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Biological and Chemical Substances as Stimulants-Inducing Growth and Oil Yield in Lemongrass Under Salinity Stress

Fawzy M. Salama¹; Sayed S.A. Abdel-Rahman^{*2}; Marwa M. Ragaey³; Noha M.M. Mostafa⁴ and Atef A.S. Abdel -Kader⁴

¹Botany Department, Faculty of Science, Assiut University, Assiut, Egypt.

²Floriculture Department, Faculty of Agriculture, Assiut University, Assiut, Egypt.

³Botany and Microbiology Department, Faculty of Science, New Valley University, New Valley, Egypt.

⁴Medicinal and Aromatic Plants Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt.

* Correspondence: shehatasayed54@yahoo.com

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Abstract

This experiment was conducted at the Experimental Farm of Floriculture, Faculty of Agriculture, Assiut University, Assiut, Egypt during the two successive seasons of 2019 and 2020 to study the effect of some natural and synthetic growth stimulants on the growth measurements, percentage and oil yield and photosynthetic pigments of lemongrass (*Cymbopogon citratus*) plants under water salinity stress. Plants were treated with four growth stimulants (control, *Moringa oleifera* leaf extract (MLE) at 5% as spray, *Bacillus subtilis* + arbuscular mycorrhiza fungi as soil drench, benzylaminopurine (BAP) at 100 mg l⁻¹ as pray). Irrigation water salinity treatments were control, 1000, 2000 and 4000 mg l⁻¹ NaCl. The results showed that the higher salinity levels (2000 and 4000 mg l⁻¹ NaCl) caused significant decreases in the morphological characteristics and oil yield, as well as the leaf content of photosynthetic pigments (chlorophyll a, b and carotenoids) of lemongrass plants compared to control and the lowest salinity level (1000 mg l⁻¹ NaCl). The highest salinity level (4000 mg l⁻¹) recorded the lowest values of these measurements. Among growth stimulants used, MLE gave the highest values of vegetative growth measurements, percentage and yield of oil/plant and chlorophyll content in fresh leaves, followed by *B. subtilis* + mycorrhiza fungi. Therefore, the study recommends spraying lemongrass plants with *M. oleifera* leaf extract at 5% or adding a mixture of *B. subtilis* with mycorrhizal fungi as natural growth stimulants to obtain the best morphological characteristics, percentage and yield of volatile oil under water salt stress conditions.

Keywords: *Cymbopogon citratus*, volatile oil, salt stress, moringa leaf extract, *Bacillus subtilis*

Introduction

There is a global trend to expand medicinal and aromatic plant production to replace chemically synthesized compounds. One of the famous plants in this regard

is lemongrass. This is due to its importance in volatile oil extracted from its herb, which is used in medicines, perfumes, and food industries. Egypt is currently expanding land reclamation to increase agricultural area, which is not proportional to population increase. These new soils often suffer from salinity in soil and irrigation water. In Asia, South America, and Africa, lemongrass (*Cymbopogon citratus* (D.C.) Stapf) is cultivated mainly for its essential oil. It is widely used in pharmaceuticals, fragrance manufacturing, perfumery, flavors, cosmetics, and detergent production. In addition to its antifungal and antibacterial properties, its oil also serves as a mosquito repellent and analgesic (Boukhatem *et al.*, 2014).

Salt sensitivity is directly linked with the lemongrass plant because it cannot grow well in salt-rich soil (Idrees *et al.*, 2010, Mukarram *et al.*, 2022 and Rehman *et al.* 2022) pointed out that water salt stress reduced lemongrass growth, and chlorophyll and carotenoids contents. However, many practical attempts exist to mitigate the harmful effects on plants from salinity, whether in soil or irrigation water. In this respect, cytokinins have important roles in alleviating abiotic and biotic stresses (Vanková, 2011). Moreover, *Rosa hybrida* plants subjected to the treatment with 6-benzyleaminopurine (BAP) showed significant upregulation in the antioxidant enzymes and downregulation in malondialdehyde activity and relative conductivity (Wang *et al.*, 2022). *Moringa oleifera* is a famous tree known for the antioxidant properties of its leaf extracts (MLE) (Ahmed, 2016). It also comprises various plant growth regulators, such as cytokinins (Yang *et al.*, 2006). It has been shown to promote different enzymes and antioxidant properties and protect the plant cells from the aging effects of the reactive oxygen species (Yasmeen *et al.*, 2013). Ibrahim and Idris (2020) investigated the influence of moringa extract on lemongrass and reported that the application of moringa extract at 8 g/l as a foliar spray enhanced growth attributes and oil content.

Grover *et al.* (2011) postulated that some microorganisms have the ability to induce plants to resist various stresses resulting from climate change. Microorganisms can alleviate the adverse effects of soil salinity on plants by enhancing biochemical and physiological processes within plant cells (Abo Nouh *et al.*, 2021 and Ali *et al.*, 2022). Mirzaei *et al.* (2020) studied the potential tolerance of lemongrass to drought stress after subjecting it to plant growth-promoting rhizobacteria (PGPR) application. It was proved that PGPR application enhances lemongrass growth, oil yield and productivity by improving antioxidant capacity. In addition, Abdel-Rahman *et al.* (2011) revealed that inoculation with *Bacillus subtilis* and/or mycorrhizal fungi showed positive effects on growth, oil %, oil yield and nutrient uptake under water salinity conditions. Therefore, the aim of the current study was to assess the efficiency of BAP, *M. oleifera* leaf extract (MLE) and *B. subtilis* + arbuscular mycorrhizal fungi (B+M) on the growth, percentage and yield of oil as well as photosynthetic pigments content of lemongrass plants under water salinity conditions.

