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Inducing Salinity Tolerance in Mango (*Mangifera indica* L.) Cv. “El-Gahrawey” by Sodium Silicate Pentahydrate and Glycine Betaine

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ABSTRACT

The investigation was done to study the effect of Sodium Silicate Pentahydrate (Si) and Glycine betaine (GB) on enhancing salt tolerance of Mango seedlings “El-Gahrawey” cultivar grown under salinity stress. The results indicated that the application of sodium chloride (NaCl) induced a significant reduction in growth parameters and plant development, increasing salinity levels decreased plant leaf number, stem diameter, and stem length as well as fresh and dry weight of leaves. Also, a reduction of chlorophyll was observed in salt-affected plants. Besides, salt stress lead to an accumulation of sodium Na^+ and a decrease in N, P, K^+ , Ca^{2+} , and Mg^{2+} concentrations in mango leaves. However, Combinations of Si and GB sprays was the most effective treatment in alleviating the negative impacts of salt stress in mango, this treatment increased significantly most of the tested parameters under salt stress.

Keywords: Glycine betaine (GB), Silicon (Si), salt stress, *Mangifera indica*

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most popular and commercially remarkable fruits in Egypt. The harvest area is around 77,895 hectares, it produced 1,703,394 tons according to (FAO, 2017). Abiotic stress such as Salinity is one of the most serious environmental factors limiting plant growth and productivity. Actually, over than 20% of the world’s agricultural irrigated land is influenced by high concentration of salts and this problem continues to become worse worldwide due to industrial pollution, poor irrigation practices, and improper application of fertilizers (Zhu and Gong, 2014). Mango is considered sensitive to salinity conditions (Maas, 1986), causing scorched of margins and leaf tips, leaf curling, and in hard cases, the growth is reduced, leaves are dropped, and the trees die (Jindal *et al.*, 1976). However, responses to salinity stress differed among mango cultivars. “El-Gahrawey” is a new cultivar its beginning was in Damietta region and it seems that it’s tolerant to salinity conditions.

Salt stress reduces the physiological activities of the cells, which involved in the photosynthesis process (Garg and Bhandari, 2016), mostly as a result of nutritional imbalance, nutritional toxicity, osmotic stress, and oxidative stress. High salinity not only reduces photosynthesis, plant development, water use efficiency, and yield but also, it led to ion toxicity and physiological drought in the plant, consequently decreasing the productivity (Shahid *et al.*, 2018). Reduction in water potential because of the high concentrations of Na^+ and Cl^- inhibits the plant development and growth (Torabi *et al.*, 2015). Therefore, the two major dangers induced by salinity are caused by ionic toxicity and osmotic stress

associated with the excessive absorption of Na^+ and Cl^- , resulting in the deficiency of K^+ and Ca^{2+} and the nutrient imbalance (Marschner, 2012), nutrient imbalances arising from the presence of salts in the soil solution resulting in obstruction of the absorption of essential nutrients (Chavarria and dos Santos, 2012).

Salinity reduces growth and productivity of fruit crops, and to overcome the negative impacts of high salinity in the soil, the use of Silicon may alleviate salinity stress in plants (Matichenkov and Bocharnikova, 2001). The external application of silicon (Si) has become a recent eco-friendly approach to promote the response of plants to salinity stress (Almeida *et al.*, 2017). Silicon is beneficial in enhancing photosynthesis, transport of water, and nutrient uptake, it also increases the tolerance of mango trees against abiotic stress (Santos *et al.*, 2014). In addition, Oliveira and Castro (2002) reported that when Silicon accumulates in the leaves it causes a decrease in the transpiration rate due to the formation of a double layer of silica, which diminishes the stomata opening, thus the loss of water is limited. The influence of Silicon when combined into the cell wall is comparable to that of lignin, which is a structural component resistant to stress. Consequently, the leaf architecture of the plant is much better, so the penetration of the sunlight is greater and the uptake of CO_2 increase, also, the rate of photosynthesis increased, and the rate of transpiration decreased (Korndorfer *et al.*, 2002; Korndorfer and Datnoff, 2000).

Problems caused by the salt are also minimized to some degree by the use of some compounds. The production of varied compatible osmolytes like glycine betaine, soluble sugars, proline, and amines increased by

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plants, these compounds help plants to resist abiotic stress through creating membrane integrity, osmotic balance, ROS detoxification, enzyme and protein stability (Blum, 2017). It is thought that glycine betaine (GB) protects the plant by balancing the water potential between the plant cell and the environment, and by stabilizing macromolecules. It localized mainly in chloroplasts and plays a pivotal role in chloroplast modification and protection of the thylakoid membrane, that way it maintains photosynthetic activity and the integrity of the plasma membrane (Tuteja *et al.*, 2012). Glycine betaine provides stress tolerance to the cells without interfering with cellular machinery (Chen and Murata, 2002). Moreover, there are two basic functions of compatible solutes, including GB, in abiotic stress tolerance, osmotic adjustment, and cellular compatibility, the osmotic changes occur by concentration-dependent effects on osmotic pressure to absorb more water from the environment, in cellular compatibility process, these compounds substitute water in biochemical reactions consequently, the metabolism maintains normal during abiotic stress (Bohnert and Jensen, 1996).

