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Research Article

Influence of nano-silicon in antioxidants enzymes, ions absorption, and biochemical indicators of King Mandarin saplings leaves (*Citrus nobilis*) under salt stress

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Abstract
High salinity levels are particularly problematic in Iraq's southern and central regions, limiting the growth and productivity of many fruit plants. One effective approach to enhance mandarins' salt tolerance is the exogenous application of Nano-Silicon. The objective of the present research was to evaluate the performance of foliar applications by potassium silicate nanoparticles (K_2SiO_3NPs) in antioxidants' enzymes, ions absorption, bio-chemical indicators of one-year-old for King Mandarin saplings grown in salt stress conditions. The experiment was conducted under controlled saline conditions and it included two factors. The first factor was three irrigation water salinity levels (0, 40 and 80 mM) using sodium chloride ($NaCl_2$) salt. The second factor was spraying with K_2SiO_3 NPs at three levels (0, 2 and 4ml L^{-1}). A randomized complete block design (RCBD) was used to execute the factorial experiment. High level of NaCl (80 mM) had a negative effect on chlorophyll, carbohydrates, N, P, K , K/Na, Fe, Ca % and Mg in leaves and increased the accumulation of Si, proline, Cl, Na, Catalase (CAT), Peroxidase (POD) and Superoxide Dismutase (SOD) in leaves, compared to the control. K_2SiO_3NPs at 4 ml L^{-1} caused significant positive findings in ions absorption and biochemical indicators (chlorophyll, carbohydrates, N, P, K, K/Na, Fe, Ca, Mg and Si) compared to control. K_2SiO_3NPs at 4 ml L^{-1} caused a positively decreased of proline , Cl, Na, CAT, POD and SOD, compared to control. The utility of the present experiment is to enhance the tolerance of King Mandarin seedlings to saline irrigation water by applying foliar K_2SiO_3 NPs.

Keywords: Nano-Silicon, Catalase(CAT), Peroxidase(POD), Superoxide Dismutase(SOD), Salt Stress

INTRODUCTION

King Mandarin (*Citrus nobilis*), belonging to the Rutaceae family and citrus genus, is a subtropical and tropical tree. It is cultivated on a limited scale in central Iraq, primarily due to soil and water salinization issues (Abdulkadhim and Mortada, 2022). King Mandarin is a notable hybrid (Tangor), resulting from the crossbreeding of oranges and mandarins. A large size, rough, bumpy peel, and polyembryonic seeds characterize its fruit. The leaves are lanceolate with serrated edges from the top to the middle of the leaf (Al- Puglisi et a1. 2017).Salinity severely restricts the expansion of cultivable areas by adversely affecting soil properties. High salinity levels are particularly problematic in Iraq's southern and central regions, limiting the growth and productivity of many fruit plants (Abdulkadhim, 2024). This issue affects 19.8% of irrigated lands and 2.2% of drylands globally (Fedae et a1., 2021; FAO, 2021).

Sustainability of fruit plants, especially citrus, is closely linked to appropriate fertilization practices. A nutrient deficiency can significantly hinder growth and productivity (Abbas and Abdulkadhim, 2024; Abdulkadhim and Hussein, 2023; Abdulkadhim, 2019; Abdulkadhim and Hadi, 2019). Various strategies are being explored to combat salinity and enhance mandarins' salt tolerance (Hashem and Abdulkadhim, 2024; Fadhil and Abdulkadhim, 2020). One effective approach is the exoge-

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nous application of potassium silicate nanoparticles (K_2SiO_3 NPs). These nanoparticles are instrumental in improving photosynthesis efficiency, carbohydrate and protein synthesis, enhancing nutrient uptake by roots, reducing sodium ion toxicity, improving the potassium-to-sodium ratio, increasing antioxidant enzyme activity, and reducing plant water loss. Collectively, these improvements help plants withstand various abiotic and biotic stressors (Abdulkadhim *et al.*, 2024; Souri *et al.*, 2021; Zhu *et al.*, 2019).

The present experiment aimed to enhance the tolerance of King Mandarin seedlings to saline irrigation water by applying foliar K_2SiO_3 NPs and assessing their effectiveness in ion absorption, biochemical markers, and antioxidant enzymes in the leaves. Due to their high sensitivity to environmental stresses, including salinity, King Mandarin plants were the focus of this study.

