, Y. (2019). Effects of 6-Benzyladenine, Aminobutyric acid, and nitric oxide on plant growth, photochemical efficiency, and ion accumulation of perennial ryegrass cultivars to salinity stress. Hortscience, 54(8), 1418-1422.
- Keutgen, A.J. & Pawelzik, E. (2009). Impacts of NaCl stress on plant growth and mineral nutrient assimilation in two cultivars of strawberry. Environmental and Experimental Botany, 65(23), 170-176.
- Kilany, A.E., El-Shenawy, I.E., Abd El-Ghany, A.A. & Ahmed, O.A. (2006). Salt tolerance of some grape rootstocks. Research Bulletin, Ain Shams Univ. pp.1-15.
- Kim, H.J., Fonseca, J.M., Choi, J.H., Kubota, C. & Kwon, D.Y. (2008). Salt in irrigation water affects the nutritional and visual properties of romaine lettuce (*Lactuca sativa* L.). Journal of Agricultural and Food Chemistry, 56, 3772-3776.
- Kunte, H.J. (2006). Osmoregulation in bacteria: compatible solute accumulation and osmosensing. Environmental Chemistry, 3, 94-99.
- Loake, G. & Grant, M. (2007). Salicylic acid in plant defence the players and protagonists. Current Opinion in Plant Biology, 10, 466-472.
- Malagoli, P., Britto, D.T., Schulze, L.M. & Kronzucker, H.J. (2008). Futile Na<sup>+</sup> cycling at the root plasma membrane in rice (*Oryza sativa* L.): Kinetics, energetics, and relationship to salinity tolerance. Journal of Experimental Botany, 59, 4109-4117.
- Mangena, P. (2020). Role of benzyladenine seed priming on growth and physiological and biochemical response of soybean plants grown under high salinity stress condition. International Journal of Agronomy, 8847098, 1-5.
- Mukarram, M., Khan, M.M.A., Zehra, A., Petrik, P. & Kurjak, D. (2022). Suffer or survive: decoding salt-sensitivity of lemongrass and its implication on essential oil productivity. Frontiers in Plant Science, 13, 903954.
- Munns, R. (2002). Comparative physiology of salt and water stress. Plant, Cell and Environment, 25(2), 239-250.
- Munns, R. & Tester, M. (2008). Mechanisms of salinity tolerance. Annual Review of Plant Biology, 59, 651-681.
- Nathawat, N.S., Kuhad, M.S., Goswami, C.L., Patel, A.L. & Kumar, R. (2005).Nitrogen-metabolizing enzymes: effect of nitrogen sources and saline irrigation. Journal of Plant Nutrition, 28, 1089-1101.
- Noctor, G., Reichheld, J.P. & Foyer, C.H. (2018). ROS-related redox regulation and signaling in plants. Semin Cell and Developmental Biology, 80, 3-12.
- Pavarini, D.P., Pavarini, S.P., Niehues, M. & Lopes, N.P. (2012). Exogenous influences on plant secondary metabolite levels. Animal Feed Science and Technology, 176, 5-16.
- Pavlovic, I., Molinari', S., Tarkowská, D., Oklestková, J., Novák, O., Lepeduš, H., Vujc'ic' Bok, V., Radic' Brkanac, S., Strnad, M.
  & Salopek-Sondi, B. (2019). Early Brassica crops responses to salinity stress: A comparative analysis between Chinese cabbage, White cabbage and Kale. Frontiers in Plant Science, 10, 450.
- Rady, M.M., Bhavya, V.C. & Howladar, S.M. (2013). Common bean (*Phaseolus vulgaris* L.) seedlings overcome NaCl stress as a result of presoaking in *Moringa oleifera* leaf extract. Scientia Horticulturae, 162, 63-70.
- Rahneshan, Z., Nasibi, F. & Moghadam, A.A. (2018). Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (*Pistacia vera* L.) rootstocks. Journal of Plant Interactions, 13(1), 73-82.