This study aimed to investigate the effect of foliar application of Sodium Silicate pentahydrate (Si) and Glycine Betaine (GB) singly or together under three levels of NaCl salinity (0, 2000 and 4000 ppm) on vegetative growth, total chlorophyll and phenol content in leaves, and nutritional behavior of “El-Gahrawey” mango cultivar.

MATERIALS AND METHODS

This investigation was conducted from March to August during 2018 and 2019 seasons at Faculty of Agriculture, Mansoura University, Egypt, in the nursery of Pomology Department, Two years old seedlings of polyembryonic mango cv. “El-Gahrawey” were selected. Thirty six nucellar plants, which were healthy, uniform in size and growth were taken for the experiment and each plant was planted in a plastic pot 33 cm in diameter and 30 cm in height, with sandy clay loamy soil (69, 26 and 5% of sand, clay and loam, respectively), containing 1.92% of organic matter, 21.72 mg/kg of N, 4.25 mg/kg of P and 397.05 mg/kg of K, pH of the soil was 9.01, and the electrical conductivity (EC) of the soil saturation extract was 0.47 dS m⁻¹. Afterward, pots were kept in the greenhouse and agricultural operations like irrigation, weeding, application of fertilizer got whenever needed for the great health of plants.

Plants were irrigated with salinized waters at three levels of NaCl salt at 0 ppm (Tap water), 2000 ppm, and 4000 ppm, four irrigation treatments were applied. The anti-stress compounds were applied singly or together eight times (15 days after the first salinity treatment was applied) on the foliage of plants every two weeks. Sodium Silicate Pentahydrate (Na₂SiO₃) was applied at the concentration of 75 ppm and Glycine Betaine (C₅H₁₁NO₂) was applied at the concentration of 250 ppm. Sampling for all parameters was taken at the end of the experiment (two weeks after the last anti-stress application).

1. Vegetative growth

Number of leaves: counted per each plant.

Stem diameter: The average diameter was estimated 15 cm from the soil surface by using a digital Vernier caliper.

Stem length: The plant height was measured from the soil surface to the top growing point.

Fresh and dry weight of leaves: Total leaves of each plant were taken to determine fresh weight as well as dry weight after oven dried at 70 °C till a constant weight.

2. Leaf chlorophyll content

The chlorophyll content was measured from the leaves middle part by using CCM-200 plus Chlorophyll Content Meter that uses transmittance to measure the chlorophyll content in leaves. The device tests the transmittance of two wavelengths and calculates a chlorophyll content index (CCI) value, which is symmetrical to the amount of chlorophyll sum in the sample (CCI = % Transmittance at 931 nm / % Transmittance at 653 nm)

3. Determination of phenolic compounds

The dry leaf samples were crushed and extracted with Ethanol (80%) according to the method described by Selim (1978) to determine total soluble phenols. Folin reagent and colorimetric method were used. Total phenols were determined spectrophotometrically at wave length 730 nm and a standard curve of pyrogallol as described by AOAC (1970) and adjusted by Daniel and George (1972).

4. Leaf mineral content

For determination of N, P, K, and Na concentrations, mature leaf samples were oven-dried at 70 °C to a constant weight and were finely ground, 0.2 g of dried powder from each sample was digested wetly according to the method stated (Jackson, 1973).

Estimation of total nitrogen was done by using the Micro Kjeldahl device according to Jones *et al.* (1991). Total phosphorus was determined spectrophotometrically according to Jackson (1973). Determination of total potassium and Sodium was done by using a Gallen Kamp flame photometer according to (Jackson, 1973). The concentration of the elements was calculated as percentages on a dry weight basis.

For estimation of Ca²⁺ and Mg²⁺, the method of microwave digestion was used. The concentration of the elements Ca²⁺ and Mg²⁺ was analyzed by electrothermal atomic absorption spectrometry, Perkin Elmer Model 5100 according to Kumpulainen *et al.*, (1983).

Statistical analysis:

The experimental design was arranged in a split plot design, since the salt stress concentrations were in the main plot and the anti-stress compounds were in the sub plot. Besides that comparison between means was achieved by using the least significant differences (LSD) at probability of 5% according to (Waller and Duncan, 1969), by using GenStat package 11 editions.

RESULTS AND DISCUSSION

Vegetative growth

Data in Table 1 show that Plants subjected to NaCl salinity at 2000 and 4000 ppm comparing with 0 ppm NaCl had a significant reduction in plant leaf number, stem diameter, and stem length in both seasons of study. The concerning results from the same Table indicate that all foliar treatments with anti-stress compounds alone or together significantly increased leaf number, stem diameter, and stem length when compared with control (foliar treatment with water) in 2018 and 2019 seasons.