Materials and Methods

A pot experiment was conducted twice at the Experimental Farm of Floriculture, Faculty of Agriculture, Assiut University, Assiut, Egypt, during the

two seasons of 2019 and 2020. This work aimed to study the effect of some natural and synthetic growth stimulants on the morphological traits, percentage and yield of volatile oil as well as photosynthetic pigments content of lemongrass (*Cymbopogon citratus*) plants under water salinity conditions. *Moringa oleifera* leaf extract (MLE) and benzylaminopurine (BAP) were added as foliar spray, while *B. subtilis* + arbuscular mycorrhizal fungi (AMF) were added as soil drench. Healthy uniform lemongrass plants were obtained from Department of Medicinal and Aromatic Plants Researches, Horticultural Research Institute, Agricultural Research Center, Egypt. Plants were transplanted in plastic pots (40 cm in diameter and 40 cm in height). Each pot was filled with 20 Kg of sandy loam soil (sand + clay 1:1), physical and chemical properties of the soil used were done according to the method of Jackson (1973) as shown in Table (1).

Table 1. Some physical and chemical analysis of soil used at the beginning of the experiment.

Texture grade	Soluble ions (meq/100 g soil)							pH†	EC ds m ⁻¹	OM%
	Cations				Anions					
	Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁼			
Sandy loam	2.24	2.14	2.21	0.05	0.54	3.80	2.30	7.80	2.16	1.22

†Soil-water suspension (1:2.5).

At the beginning of May, in both seasons, two seedlings were planted in each pot. Two weeks after transplanting, plants were treated with BAP and *M. oleifera* leaf extract as a foliar application or inoculated with *B. subtilis* plus mycorrhizal fungi as soil drench three times (two weeks after transplanting, three weeks later and two weeks after the 1st cut). The active strain of *B. subtilis* (10⁸ CFU/ml) and arbuscular mycorrhizal fungi (*Glomus irradicans*) was provided by the Unit of Biofertilizers, Faculty of Agriculture, Ain Shams University, Shobra El-Kheima, Egypt. The soil was inoculated with the combined treatment of both microorganisms (*B. subtilis* + 25 spores/ pot of *G. irradicans* at 20 ml/pot (Demir and Onogur, 1999). Meanwhile, uninoculated plants served as a control.

BAP was purchased from El-Gomhorya Company, Egypt. It was applied at a rate of 100 mg l⁻¹, while *M. oleifera* leaf extract was used at 5% for foliar applications. Also, untreated plants were involved as a control. Plants were irrigated regularly with tap water for three weeks after transplanting, and then plants were subjected to different salinity levels (control, 1000, 2000 and 4000 mg l⁻¹ NaCl) and foliar application of control “tap water”.

Preparing *M. oleifera* leaf extract (MLE)

Young fresh leaves of *Moringa oleifera* were collected from the Farm of Floriculture, Faculty of Agriculture, Assiut University from matured trees. Aqueous extract of moringa was prepared by mixing 100 g of fresh leaves with 1 liter of distilled water. The obtained suspension was homogenized using a household blender for 15 min. Then, the solution was filtered by wringing using a mutton cloth. Finally, the solution was re-filtered using Whatman filter paper No. 2 (Fuglie, 2000). The extract was diluted with distilled water at 1:1 v:v (5%) and

then sprayed directly onto plants. The extract was used within five hours from cutting and extracting.

Experiment Design

The experiment included 16 combined treatments of four salinity levels (0 “tap water”, 1000, 2000 and 4000 mg l⁻¹ NaCl) and four synthetic and natural growth substances (control, 100 ppm BAP, 5% MLE and *B. subtilis* + AMF). The treatments were arranged as split-plot in Randomized Complete Blocks Design (RCBD) with three replicates. The four salinity levels represented the main plots, while the four synthetic and natural growth substances were assigned for the sub-plots.

1. Morphological characteristics

Data on morphological characteristics were collected for two cuts, the 1st cut after three weeks from the last application treatments and the 2nd cut after 45 days from the first cut. The following morphological characteristics were assessed for each cut: plant height (cm), leaves no per plant, tillers no per plant, and fresh and dry weights of herbs per plant (g).

2. Volatile oil yield and percentage

The essential oil percentage and yield (ml/ plant) and were determined in the fresh herb by hydrodistillation for 3 hours using a Clevenger apparatus. Calculations of oil percentage and yield were calculated according to British Pharmacopoeia (1963).

3. Photosynthetic pigments determination

Extraction was done from fresh lemongrass leaves with dimethyl formamide solution [HCON (CH₃)₂] and left overnight at 5°C. Absorbance was recorded by spectrophotometer (Shimadzu UV-12002) at the wavelengths of 663, 647 and 470 nm, respectively. Chlorophylls a and b and carotenoids were calculated according to Nornai (1982).

Statistical analysis

Statistix 8.1 analytical software was used to analyze the data obtained during the two seasons, and the means were compared using the least significant difference (LSD) test, according to Dowdy and Wearden (1983).