MATERIALS AND METHODS

Study area

An experiment was executed during the 2023 growing season in the General Directorate of Horticulture and Forestry, Holy Karbala City, Iraq (Lat 32.6° North, long 44.1° East), utilizing one-year-old King Mandarin saplings. Uniformly grown saplings (Approximately 80 cm height) were selected and transplanted into 8 kg plastic pots filled with sandy soil inside a fabric canopy. The saplings were placed in a lathe house. Service operations such as weeding, irrigation, insecticides and fungicides were performed throughout the experiment. The experiment included 135 King Mandarin saplings grafted on Citrus aurantium rootstock. The study used a randomized complete block design (RC.BD) with two factors (3ˣ 3) and three replications. The study contained nine experimental units (five saplings per experimental unit). The first factor was irrigation with three levels of salt water (0, 40, and 80 mM $NaCl_2$) by dissolving $NaCl_2$ salt in distilled water according to the levels required in the experiment. The pots were watered with 500 ml/ sapling of salty irrigation water twice weekly. The second factor was foliar application with potassium silicate nanoparticles (K_2SiO_3 NPs), consisting of 35% potassium silicate and 12% potassium oxide dissolved in water, applied at three levels (0, 2 and 4 ml L^{-l}) 8 times (15 days between one spray and another) and the first spray was scheduled for 15/2/2023.

Indicators studied

Ions absorption and biochemical indicators

Chlorophyll (Spad Unit): It was measured using a chlorophyll measuring device type SPAD-502 as mentioned by (Ranganna¸ 1977). Carbohydrates (mg kg^{-1} dw): It was determined using the method of Joslyn (1970). Nitrogen (N) percentage: It was measured according to the modified Kjeldahl method using a micro-kjeldahl device (Novamsky *et al.*, 1974). Phosphorus (P) percentage: This was determined using the soft digestion method. The colour was developed by ascorbic acid (AsA) and ammonium molybdαte. Measurements were taken by Spectro- photometer at wavelengths of 620 nm. (Johns, I970). Potassium (K) and sodium (Na): These were determined in the digested sample by flame photometers (Hornecke and Hensson¸ 1998). Iron (Fe) (mg Kg^{-1} dw): It was determined by Absorption Spectrophotometer as reported (Allan¸ 1961). Calcium (Ca) and magnesium (Mg) percentage: These were measured by eluting with Ethylene Di-amine Tetra Acetic Acids (2Na-EDTA) Di-sodium according to the method of Richards (1954). Silicon (Si) percentage was estimated using the method described by Elliott and Synderr (1991). Chloride (Cl) percentage: It was estimated by titration of a certain volume of digested plant extract with 0.05 ml silver nitrate solution using potassium chromate reagent as described by Kalra and Maynard (1991). Potassium/sodium ratio (K/Na): Estimated by dividing K results by Na results. Proline acid (mg g^{-1} dwt): This was estimated using a Spectrophotometer at wavelength 520 nm (Bates *et al.*, 1973).

Estimating the effectiveness of antioxidant enzymes:

Catalase (CAT) enzyme activity (unit mol $^{-1}$): This was estimated according to method described by Beers and Seizer (I952) and modified by (Gogorcena *et al.*, 1995). Peroxidase (POD) enzyme activity (unit mol^{-1}): It was estimated as described (Nezih, 1985). Superoxide (SOD) Dismutase enzyme activity (unit mol^{-1}):This was estimated according to the method of Marklund and Marklund (I974) and calculated based on the equation described by Frary *et al.* (2010).

Data analysis

Statistical analysis of the data was performed using Gen.Stat. (Version 12) software. Means were compared to determine the significance status between coefficients by Least Significant Difference (LSD.) with a probability < 0.05. (Al-Rawi and Khalafalla, 2000).