Also, it was clear that the combination treatment Si + GB recorded the highest values of leaves number (47.33 & 50.44), stem diameter (15.93 & 16.08 mm), and Stem length (86.67 & 86.56 cm) in both seasons, respectively.

Data from the interaction effects of anti-stress compounds and NaCl treatments clearly indicate the role of anti-stress compounds in improving the number of leaves, stem diameter, and stem length of salinized plants (2000 and 4000 ppm NaCl) over non treated ones. Moreover, plants under 2000 ppm NaCl salinity treated with Si + GB significantly had a higher number of leaves (46.67 & 49.33), stem diameter (13.88 & 15.62 mm), and stem length (87.67 & 88.33 cm) during 2018 and 2019 seasons, respectively compared with untreated plants. In the same

line, the combination treatment Si + GB under 4000 ppm NaCl salinity gave the highest significant values of leaves number (40.33 & 41.67), stem diameter (15.62 & 15.94 mm) and stem length (74.67 & 75.00 cm) comparing with its control which recorded the lowest values of leaves number (19.33 & 22.33), stem diameter (10.70 & 10.83 mm) and stem length (55.00 & 56.67 cm) in both seasons, respectively under all salinity levels. In addition, the combination treatment of Si and GB under 0 ppm NaCl salinity significantly increased stem length (97.67 & 96.33 cm) in 2018 and 2019 seasons, respectively compared with all other foliar treatments including plants subjected to 2000 and 4000 ppm NaCl salinity.

Table 1. Impact of salinity levels, anti-stress compounds and their interactions on number of leaves, stem diameter (mm) and stem length (cm) of "El Gahrawey" cultivar during 2018 and 2019 seasons.

Treatments		Number of leaves		Stem diameter (mm)		Stem length(cm)	
Salinity levels		2018	2019	2018	2019	2018	2019
0 ppm		53.83	56.75	15.07	15.13	83.33	86.92
2000 ppm		39.50	41.67	13.03	14.20	78.83	79.58
4000 ppm		31.33	33.42	12.28	13.12	65.58	67.33
LSD at 5%		2.65	2.94	0.58	0.63	4.42	2.37
Anti-stress compounds							
Without (water only)		32.67	35.33	11.49	11.78	60.78	63.11
Sodium Silicate pentahydrate (Si)		41.67	44.22	12.79	14.28	75.78	81.33
Glycine betaine (GB)		44.56	45.78	14.18	14.46	80.44	80.78
Si + GB		47.33	50.44	15.38	16.08	86.67	86.56
LSD at 5%		3.00	3.80	0.47	0.48	4.79	2.85
Salinity levels	Anti-stress						
0 ppm	Without	52.67	53.67	12.94	13.14	67.33	70.33
	Si	50.67	55.33	13.35	15.02	83.00	91.33
	GB	57.00	57.67	17.37	15.68	85.33	89.67
	Si + GB	55.00	60.33	16.63	16.68	97.67	96.33
2000 ppm	Without	26.00	30.00	10.84	11.37	60.00	62.33
	Si	41.00	42.33	13.82	14.72	78.33	84.00
	GB	44.33	45.00	13.57	15.09	89.33	83.67
	Si + GB	46.67	49.33	13.88	15.62	87.67	88.33
4000 ppm	Without	19.33	22.33	10.70	10.83	55.00	56.67
	Si	33.33	35.00	11.20	13.10	66.00	68.67
	GB	32.33	34.67	11.59	12.62	66.67	69.00
	Si + GB	40.33	41.67	15.62	15.94	74.67	75.00
LSD at 5%		4.84	6.02	0.82	0.85	7.77	4.56

Regarding the effect of salinity levels on leaves fresh (FW) and dry weight (DW) data in Table 2 show that FW and DW were significantly reduced gradually with increasing the salinity level. Concerning the effect of foliar spray with anti-stress compounds on leaves fresh weight, the application with Si plus GB significantly recorded the highest fresh weight values (42.04 & 44.90 g) and dry weight (13.48 & 16.52 g) comparing to other treatments in both seasons, respectively. In case of the effect of interaction between salinity levels and anti-stress compounds, data in the same Table clearly show that leaf FW and DW decrease was less severe in plants treated with Si and GB alone or together at all salinity levels in comparison to untreated plants.