Results

1. Morphological features

Results presented in the current study demonstrated the depressive influence of salt stress and the importance of growth stimulants for improving plant growth characteristics, including the plant height, leaves no/ plant, tillers no/ plant, herb fresh weight and herb dry weight of lemongrass during the first and second cuts for both seasons (Table 2 and Figure 1). However, the plant growth reduction was more pronounced in lemongrass subjected to salt stress without the external

addition of growth stimulants. Morphological traits were significantly affected by exposure of lemongrass plants to salinity stress imposed by irrigation. The vigor plants of lemongrass were produced with non-salinized plants, followed by those treated with the lower concentration. Meanwhile, the lowest values of morphological parameters were produced with those treated with the higher concentration. There were significant differences in the morphological traits as a result of growth stimulants on lemongrass. Among the growth stimulants, foliar spraying with MLE increased plant height, leaves number/ plant, number of tillers/plants, herb fresh weight and herb dry weight compared to the other treatments in the 1st and 2nd cuts for both seasons. Also, microorganisms' treatment (B+M) was in the second rank, while the lower values of these traits were produced with the control, followed by BAP. Regarding the combination effect between salinity levels and growth stimulants on the growth traits, non-salinized plants treated with moringa leaf extract showed higher growth parameters than the other combinations. At the same time, the lowest ones appeared by applying 4000 mg l⁻¹ without adding any growth stimulants for both seasons.

Table 2. Mean number of tillers in lemongrass as affected by growth stimulants under salt stress conditions during the two seasons of 2019 and 2020

Salinity levels “mg l ⁻¹ NaCl” (A)	1 st season (2019)										
	1 st cut					2 nd cut					
	Growth stimulants (B)										
	Cont.	MLE	BAP	B+M	Mean	Cont.	MLE	BAP	B+M	Mean	
Control	29.00	38.23	34.50	36.00	34.43	25.00	35.67	32.00	31.00	30.92	
1000	28.67	35.97	32.90	34.33	32.97	23.00	31.67	24.67	29.67	27.25	
2000	23.83	30.70	28.30	30.07	28.23	18.67	26.00	22.00	27.00	23.42	
4000	17.43	23.87	22.07	22.97	21.58	21.67	14.67	18.33	20.33	18.75	
Mean	24.73	32.20	29.44	30.84		22.08	27.00	24.25	27.00		
L.S.D. 5%	A= 0.618		B= 0.634		AB= N.S		A= 3.308		B= 1.764		AB= N.S
	2 nd season (2020)										
Control	23.33	34.67	29.00	29.67	29.17	23.07	30.57	26.97	27.87	27.12	
1000	22.00	29.67	23.33	27.33	25.58	19.63	25.93	24.27	25.00	23.71	
2000	16.67	23.33	20.00	23.67	20.92	15.90	20.80	18.27	19.27	18.56	
4000	11.33	18.67	15.67	18.00	15.92	10.77	16.87	13.47	13.90	13.75	
Mean	18.33	26.59	22.00	24.67		17.34	23.54	20.74	21.51		
L.S.D. 5%	A= 1.688		B= 1.308		AB= N.S		A= 0.836		B= 1.082		AB= N.S