RESULTS AND DISCUSSION

Ions absorption and biochemical indicators

The results of ions and biochemical parameters of King Mandarin saplings are summarized in Tables 1 and 2. A salinity level of 80 mM caused a negative decrease in chlorophyll , carbohydrates, N , P, K , K/Na ratio , Fe, Ca % and Mg % in leaves (26.79 spad unit, 4.27 mg kg $^{-1}$ DW , 1.88%, 0.30%, 2.30%, 3.83%, 95.35mg Kg^{-1} DW , 2.38% and 0.21 %). Also, the leaves' contents of Si% , proline , Cl%, and Na% (3.03% , 2.80 mg g^{-1} DW, 1.80%, 0.60%) were augmented consecutively. Salinity level 0 mM achieved the highest means in chlorophyll, carbohydrates, N, P, K, K/Na ratio and Fe , Ca and Mg in leaves (31.27 Spad Unit, 5.49 mg kg $^{-1}$ DW, 2.04% , 0.32% , 2.46% , 4.24% , 114.19 mg Kg^{-1} DW, 2.44% and 0.23 %). Also, the leaves' contents of Si , proline, Cl%, and Na% (2.63% , 2.67 mg g^{-1} DW , 1.67% and 0.57 %) were reduced consecutively. These findings are consistent with Abdulkadhim and Mortada (2022), which found that wonderful pomegranate seedlings irrigated with saline irrigation water at (9, 6, 3, 1.3 dSm^{-1}), caused a negative decrease in some chemical traits (nitrogen, phosphorous, potassium) at 9 dSm^{-1} compared with the other dosages. Foliar applications by K_2SiO_3 NPs at 4 ml L^{-1} achieved positive findings in biochemical indicators. Chlorophyll, Carbohydrates , N, P, K, K/Na , Fe, Ca , Mg and Si in leaves achieved the highest means (28.79 Spad Unit , 5.80 mg kg^{-1} DW, 2.07% , 0.32%, 2.55%, 4.47 %, 109.06 mg Kg^{-1} DW , 2.35%, 0.22% and 3.79%) , compared to control (28.60 Spad Unit, 4.02 mg kg-1 DW, 1.85%, 0.28%, 2.37% , 4.02, 97.66 mg Kg^{-1} DW , 2.35%, 0.20%, 1.85%) consecutively. K2SiO3 NPs at 4 ml L^{-1} caused a positive decrease in Proline, Cl and Na (2.56 mg g-l D.W., 1.62%, 0.56%) compared to control (3.00 mg g^{-l} D.W, 1.78 %, and 0.61 %) consecutively. These findings are consistent with Hassan, *et al.* (2022), who found that utilization of nano-silicon by foliar application enhanced the biochemical characteristics of olive. Mahmoud *et al.* (2022) found that nano silicon enhanced salt tolerance in Valencia orange plants by enhancing the biochemical characteristics, and El-Dengawy *et al.* (2021) found that nano silicon enhanced biochemical characteristics of two mango rootstocks under salinity stress.

Increased levels of $NaCl_2$ caused a decrease in the absorption of ions due to the increased osmotic stress of the soil solution, and difficulty in absorption of nutrients and water by plant roots. Direct harmful effect of salts is through the antagonism between No_3 and Cl^{-1}, which prevents the absorption of nitrates. Also, the antagonism between Mg^{+2} and Na^{+1} leads to a decrease in the absorption of Mg^{+2}, and since N and Mg ions are involved in the synthesis of chlorophyll, therefore the chlorophyll content of the leaves decreases. The indirect harmful effect of salts occurs by reducing the permeability of plasma membranes. (Zhou MX *et al.*, 2020). Cl^{-1} ions compete with $HPO4^{-2}$ ions, reducing P absorption. Antagonism between Na^+ ions and both of K^+ , Mg^{+2} and Ca^{+2} ions on adsorption positions in roots leads to decreased uptake of K^+ ions , K/ Na ratio, Mg^{+2}, and Ca^{+2}. Excess salts in irrigation wa-

Table 1. Influence of K_2SiO_3 NPs in some Ions absorption and biochemical indicators of King Mandarin saplings leaves under salt stress

Salt level (mM)	K_2SiO_3 NPs	Total chlorophyll (Spad Unit)	Carbohydrate ((mg kg^{-1} dw))	N (%)	P (%)	K (%)	K/Na (%)	Fe(mg Kg^{-1}dw
0	0	31.40	4.51	1.98	0.30	2.55	4.32	104.33
	2	30.82	5.33	2.02	0.31	2.37	4.28	115.31
	4	31.60	6.62	2.12	0.35	2.44	4.28	122.92
40	0	26.99	3.92	2.06	0.25	2.20	3.93	96.53
	2	28.98	4.66	2.07	0.29	2.16	3.60	104.44
	4	28.08	5.72	2.08	0.30	2.74	5.07	106.33
80	0	27.40	3.64	1.52	0.29	2.37	3.76	92.12
	2	26.28	4.12	2.11	0.31	2.07	3.63	96.00
	4	26.69	5.07	2.01	0.30	2.45	4.15	97.92
LSD. at $p< 0.05$		2.81	0.37	0.18	0.02	0.15	1.67	4.95
Salt level mM	0	31.27	5.49	2.04	0.32	2.46	4.24	114.19
	40	28.1	4.77	2.07	0.28	2.37	4.23	102.43
	80	26.79	4.27	1.88	0.30	2.30	3.83	95.35
LSD. at $p< 0.05$		1.63	0.22	0.10	0.01	0.09	0.97	2.78
K_2SiO_3 NPs ml L^{-l}	0	28.60	4.02	1.85	0.28	2.37	4.02	97.66
	2	28.69	4.70	2.06	0.30	2.20	3.79	105.25
	4	28.79	5.80	2.07	0.32	2.55	4.47	109.06
LSD. At $p< 0.05$		1.63	0.22	0.10	0.01	0.09	0.97	2.78