The decline in vegetative growth in this study correlated with increasing levels of salinity agrees with results of Duran Zuazo *et al.* (2003), on Osteen cultivar, grafted onto Gomera-3 and Gomera-1 rootstocks, Pandey (2013) on two mango genotypes *viz.*, Kurukkan and Olour, Roy *et al.* (2014) on four mango rootstock lines collected from Kuakata, Khulna, Dumki, and Rangpur, and (Rashedy and Abd Allatif, 2017) on Ewais, Hindi

Besinnara, Misk, Zebda, Sukkary, and Golek cvs. They reported that the saline conditions reduced the number of leaves, stem diameter, plant height, and leaves fresh and dry weight of plants compared to the control treatment. In addition, Munns and Tester (2008) mentioned that the high concentration of salt in the irrigation water results in a reduction in the plant growth. Munns (1992) concluded that growth is first decreased by a reduction in the soil water potential (osmotic phase), and subsequently, a specific damage effect in leaves occur, it dies due to the rapid increase of salts in the cytoplasm or cell wall, so the vacuoles can no longer insulate incoming salts (ionic phase). Also, he mentioned that this accumulation of salts in the old leaves hastens their death, consequently, the growth hormones and carbohydrates supply to the meristematic parts decreases, resulting in growth inhibition.

The anatomical disorders caused by salts such as NaCl, CaCl₂, and KCl were studied by Romero-Aranda *et al.* (1998) in citrus varieties, which are tolerant (Cleopatra mandarin) and sensitive (Carrizo citrange). They observed changes in leaf anatomical characteristics, such as the lower area/volume ratio of mesophyll cells, and the

increase in leaf thickness. Also, salinity increased the succulence of leaves and decreased tissue density, the intercellular air spaces, and the cells surface/volume ratio. They suggested that under salinity stress, the increase in leaf thickness, in integration with different metabolic components like an accumulation of Cl⁻, a decrease of Magnesium, chlorophyll loss, and stomatal closure, may participate, among other factors, to the photosynthesis reduction. The main reduction in the FW and DW of leaves, roots, and stems was observed as a result of salt stress (AliDinar *et al.*, 1999). In the same line, (Tudela and Primo-Millo, 1992; Gómez-Cadenas *et al.*, 1998) observed a comprehensive reduction in fresh and dry weight in all plant tissues submitted to salinity stress, however, it is particularly remarkable in the aerial part, and this reduction was associated with a decrease in leaves number and leaves abscission, to explain this, experiments on salt-stressed citrus plants showed that a specific chloride build-up in the leaf stimulates 1-aminocyclopropane -1-carboxylic acid (ACC) synthesis and its conversion to ethylene with high efficacy, thus, releasing enough hormone to make the abscission of leaves.

Plant growth depends largely on photosynthesis. Salinity stress results in a reduction of the photosynthetic rate in plants due to ion toxicity, the adjustment of the structure and function of organelles, which are responsible for photosynthesis and oxidative stress to thylakoid membranes and other components of the cell. Besides, osmotic stress stimulates a reduction in the rate of CO₂ assimilation, which promotes stomatal closure and the availability of CO₂, and thus the transfer of assimilation products inhibited (Chaves *et al.*, 2008; Yin *et al.*, 2013; Yamori, 2016). Consequently, the addition of Silicon under salinity stress improves plant photosynthesis by decreasing ROS aggregation and ion toxicity to maintain the structure and performance of organelles that are mainly responsible for the photosynthesis process (Liang, 1999 and Liang *et*

al., 2003). Also, Helaly *et al.* (2017) suggested that the Silicon application under water stress conditions can increase mango plants ability to survive.

Chen and Murata (2008) mentioned that, glycine betaine could be concerned in protection of photosynthesis, inhibiting ROS accumulation, activation of some genes related to stress, and membrane preservation, it also involved in protection of quaternary structure of proteins that way, it maintains the enzyme activity from damaging effects due to the high concentration of sodium chloride (Sakamoto and Murata, 2002). Also, GB protected the photosynthesis machinery by stabilizing the activity of repair proteins under salinity stress (Murata *et al.*, 2007).

Total chlorophyll content

Results illustrated in Table 2 show that leaf chlorophyll content decreased with increasing levels of salinity. NaCl salinity at 4000 ppm significantly reduced the concentration of chlorophyll in leaves in both seasons of study comparing with its value under both 0 and 2000 ppm NaCl salinity. Considering the impact of anti-stress compounds, data reveal that the concentration of chlorophyll in leaves was highly significant in plants foliar treated with both Si and GB together compared with the other treatments in both seasons. Furthermore, the interaction between salinity levels and anti-stress compounds showed that salt stress (4000 ppm) with or without anti-stress compounds treatment significantly reduced leaves chlorophyll contents compared to the non-salt-stressed control (0 ppm). The application of Si + GB improved chlorophyll content under all salinity levels. In 2000 ppm treatment with Si + GB, the highest chlorophyll content was observed as it recorded 38.20 & 37.35 % in 2018 and 2019 seasons, respectively, and the lowest ones were recorded under higher salinity (4000 ppm) treatment without anti-stress compounds, it recorded 11.57 & 12.69 % in 2018 and 2019 seasons respectively.

Table 2. Impact of salinity levels, anti-stress compounds and their interactions on Leaves Fresh and dry weights (g), leaf chlorophyll content (%) and total soluble phenols (mg/g DW) of "El Gahrawey" cultivar during 2018 and 2019 seasons.