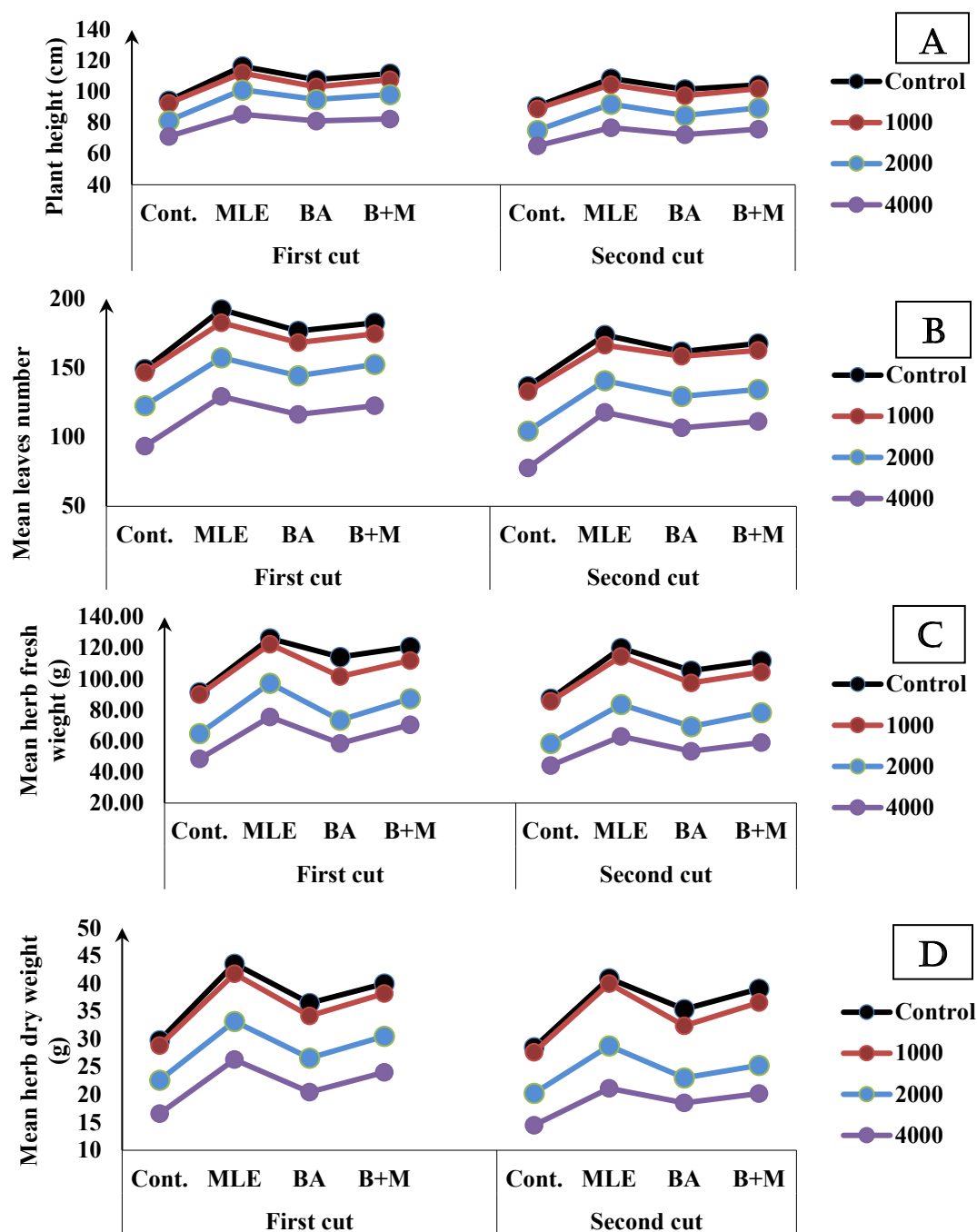


Figure 1. Effect of moringa leaves extracts (MLE), benzylaminopurine (BAP) and *Bacillus subtilis* + arbuscular mycorrhizal (B+M) under irrigation water salinity, as average seasons of 2019 and 2020 on A (plant height), B (number of leaves/ plant), C (herb fresh weight) and D (herb dry weight).

2- Oil percentage

As shown in Table 3, lemongrass plants irrigated with saline water at the medium and lower levels (2000 and 1000 mg l⁻¹ NaCl) produced higher volatile oil percent than those irrigated without salinity, or that received 4000 mg l⁻¹ NaCl in the two cuts during the two studied seasons. Regarding the effect of the used

growth substances, it is clear that all applied substances increased volatile oil percent more than control. However, the higher volatile oil percent was obtained with MLE, followed by BAP in the studied seasons. Interaction between the two studied factors was not significant for oil % in this study; the highest value in this respect was registered in the lemongrass plants irrigated with 2000 mg l⁻¹ NaCl and sprayed with MLE.

Table 3. Mean essential oil percentage in fresh leaves of lemongrass as affected by growth stimulants under salt stress conditions during the two seasons of 2019 and 2020

Salinity levels “mg l ⁻¹ NaCl” (A)	1 st season (2019)												
	1 st cut					2 nd cut							
	Growth stimulants (B)												
	Cont.	MLE	BAP	B+M	Mean	Cont.	MLE	BAP	B+M	Mean			
Control	0.280	0.363	0.347	0.327	0.330	0.310	0.390	0.370	0.360	0.360			
1000	0.303	0.377	0.363	0.337	0.350	0.333	0.417	0.387	0.373	0.378			
2000	0.340	0.410	0.410	0.360	0.380	0.357	0.460	0.430	0.430	0.420			
4000	0.273	0.373	0.337	0.327	0.330	0.300	0.367	0.347	0.350	0.340			
Mean	0.299	0.381	0.364	0.338		0.325	0.408	0.383	0.378				
L.S.D. 5%	A= 0.035			B= 0.015		AB= N.S		A= 0.034		B= 0.015		AB= N.S	
2 nd season (2020)													
Control	0.260	0.347	0.327	0.303	0.309	0.290	0.380	0.350	0.350	0.340			
1000	0.270	0.373	0.357	0.353	0.338	0.300	0.390	0.370	0.360	0.360			
2000	0.320	0.397	0.393	0.383	0.373	0.350	0.430	0.410	0.410	0.400			
4000	0.273	0.350	0.330	0.320	0.318	0.280	0.360	0.340	0.350	0.330			
Mean	0.281	0.367	0.352	0.340		0.305	0.390	0.368	0.368				
L.S.D. 5%	A= 0.024			B= 0.022		AB= N.S		A= 0.033		B= 0.024		AB= N.S	

3- Oil yield (ml/ plant)

The effect of salinity, growth substances and their combination on oil yield of lemongrass is shown in Table 4. The impact of the two factors was significant, while the effect of their interaction on oil yield was not significant. Lemongrass plants irrigated with saline water at the lower level (1000 mg l⁻¹ NaCl) or irrigated without salinity produced higher volatile oil yield than those irrigated with the higher salinity levels. Concerning the effect of the used growth substances, it is clear that all applied growth stimulants increased volatile oil yield more than the control. However, the higher volatile oil yield was attained with MLE in the two cuts during both seasons. Interaction between the two studied factors was not significant for oil yield in this study; the higher yield was recorded in the lemongrass plants irrigated with the low salinity level or without salinity and sprayed with MLE.