- Rehman, Z., Hussain, A., Saleem, S., Khilji, S.A. & Sajid, Z.A. (2022). Exogenous application of salicylic acid enhances salt stress tolerance in lemongrass (*Cymbopogon flexuosus* Steud. Wats.). Pakistan Journal of Botany, 54(2), 371-378.
- Rodríguez-Navarro, A. (2000). Potassium transport in fungi and plants. Biochimica et Biophysica Acta, 1469, 1-30.
- Semida, W.M., Taha, R.S., Abdelhamid, M.T. & Rady, M.M. (2014). Foliar-applied α-tocopherol enhances salt-tolerance in *Vicia faba* L. plants grown under saline conditions. South African Journal of Botany, 95, 24-31.
- Shabala, S. & Cuin, T.A. (2008). Potassium transport and plant salt tolerance. Plant Physiology, 133, 651-669.
- Shahbaz, M. & Ashraf, M. (2013). Improving salinity tolerance in cereals. Critical Reviews in Plant Sciences, 32, 237-249.
- Shrivastava, P. & Kumar, R. (2015). Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi Journal of Biological Sciences, 22(2), 123-131.

- Siddhuraju, P. & Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. Journal of Agricultural and Food Chemistry, 51, 2144-2155.
- Siddiqui, M. H., Khan, M. N., Mohammad, F. & Khan, M.M.A. (2008). Role of nitrogen and gibberellin (GA3) in the regulation of enzyme activities and in osmoprotectant accumulation in *Brassica juncea* L. under salt stress. Journal of Agronomy and Crop Science, 194, 214-224.
- Taiz, L. & Zeiger, E. (2010). Responses and Adaptations to Abiotic Stress. In Plant Physiology; Sinauer Associates, Inc.: Sunderland, MA, USA.
- Vanková, R. (2011). Analysis of cytokinin mutants and regulation of cytokinin metabolic genes reveals important regulatory roles of cytokinins in drought, salt and ABA responses, and ABA biosynthesis. Plant Cell, 23, 2169-2183.
- Vasco, C., Ruales, J. & Kamal-Eldin, A. (2008). Phenolic compounds and antioxidant capacities of major fruits from Ecuador. Food Chemistry, 111, 816-823.
- Wang, K., Shen, Y., Wang, H., He, S., Kim, W.S., Shang, W., Wang, Z. & Shi, L. (2022). Effects of exogenous salicylic acid (SA), 6-benzylaminopurine (6-BA), or abscisic acid (ABA) on the physiology of *Rosa hybrida* 'Carolla' under hightemperature stress. Horticulturae, 8(9), 851.
- Yang, R.Y., Tsou, S.C.S., Lee, T.C., Chang, L.C., Kuo, G. & Lai, P.Y. (2006). Moringa, a novel plant rich in antioxidants, bioavailable iron, and nutrients. In Proceedings of the ACS Symposium Series; ACS Publication: Washington, DC, USA, 925, 224-239.
- Yasmeen, A., Basra, S., Farooq, M., Ur Rehman, H. & Hussain, N. (2013). Exogenous application of moringa leaf extract modulates the antioxidant enzyme system to improve wheat performance under saline conditions. Plant Growth Regulation, 69, 225-233.
- Zrige, A., Najar, B., Korany, S.M., Hassan, A.H.A., Alsherif, E.A., Shah, A.A., Fahad, S., Selim, S. & AbdElgawad, H. (2022). The interaction effect of laser irradiation and 6-benzylaminopurine improves the chemical composition and biological activities of linseed (*Linum usitatissimum*) Sprouts. Biology, 11, 1398.
- Zulfiqar, F., Casadesús, A., Brockman, H. & Munné-Bosch, S. (2020). An overview of plant-based natural biostimulants for sustainable horticulture with a particular focus on moringa leaf extracts. Plant Science, 295, 110194.



Copyright: © 2023 by the authors. Licensee Multidisciplines. This work is an open-access article assigned in Creative Commons Attribution (CC BY 4.0) license terms and conditions (http://creativecommons.org/licenses/by/4.0/).