Treatments	Leaf fresh weight (g)		Leaf dry weight (g)		Leaf chlorophyll content (%)		Total Phenols (mg/g DW)		
	2018	2019	2018	2019	2018	2019	2018	2019	
Salinity levels									
0 ppm	52.27	54.72	15.66	17.33	35.00	35.58	2.68	2.74	
2000 ppm	31.03	32.50	9.53	12.50	26.47	29.95	1.75	1.93	
4000 ppm	22.58	24.01	7.65	8.59	19.50	20.91	1.99	1.95	
LSD at 5%	2.29	1.60	1.29	0.39	0.61	0.31	0.16	0.08	
Anti-stress compounds									
Without (water only)	21.50	23.03	6.61	8.29	22.88	24.25	1.84	1.77	
Sodium Silicate pentahydrate (Si)	39.07	41.16	12.04	13.40	24.63	27.51	2.74	2.75	
Glycine betaine (GB)	38.56	39.21	11.64	13.02	27.29	29.88	1.91	1.94	
Si + GB	42.04	44.90	13.48	16.52	33.14	33.60	2.08	2.37	
LSD at 5%	2.09	1.00	1.00	0.34	0.61	0.23	0.11	0.08	
Salinity levels	Anti-stress								
0 ppm	Without	38.86	39.54	11.77	12.52	32.20	34.43	2.69	2.49
	Si	58.36	60.67	17.39	19.85	36.47	35.41	3.08	3.16
	GB	49.91	51.04	14.66	16.12	35.30	35.75	2.34	2.35
	Si + GB	61.96	67.62	18.80	20.82	36.03	36.71	2.63	2.97
2000 ppm	Without	13.98	15.73	3.91	7.53	24.87	25.64	1.32	1.46
	Si	38.10	39.84	11.41	11.67	19.07	26.46	2.54	2.53
	GB	36.38	36.78	11.28	12.77	23.73	30.35	1.65	1.84
	Si + GB	35.66	37.65	11.51	18.03	38.20	37.35	1.51	1.89
4000 ppm	Without	11.66	13.83	4.16	4.82	11.57	12.69	1.52	1.35
	Si	20.77	22.96	7.33	8.67	18.37	20.67	2.61	2.56
	GB	29.40	29.82	8.99	10.16	22.85	23.55	1.75	1.63
	Si + GB	28.48	29.43	10.12	10.72	25.20	26.75	2.09	2.25
LSD at 5%		3.51	1.91	1.76	0.57	1.01	0.42	0.20	0.13

Reduction of chlorophyll concentration at high salinity levels could happen as a result of the changes in the thylakoid membrane structure or destruction of chloroplasts (Iyengar and Reddy, 1996). Also, the leakage of particular enzymes, which plays a great function in the biosynthesis of chlorophyll pigments (Murkute *et al.*, 2006). In addition, the great activity of chlorophyllase under stress conditions may reduce chlorophyll content (Woodward and Bennett, 2005), this is responsible for chlorophyll degradation (Sabater and Rodriguez, 1978).

The results of this study are corresponding to those of Pandey (2013), who mentioned that total chlorophyll decreased with increasing level of salinity. Abd Allatif *et al.* (2015) reported that the salinity treatment at 2000 ppm slightly reduced the chlorophyll content of all the studied mango cultivars. Golek had the lowest chlorophyll content, while Misk had the highest chlorophyll content compared with the other cultivars. In addition, the results suggest the role of Si and GB in ameliorating the inhibitory effects of NaCl on chlorophyll content in leaves. Guevel *et al.* (2007) reported that Silicon treatment increased chlorophylls and content of nutrients in various plants, that might cause an increase in photosynthetic rate and they mentioned that the enhancement effect of potassium silicate on photosynthetic pigments may be due to the suitable effect of potassium silicate to increase the biosynthesis of chlorophyll through its effect on improving absorption of ions such as N and Fe, which are included in the formation of the chloroplast. Furthermore, in an experiment with "Blue Bangalore" a grapevine cultivar, plants subjected to 4mL/L of SiO₂ presented a greater leaf area, higher chlorophyll content, and a longer leaf length (Bhavya *et al.*, 2011).

The application of some organic solutions, such as Glycine betaine enhanced plant tolerance to the stress conditions. (Makela *et al.*, 2000) reported that GB plays an essential role comparable to cytokinins in improving the concentration of chlorophyll. Besides, Genard *et al.* (1991) mentioned that GB has the main role in protecting the thylakoid membrane and chloroplasts from oxidative damage and improvement of the stability of some cytoplasmic and mitochondrial enzymes under conditions of salt stress. Denaxa *et al.* (2012) mentioned that, Glycine betaine application increased the total Chl and Chl *a*, *b* contents in olive trees subjected to drought stress.