Table 4. Mean oil yield (ml/ plant) of lemongrass plant as affected by growth stimulants under salt stress conditions during the two seasons of 2019 and 2020

Salinity levels “ppm” (A)	1 st season (2019)									
	1 st cut					2 nd cut				
	Growth stimulants (B)									
	Cont.	MLE	BAP	B+M	Mean	Cont.	MLE	BAP	B+M	Mean
Control	0.264	0.489	0.420	0.419	0.398	0.286	0.486	0.396	0.406	0.394
1000	0.281	0.490	0.387	0.400	0.389	0.306	0.489	0.391	0.397	0.396
2000	0.249	0.453	0.337	0.362	0.350	0.245	0.416	0.346	0.375	0.345
4000	0.148	0.317	0.217	0.256	0.234	0.157	0.254	0.203	0.222	0.209
Mean	0.236	0.437	0.341	0.359		0.248	0.412	0.334	0.349	
L.S.D. 5%	A= 0.043 B= 0.020 AB= N.S			A=0.042 B=0.016 AB=0.032						
2 nd season (2020)										
Control	0.231	0.409	0.350	0.346	0.334	0.239	0.439	0.363	0.388	0.357
1000	0.235	0.429	0.344	0.374	0.346	0.239	0.436	0.347	0.370	0.348
2000	0.181	0.334	0.255	0.284	0.264	0.169	0.330	0.238	0.280	0.254
4000	0.117	0.232	0.173	0.200	0.181	0.101	0.205	0.165	0.192	0.166
Mean	0.191	0.351	0.281	0.301		0.187	0.352	0.278	0.308	
L.S.D. 5%	A=0.018 B= 0.025 AB= N.S			A=0.031 B=0.021 AB=N.S						

4. Photosynthetic pigments

The presented results in Figure 2 showed that exposure of lemongrass plants to salinity stress imposed by irrigation with different concentrations significantly affected chlorophyll ‘a’ and ‘b’ and total carotenoid content for the two cuts during the two seasons of 2019 and 2020. It is proved that non-salinized plants, followed by application 1000 ppm, registered the highest chlorophyll content values compared with 4000 and 2000 mg l⁻¹ NaCl in the two cuts during both seasons. The lowest value of chlorophyll content was obtained, with the highest salinity level in the two cuts for both seasons. Photosynthetic pigments were gradually decreased with increasing salinity levels for the two cuts in the two seasons. Statistical analysis pointed out that external supplementation of all growth stimulants caused significant increases in chlorophyll a and b and total carotenoid content compared to the control in the two cuts during both seasons. Among growth stimulant treatments, MLE was more effective in increasing chlorophyll content as compared to the other ones in the two cuts for both seasons. In this regard, the maximum reduction in chlorophylls was registered with untreated plants, followed by that sprayed with BAP for both seasons. In connection with the combination effects between salinity levels and growth stimulant treatments, there was a significant effect on chlorophylls only in the 2nd season. Generally, it turns out that a maximum increment of chlorophylls was observed in plants developed from non-salinized treatment and those sprayed with MLE compared to the other treatments in the two cuts during both seasons.

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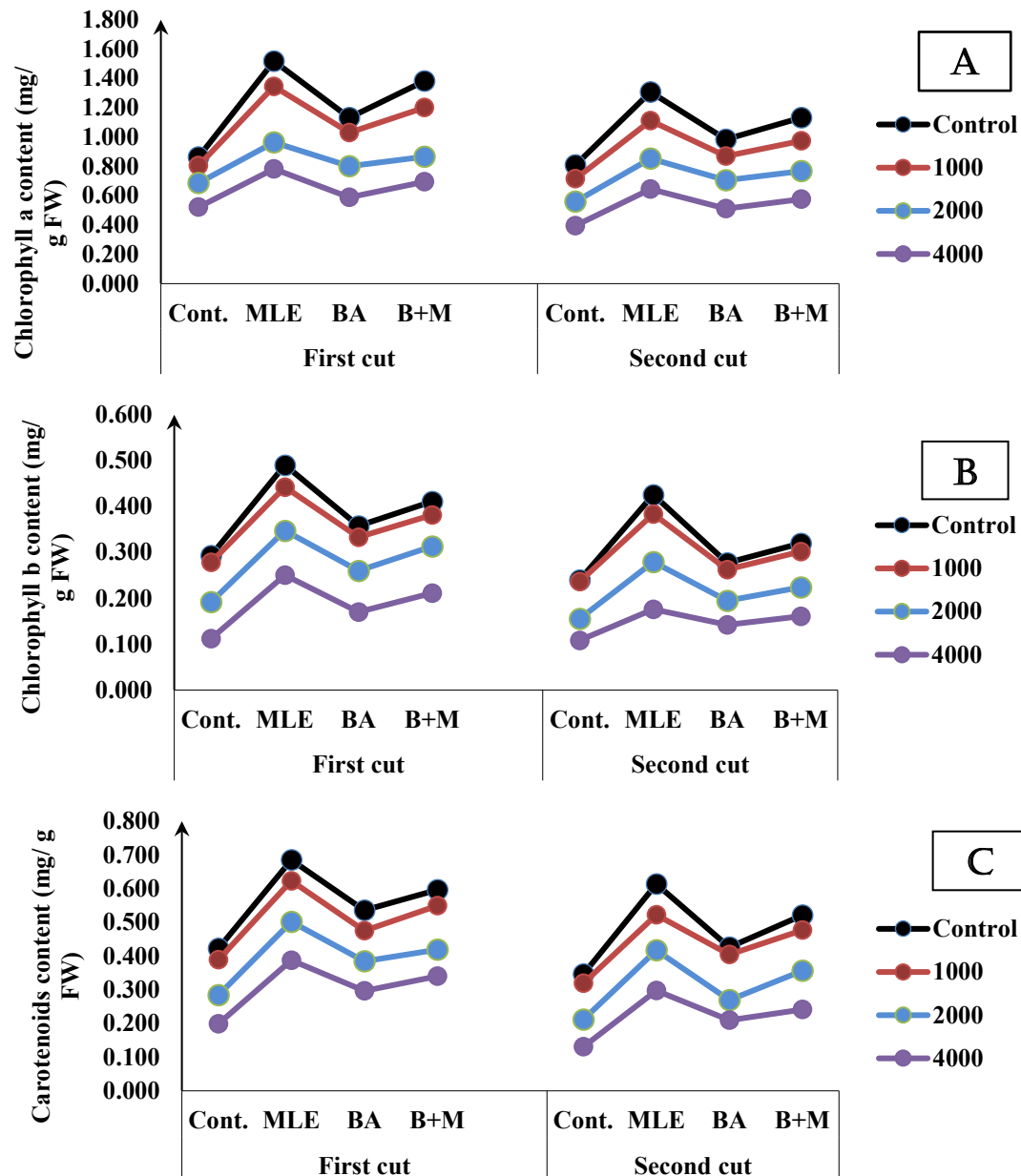


Figure 2. Effect of moringa leaves extracts (MLE), benzylaminopurine (BAP) and *Bacillus subtilis* + arbuscular mycorrhizal (B+M) under irrigation water salinity, as average seasons of 2019 and 2020 on; A (chlorophyll a), B (chlorophyll b) and C (carotenoids).

Discussion

The presented results clearly showed that morphological traits and chlorophylls of lemongrass plants were negatively affected by salt stress, particularly the higher concentration. It is also clear that the exogenous application of various growth promoters improved the growth characteristics, oil yield and chlorophylls of plants exposed to salt stress.

Our results proved that salinity levels showed significant differences in plant height, number of leaves and tillers/plant, as well as herb fresh and dry weight of lemongrass in the 1st and 2nd cuts for both seasons. These morphological traits were gradually reduced with increasing salinity concentration from 1000 to 4000 mg l⁻¹ NaCl. The lowest values of the studied traits were registered from plants exposed to the highest salinity level in the two cuts during both seasons. Recently, Mukarram *et al.* (2022) found that salt treatments ranging from 40 to 240 mM NaCl reduced the morphological traits of the treated plants. Although the damage in the morphological characteristics of lemongrass was high at 4000 mg l⁻¹ NaCl, it was less at 1000 and 2000 mg l⁻¹ NaCl. In addition, Ullah *et al.* (2020) also observed moderate resistance of the plant to salt stress reflected in its stable biomass production. Several plant species have shown similar responses to salinity stress, such as tomato (Diouf *et al.*, 2018); maize (Hessini *et al.*, 2019); barley (Zeeshan *et al.*, 2020); wheat (Saddiq *et al.*, 2021). The formation of new leaves, buds, and shoots is adversely regulated by osmotic pressure, which is created by harmful amounts of salt ions. Meanwhile, the higher accumulation rate of sodium ions in leaves cannot alleviate salt stress effects due to lack of expansion and eventually results in their death. Also, other plant species are susceptible to the higher chloride concentration; this results in a decrease in photosynthetic rate and a reduction in their growth rate (Ahmad *et al.*, 2022). Moreover, Na⁺ is a toxic ion that is quickly absorbed by root cells (Malagoli *et al.*, 2008). Thus, under salt stress, disturbance in the Na⁺ exclusion pathway at the root level caused this ion to be enhanced in the roots and leaves of lemongrass plants (Ghassemi-Golezani *et al.*, 2022).

Aiming to ameliorate the adverse effect of salinity stress on lemongrass, the plants were treated with three growth promoters: MLE, microorganisms (B+M) and BAP. Our results proved that the external application of these growth stimulants led to remarkable increases in the morphological traits compared to untreated plants. Generally, MLE was superior to the others for producing the best results of plant height, number of leaves and tillers/plant, and herb fresh and dry weights, followed by B+M and BAP in the 1st and 2nd cuts for both seasons. It is evident from the positive results obtained with MLE addition that MLE is a highly promising growth stimulant, which supports previous reports from other plants (Alkuwayti *et al.*, 2020; Ngcobo and Bertling, 2021). MLE contains diverse compositions, including essential macro- and micronutrients, antioxidants, and phytohormones (Zaki and Rady, 2015), indicating that this extract can be applied for growth promotion. Various publications emphasized the role of MLE in inducing plant growth, development and production in different plants (Rehman *et al.* 2014 and Yap *et al.*, 2021). Additionally, the higher IAA, GAs, and zeatin concentrations in MLE under salt stress conditions enhanced plant growth and production (Rehman *et al.*, 2014). The enhanced content of MLE of crude proteins and growth-promoting hormones like auxins and cytokinins may cause this growth acceleration (Moyo *et al.*, 2011). On the other hand, the application of MLE showed the best results under saline conditions for ameliorating the negative effect

of salt stress by preventing the decrease in growth traits, leaf photosynthetic pigments, and nutrient elements.