Total Soluble phenols

Data presented in Table 2 show that phenol content decreased with salt treatment. Regardless of salinity, foliar application with silicon caused a significant increase in total phenols compared to other treatments during the two seasons of study. The interaction between salinity and anti-stress compounds also indicated a significant increase of total phenols when plants were treated with silicon under all salinity levels, maximum significant phenol content (3.08 & 3.16 mg/g DW in 2018 and 2019 seasons, respectively) was recorded when seedlings were foliar applied with Si in absence of NaCl, while the lowest values were observed at 2000 and 4000 ppm NaCl without anti-stress compounds (1.32 & 1.46 mg/g DW at 2000 ppm and 1.52 & 1.35 mg/g DW at 4000 ppm in 2018 and 2019 seasons, respectively)

This result is supported by the conclusions of Helaly *et al.* (2017), they reported that the silicon treatment and its combination with drought stress presented a significant increase in the content of total phenols. Some reports demonstrated the increase of phenolic compounds in different plant structures and organ systems under salt stress conditions (Valifard *et al.*, 2014; Akbari *et al.*, 2018). The moderate salinity stress may encourage the normal saline tolerance pathway by increasing the content of total phenolic compounds (Salem *et al.*, 2014). Navarro *et al.* (2006) observed in red pepper that total phenolic content increased under the conditions of moderate salinity stress. In fact, the phenolic concentrations are affected by environmental and genetics factors (Awika and Rooney, 2004). Moreover, Qin and Tian (2009) reported that Silicon is useful in saving plants from abiotic stress by stimulating a natural defense reaction and producing phenolic compounds which act as antioxidants, due to their characteristics to act as reducing agents, hydrogen donors, and quenchers of singlet O₂, thus preventing subcellular damage during stress (Rice-Evans *et al.*, 1997).

Leaf mineral content

Data in Table 3 reveal that salinity stress decreased K, P, and N content in leaves with increasing the level of NaCl concentration. However, foliar application of Si plus GB showed the highest significant content of N, P, and K (4.31 & 4.56% for N, 0.129 & 0.152% for P, and 0.64 & 0.66% for K during 2018 and 2019 seasons, respectively) as compared to the foliar application with water or Si and GB each of them alone. In case of the effect of interaction treatments between salinity levels and anti-stress compounds, salt treatment (4000 ppm) significantly reduced leaves N and P % compared to salt-free control plants. Furthermore, the effect of Si + GB was more pronounced than each of them alone in increasing K, P, and N % estimated in mango leaves under salt stress. On the other hand, at higher NaCl concentration (4000 ppm without anti-stress compounds), maximum reduction of leaf N, P, and K % was found, as it recorded the lowest values (1.70 & 1.87% for N, 0.045 & 0.066 % for P, and 0.30 & 0.31% for K, in both seasons, respectively).

It reveals from the data presented in Table 4 that both Calcium and Magnesium content in leaves decreased with increasing concentrations of sodium chloride. Maximum significant reduction was found in leaves of plants treated with 4000 ppm (3.68 & 3.72 % in leaf Ca²⁺ and 0.20 & 0.20 % in leaf Mg²⁺ during 2018 and 2019 seasons, respectively), as compared to other salinity levels. Furthermore, exogenous application of Si and GB affected the leaf Ca²⁺ and Mg²⁺ contents under saline and non-saline conditions. However, the NaCl treatments with or without anti-stress compounds decreased Ca²⁺ and Mg²⁺ % in comparison to non-salt-stressed control during the both seasons of study. Data in the same Table show that NaCl stress influenced Na⁺ accumulation in leaf significantly. Without considering salinity, leaf Na⁺ accumulation was significantly higher (0.96 & 0.98% during both seasons, respectively) in plants that were not given anti-stress compounds. Regarding the effect of the interaction, data reveal that salt treated plants showed significantly higher values of Na⁺ than non-salt treated

plants. However, it was observed that anti-stress compounds treatment under salinity stress significantly reduced the adverse effect of salinity, especially when plants were treated with both Si and GB together, the values were (0.88 & 0.89%) under 2000 ppm NaCl and (1.21& 1.25%) under 4000 ppm NaCl in 2018 and 2019 seasons, respectively. On the other hand, the highest significant value of Na⁺ (1.41 & 1.44 % in both seasons, respectively) was recorded under NaCl salinity at 4000 ppm without foliar application of anti-stress compounds.