On the other hand, our results proved that the exogenous application of beneficial microorganisms (B+M) has also been demonstrated to enhance the morphological traits of lemongrass under salt stress. These results are in agreement with those obtained by Farhangi-Abriz and Ghassemi-Golezani (2019) and Mukhtar *et al.* (2019). However, the utilization of beneficial microorganisms that promote the growth of plants appear to be highly promising as a solution for ecologically sound and sustainable agriculture (Mokrani *et al.*, 2022). Abdel-Rahman *et al.* (2011) reported that dual inoculation with *Bacillus subtilis* and mycorrhizae provided higher tolerance to salinity compared with the individual treatment and untreated ones, where basil plants treated with *B. subtilis* + AMF showed greater plant height, number of branches, fresh and dry weight, oil % and yield as well as N, P, K % and lower Na % compared to the other treatments under saline conditions. These results may be due to the ability of plants in associations with AMF to uptake some nutrients efficiently (Smith *et al.*, 1992), AMF in saline conditions could have partly increased the uptake of some macro- and micronutrients such as P, N, K, Ca, Zn, Cu, this may be due to the soil pores that can be penetrated by AMF hyphae are perhaps an order of magnitude smaller than those available to roots (Smith and Read, 1997). The effect of AMF on plant Na content was clearly observed that AMF decreased the Na uptake of plants (Rabie and Almadini, 2006). Also, *Bacillus subtilis* improved photosynthesis under salt stress by increasing the net photosynthetic rate and the stomatic conductance. *B. subtilis* can produce bacterial exopolysaccharides (EPSs) that bind cations, including Na⁺ (Geddie and Sutherland, 1993), it may be envisaged that increasing the population density of EPS-producing bacteria in the root zone would decrease the content of Na⁺ available for plant uptake and thus help to alleviate salt stress in plants growing in saline environments (Ashraf *et al.*, 2004).

In comparison, foliar application by BAP improved morphological traits compared to the control. The positive effect of BAP has already been suggested by Abdel Latef *et al.* (2021) and Zrig *et al.* (2022) have shown that application of BAP increases the growth, yield, and plant biomass under salt stress. Moreover, the exogenous application of BAP regulates cellular pathways and preserves them from Na⁺ toxicity with an increment in K⁺ uptake.

Lemongrass plants exposed to irrigation with saline water at 2000 and 1000 mg l⁻¹ yielded higher oil percentages than those irrigated without salinity or that received the higher saline level in the two cuts of both seasons. Salinity treatments similarly affected oil yield/ plant, where the plants irrigated with saline water at 1000 ppm or without salinity produced higher volatile oil yield than other treatments. These results are supported by previous findings of Mukarram *et al.* (2022) on lemongrass. Due to the antioxidant and ant biological activities of the lemongrass plant, the antioxidant system is improved as a mechanism of defense at the onset of stress in the environment (Mirzaei *et al.*, 2020 and Mukarram *et al.*, 2021). The reduction in oil percentage could be attributed to the reduction of

vegetative growth and chlorophyll content. So, the decrease in oil percentage and yield/ plant may be due to the decrease in sugar synthesis as a reduction in the dry weight of plant organs via chlorophyll reduction and photosynthetic activity (El-Shafey *et al.*, 1991). Similarly, the result of Idrees *et al.* (2012) found that exposing lemongrass to higher salinity levels led to a reduction of essential oil content. In this respect, Mukarram *et al.* (2022) suggested that the decrease in enzyme activity could be related to the reduction of volatile oil content obtained during the extreme salt level.

An increasing trend of volatile oil by growth stimulants was arranged as follows: MLE> BAP>B. *subtilis* + arbuscular mycorrhiza (B+M). A potential reason for this increment of essential oil may be ascribed to the contents of crude proteins, auxins and cytokinin in MLE (Zaki and Rady, 2015). Moreover, the application of cytokinin under non-saline and low-saline conditions showed a significant improvement in essential oil in dill seeds (Ghassemi-Golezani *et al.*, 2022). Also, the exogenous application of BAP on wheat (Yasmeen *et al.*, 2013) and the perennial ryegrass plants (Ma *et al.*, 2016) also improved antioxidant enzyme activities under salinity. On the other hand, microorganisms have resulted in the growth improvement of several plant species exposed to osmotic or salt stress (Jalili *et al.*, 2017). For example, *Arabidopsis thaliana* plants were exposed to the treatment with *Trichoderma koningii* tolerated 100 mM sodium chloride (Jamilet *et al.*, 2011). *Pseudomonas simiae* improved soybean tolerance to salinity stress at 150 mM (Vaishnav *et al.*, 2016). The authors ascribed these effects to the reduction of sodium ions and the improvement of phosphorus and potassium levels in soybean plants (Vaishnav *et al.*, 2015). Moreover, *B. amyloliquefaciens* enhanced volatile oil yield in pepper mint plants exposed to salt stress. Fortunately, microorganisms are nontoxic and biodegradable, which are needed at lower levels to enhance plant growth (Fincheira *et al.*, 2021). Salt tolerance is attained by several mechanisms, including controlling the transportation of sodium and potassium ions, the accumulation of various osmoprotectants, the decrement of toxicity through the production of antioxidants, and maintenance of hormone homeostasis that permit appropriate growth and development under opposing conditions (Wani *et al.*, 2020).

The results showed that exposure of lemongrass plants to salinity stress imposed by irrigation with different concentrations affected photosynthetic pigment concentrations (chlorophyll a & b and carotenoids) for the two cuts during the two seasons. It is proved that non-salinized plants, followed by the lowest salinity level, registered the highest values of these pigments compared to 4000 and 2000 ppm NaCl in the two cuts during both seasons. These results are in agreement with those reported by Bekhradi *et al.* (2015), who attributed the reduction in photosynthetic pigments content to the increase of salinity levels, which inhibited the synthesis of pigments. Jaleel *et al.* (2007) suggested that the decrease in leaf chlorophylls under high salinity has been attributed to the destruction of pigments and the instability of the pigment-protein complex.