In this investigation, accumulation of P, N, K⁺, Ca²⁺, and Mg²⁺ in the leaves of "El Gahrawey" cultivar decreased significantly due to salt stress, while the levels of Na⁺ increased. These results can be supported by de Lucena *et al.* (2012), who reported that the levels of N, P, K⁺, Ca²⁺, and Mg²⁺ in mango leaves of 'Tommy Atkins', 'Haden', 'Ubá', and 'Palmer' cultivars grafted on 'Imbu' rootstock reduced with the increase of salinity in the root system, because of the external concentrations of the Na⁺ ion, which occupied the place of absorption of K⁺ and Mg²⁺, also, the Cl⁻ ion acted at the absorption site of

nitrogen and phosphorous and inhibited their absorption due to competitive mechanisms. In addition, according to Pandey (2013) salinity stress increased Na⁺ concentrations in leaf tissues of mango (*Mangifera indica* L.) rootstocks, while inhibited the concentration of Mg²⁺, K⁺, and Ca²⁺ in the tissues. Moreover, K⁺ and Ca²⁺ concentrations of leaf tissues decreased significantly under NaCl stress in all the rootstocks. Reduced nitrogen in mango seedlings exposed to salt stress, were also reported by Rashedy and Abd Allatif (2017).

Salinity stress caused ion toxicity, which exists due to the toxic effects of salt ions such as Cl⁻ and Na⁺ inside the plant cells, this too much accumulation of intracellular salt ions results in nutritional imbalances and metabolic disturbances in the plant tissues (Ferguson and Grattan, 2005). In addition, as a result of the osmotic and ionic stresses due to salinity, toxic compounds such as ROS accumulate and imbalance of nutrients in plants happens. For instance, under high concentrations of salt, Na⁺ competes with K⁺ and Ca²⁺ in the cell membrane, resulting in reproductive disturbances (Zhu, 2016).

Table 3. Impact of salinity levels, anti-stress compounds and their interactions on Nitrogen, Phosphorous and Potassium (%) in leaves of "El Gahrawey" cultivar during 2018 and 2019 seasons.

Treatments	N (%)		P (%)		K (%)		
	2018	2019	2018	2019	2018	2019	
Salinity levels							
0 ppm	4.35	4.75	0.171	0.192	0.65	0.74	
2000 ppm	3.81	4.22	0.081	0.144	0.55	0.59	
4000 ppm	2.06	2.18	0.065	0.081	0.40	0.43	
LSD at 5%	0.15	0.22	0.005	0.004	0.03	0.03	
Anti-stress compounds							
Without (water only)	2.72	2.94	0.091	0.123	0.42	0.49	
Sodium Silicate pentahydrate (Si)	3.54	3.76	0.099	0.142	0.52	0.60	
Glycine betaine (GB)	3.04	3.60	0.104	0.141	0.56	0.60	
Si + GB	4.31	4.56	0.129	0.152	0.64	0.66	
LSD at 5%	0.19	0.20	0.003	0.003	0.04	0.02	
Salinity levels	Anti-stress						
0 ppm	Without	3.77	3.97	0.159	0.175	0.53	0.66
	Si	4.37	4.77	0.168	0.193	0.65	0.74
	GB	4.17	4.50	0.165	0.196	0.66	0.75
	Si + GB	5.10	5.77	0.193	0.206	0.77	0.81
2000 ppm	Without	2.70	3.00	0.069	0.127	0.42	0.51
	Si	4.10	4.27	0.066	0.149	0.56	0.61
	GB	3.07	4.30	0.078	0.146	0.59	0.59
	Si + GB	5.37	5.30	0.113	0.156	0.63	0.64
4000 ppm	Without	1.70	1.87	0.045	0.066	0.30	0.31
	Si	2.17	2.23	0.063	0.084	0.35	0.43
	GB	1.90	2.00	0.070	0.081	0.44	0.47
	Si + GB	2.47	2.60	0.081	0.094	0.53	0.52
LSD at 5%		0.30	0.34	0.006	0.005	0.06	0.04

The high concentration of salt ions, especially Cl⁻ and Na⁺ ions affect the absorption of the plant to other nutrients like K⁺ and Ca²⁺ and cause increased cell membrane permeability, resulting in metabolic disturbances (Xu *et al.*, 2015). Salinity stress also causes the osmotic effect, ionic imbalances, nutrient (e.g. N, P, K, Zn, Fe, and Ca) deficiency, and water use deficiency, which finally results in oxidative stress in plants (Rehman *et al.*, 2019). Besides, other studies showed that the antagonistic relation between Ca²⁺ and Na⁺ takes place, which resulted in damaged cell architecture and function of Ca²⁺, as a secondary messenger (Hansen and Munns, 1988). The reduction of the absorption of water and nutrients due to salinity stress exists as a result of changing the operating root ion channel, such as K⁺ channels, or

prevents root development via the osmotic effect of Na⁺ (Parida and Das 2005, Zhu 2007).

Silicon may relieve salt stress in plants by improving photosynthetic activity, increasing enzyme activity, enhancing K⁺/Na⁺ selectivity ratio, and increasing the concentration of soluble substances in the xylem, resulting in limited Na⁺ absorption by plants (Bradbury and Ahmad, 1990; Ahmad *et al.*, 1992; Liang, 1999). In addition, Moawad *et al.* (2015) reported that potassium silicate increased K, P, and N in 'Succary' mango leaves compared to the control plants. Glycine betaine treatment stimulated a generation of additional vacuoles within the root cells, resulting in the accumulation of Na⁺ in the roots and a reduction in its transmission to the shoots (Rahman *et al.* 2002).

Table 4. Impact of salinity levels, anti-stress compounds and their interactions on Ca, Mg and Na (%) in leaves of "El Gahrawey" cultivar during 2018 and 2019 seasons.

Treatments		Ca (%)		Mg (%)		Na (%)	
		2018	2019	2018	2019	2018	2019
Salinity levels							
0 ppm		6.64	7.24	0.27	0.28	0.27	0.26
2000 ppm		4.43	4.54	0.23	0.25	1.04	1.05
4000 ppm		3.68	3.72	0.20	0.20	1.28	1.31
LSD at 5%		0.30	0.23	0.01	0.01	0.02	0.02
Anti-stress compounds							
Without (water only)		4.31	4.47	0.22	0.23	0.96	0.98
Sodium Silicate pentahydrate (Si)		4.96	5.31	0.23	0.24	0.85	0.87
Glycine betaine (GB)		5.16	5.24	0.23	0.24	0.85	0.86
Si + GB		5.24	5.64	0.24	0.27	0.78	0.79
LSD at 5%		0.40	0.15	0.01	0.01	0.01	0.01
Salinity levels	Anti-stress						
0 ppm	Without	5.30	5.80	0.26	0.26	0.31	0.31
	Si	6.80	7.63	0.27	0.29	0.26	0.25
	GB	7.20	7.47	0.27	0.27	0.27	0.25
	Si + GB	7.27	8.07	0.28	0.32	0.25	0.23
2000 ppm	Without	4.23	4.07	0.22	0.23	1.16	1.18
	Si	4.37	4.57	0.23	0.24	1.06	1.08
	GB	4.50	4.67	0.23	0.25	1.03	1.06
	Si + GB	4.60	4.87	0.24	0.27	0.88	0.89
4000 ppm	Without	3.40	3.53	0.19	0.19	1.41	1.44
	Si	3.70	3.73	0.20	0.20	1.22	1.29
	GB	3.77	3.60	0.19	0.21	1.26	1.27
	Si + GB	3.87	4.00	0.21	0.22	1.21	1.25
LSD at 5%		0.63	0.29	0.02	0.02	0.02	0.02

CONCLUSION

From this study, it could be concluded that Salinity had a negative impact on plant growth, however, the vegetative growth parameters of salt-stressed seedlings of "El Gahrawey" mango cultivar, as well as the leaf mineral content (N, P, K, Ca²⁺ and Mg²⁺), were significantly improved by the foliar application of Silicon (75 ppm) and Glycine betaine (250 ppm) together, and they were more effective in reducing the adverse effects of salinity on plants in terms of growth and leaf mineral content. Also, Si treatment gave the best values of total phenols under all salinity levels when compared with the other treatments. We suggest that the foliar application of Silicon and osmoprotectants like Glycine betaine may alleviate salt stress as they play an important role in response and adaptation of mango plants when cultivated under salinity soil conditions.

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تعزيز تحمل شتلات المانجو صنف "الجراروي" للملوحة بواسطة سليكات الصوديوم والجليسين بيتين. دعاء مصطفى حمزه وأمير محمد شعلان قسم الفاكهة – كلية الزراعة – جامعة المنصورة – مصر

تم اجراء هذا البحث على شتلات مانجو صنف "الجراروي" عمر سنتين لدراسة تأثير الرش الورقي بسليكات الصوديوم والجليسين بيتين بشكل منفرد أو معا تحت تأثير ظروف الاجهاد الملحي حيث تم تعريض الشتلات لثلاثة مستويات من ملوحة كلوريد الصوديوم (0 و 2000 و 4000 جزء في المليون) على النمو الخضري ومحتوى الأوراق من الكلوروفيل والفينول والعناصر المعدنية، أظهرت النتائج أن الملوحة أثرت على خصائص النمو وزيادة مستوى الملوحة لوحظ انخفاض في عدد أوراق النبات، وقطر الساق، وطول الساق وكذلك الوزن الطازج والجاف للأوراق كما لوحظ انخفاض الكلوروفيل في النباتات المتأثرة بالملوحة. إلى جانب ذلك، أدى الإجهاد الملحي إلى تراكم الصوديوم وانخفاض في تركيزات كل من N و P و K و Ca و Mg في الأوراق. وتشير نتائج الدراسة إلى زيادة محتوى الأوراق من الفينولات عند معاملة النباتات بالسليكات تحت جميع مستويات الملوحة كما أدى الرش الورقي بسليكات الصوديوم والجليسين بيتين معا على تعزيز تحمل شتلات المانجو للملوحة حيث كانت هذه المعاملة هي الأكثر فعالية في التخفيف من الآثار السلبية للإجهاد الملحي على شتلات المانجو.