Concerning the effect of external growth stimulants on photosynthetic pigments, it is clearly proved that these growth promoters resulted in significant improvements in pigments as compared to untreated plants. In addition, an increasing trend of photosynthetic pigments by growth stimulants was noticed as follows: MLE>*B. subtilis* + arbuscular mycorrhiza (B+M) > BAP. Synthesis of pigments in terms of chlorophyll a and b and carotenoids requires vital factors such as light, temperature, nitrogen, magnesium, iron, water, and trace elements (Cu, Mn and Zn). If any of these factors are incomplete, the synthesis of the chlorophylls will be affected (Setiawati *et al.*, 2021). However, the foliar spray with MLE showed the best results under salt stress and alleviating the negative effects of salinity by preventing reduction in leaf photosynthetic pigments by inhibiting increases in leaf electrolyte leakage or might result in the synthesis of substances with protective effects on plants under salinity conditions (Zaki and Rady, 2015). Maintenance of morphological traits as number of leaves per plant maximized photosynthesizing leaves through the supply of photo-assimilates from stayed green leaves (Thomas and Howarth, 2000). Also, the presence of zeatin-like cytokinin in MLE or BAP delays premature senescence of leaves and maintains higher leaf area for photosynthetic activity. Also, our results showed that exogenously applied microorganisms such as bacteria and mycorrhiza alleviated salinity stress and increased photosynthetic pigments in lemongrass. Our results agree with those of Setiawati *et al.* (2021), who stated that the highest photosynthetic pigment contents were discovered in the application of the isolates of endophytic bacteria in rice plants. Moreover, Srividhya *et al.* (2020) found that utilizing endophytic bacteria, which infiltrate plant tissue without harming the host plant and may even benefit it, was effective.

Conclusions

It is clear from this experiment that treating lemongrass plants with growth stimulants; including MLE, microorganisms (B+M) and BAP could improve the morphological traits and photosynthetic pigments under salt stress conditions. Foliar application of MLE at 5%, followed by the inoculation with B+M was the most effective treatment for inducing growth and yield of lemongrass under salinity conditions, especially with lower salinity levels (1000 and 2000 mg l⁻¹ NaCl). Therefore, it can be recommended when planting lemongrass, especially under salinity conditions, to apply MLE at 5% or inoculation with B+M to obtain higher vegetative growth and productivity.

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مواد النمو الطبيعية والكيميائية كمحفزات لتنشيط النمو ومحصول الزيت لنباتات حشيشة الليمون تحت ظروف الإجهاد الملحي

فوزي محمود سلامة¹، سيد شحاته عابدين²، مروة محمد رجائي³، نها مدحت مصطفى⁴، عاطف عبده سيد⁴

- ¹ قسم النبات، كلية العلوم، جامعة أسيوط، أسيوط، مصر.
- ² قسم الزينة، كلية الزراعة، جامعة أسيوط، أسيوط، مصر.
- ³ قسم النبات والميكروبيولوجي، كلية العلوم، جامعة الوادي الجديد، الوادي الجديد، مصر.
- ⁴ قسم بحوث النباتات الطبية والعطرية، معهد بحوث البساتين، مركز البحوث الزراعية، الجيزة، مصر.

الملخص

أجريت هذه التجربة بمزرعة قسم الزينة بكلية الزراعة جامعة أسيوط خلال موسمي 2019 و 2020 م وذلك لدراسة تأثير الرش بمستخلص أوراق المورينجا أوليفيرا بمعدل 5 % وإضافة بكتيريا *Bacillus subtilis* مع فطر الميكورهيذا وكذلك الرش بالبنزول أمينو بيورين (100 ملجم/لتر)، بالإضافة الي معاملة الكنترول (غير المعاملة) وذلك علي قياسات النمو الخضري، نسبة ومحصول الزيت الطيار، محتوى صبغات التمثيل الضوئي لنباتات حشيشة الليمون النامية في أصص تحت أربعة مستويات مختلفة لملوحة ماء الري هي الكنترول (ماء الصنبور)، 1000، 2000، 4000 ملجم/لتر من كلوريد الصوديوم . أظهرت النتائج المتحصل عليها أن ملوحة ماء الري أثرت معنوياً على الصفات المورفولوجية، نسبة ومحصول الزيت الطيار وكذلك محتوى الأوراق من صبغات التمثيل الضوئي (كلوروفيل أ، ب والكاروتينيدات) لنباتات حشيشة الليمون. تم الحصول على أفضل النتائج من القياسات المورفولوجية، نسبة ومحصول الزيت، محتوى الأوراق من صبغات التمثيل الضوئي كنتيجة لري النباتات بماء الصنبور، يليها الري بالمستوي المنخفض من الملوحة (1000 ملجم/لتر). أقل القيم من تلك القياسات سجلت مع النباتات التي تم ريها بالمستويات العالية من الملوحة (2000 و 4000 ملجم/لتر). أدت إضافة جميع محفزات النمو المستعملة إلى تحسن واضح في جميع الصفات المدروسة، خاصة مستخلص أوراق المورينجا بمعدل 5 % والذي سجل أعلى القيم لقياسات النمو الخضري، نسبة ومحصول الزيت/نبات ومحتوي أوراق النباتات من صبغات التمثيل الضوئي، يليه التلقيح ببكتيريا *Bacillus subtilis* بالاشتراك مع فطر الميكورهيذا. لذا توصي الدراسة برش نباتات حشيشة الليمون بمستخلص أوراق المورينجا أوليفيرا بمعدل 5 % أو التلقيح بخليط من بكتيريا *Bacillus subtilis* وفطر الميكورهيذا كمحفزات نمو طبيعية وذلك للحصول على أفضل النتائج تحت ظروف ملوحة ماء الري.