Table 2. Influence of K_2SiO_3 NPs in some ions absorption and biochemical indicators of King Mandarin Saplings leaves under salt stress

Salts level	K_2SiO_3 NPs ml L^{-l}	Ca (%)	Mg (%)	Si (%)	Proline (mg g^{-1} dw)	Cl (%)	Na (%)
0 mM	0	2.37	0.22	1.54	3.12	1.84	0.58
	2	2.52	0.21	2.44	2.66	1.65	0.57
	4	2.43	0.26	3.93	2.23	1.53	0.55
40 mM	0	2.35	0.17	1.71	3.00	1.77	0.61
	2	2.47	0.21	2.74	2.69	1.66	0.57
	4	2.56	0.20	3.67	2.66	1.57	0.56
80 mM	0	2.33	0.20	2.30	2.88	1.74	0.63
	2	2.31	0.21	3.02	2.74	1.89	0.59
	4	2.51	0.21	3.78	2.78	1.77	0.57
LSD. at p< 0.05		0.12	0.2	0.23	0.25	0.14	0.5
Salts levels mM	0	2.44	0.23	2.63	2.67	1.67	0.57
	40	2.46	0.19	2.71	2.78	1.67	0.58
	80	2.38	0.21	3.03	2.80	1.80	0.60
LSD. at p< 0.05		0.07	0.01	0.13	0.15	0.08	0.03
K_2SiO_3 NPs ml L^{-l}	0	2.35	0.20	1.85	3.00	1.78	0.61
	2	2.43	0.21	2.73	2.70	1.73	0.58
	4	2.50	0.22	3.79	2.56	1.62	0.56
LSD. at p< 0.05		0.07	0.01	0.13	0.15	0.08	0.03

ter lead to iron deposition in the soil, which restricts its absorption. It also leads to the accumulation of nitrogenous compounds (proline) as an adaptive means to protect plant cells from destruction (Khalil *et al.*, 2022; Boguszewska and Zagdańska, 2012; Zhu *et al.*, 2019).

In present study, foliar applications with K_2SiO_3 NPs were superior in most biochemical indicators, except for proline , Cl and Na. Increased chlorophyll was due to the role of silicon in increasing the absorption of N, Fe, and Mg which are involved in the synthesis of chlorophyll (Zhu *et al.*, 2019; Liu *et al.*,2019). Silicon increases the percentage of carbohydrates in leaves due to its role in enhancing the efficiency of photosynthesis. Also, it regulates the rate of transpiration and plays a major role in reducing the permeability of Na^+ ions into the plant cells. It also raises the K^+/Na^+ ratio inside the plant cell, which reduces the toxicity of Na^+ ions and thus increases plants' tolerance to salt stress. (Avestan *et al.*, 2019). Silicon also has an important mechanism in increasing Ca^{++}ions by increasing the effectiveness of proteins in the plasma membrane of roots. (Hoffmann *et al.*, 2020). These findings are in agreement with Abdulkadhim *et al.* (2024); Mahmoud *et al.* (2020), who observed the impact of silicon NPs by foliar application to enhance the biochemical characteristics of papaya and banana plants under salinity stress consecutively .

Estimating of Catalase (CAT), Peroxidase (POD) and Superoxide Dismutase (SOD) effectiveness

Findings in Table 3 shows that the salinity level of 80 mM increased the effectiveness of antioxidant enzymes (CAT, POD, and SOD), reaching 8.37, 11.41, 31.45 unit mol $^{-1}$, compared to salinity levels 0 mM (7.43, 10.94, 15.15 unit mol $^{-1}$) consecutively. Foliar applications by K_2SiO_3 NPs at 4 ml L^{-1} caused a positively decreased in the effectiveness of antioxidant enzymes (CAT, POD and SOD) which scored 6.14, 8.43, 21.01 unit mol^{-1}, contrast to 0 ml L^{-l} (9.68, 13.41, 24.85 unit mol^{-1}) consecutively.

Exposure of the plant to salt stress leads to the production of reactive oxygen radicals (ROS), which stimulates the plant to produce the enzymes SOD and POD as a defensive means to reduce levels of ROS that are harmful to the plant. The plant also produces the enzyme CAT, which is important in removing toxic hydrogen peroxide (H_2O_2) by decomposing it into water and oxygen (Ashraf, 2009; Harinasut *et al.*, 2000). ROS synthesis within plant cells is the first response to plant exposure to stress, and antioxidant enzymes are the organic key in the plant's protection system and increasing its tolerance to salt stress (Elsheery *et al.*, 2020). The decrease in the effectiveness of the antioxidants (POD, CAT, and SOD) is due to the role of K_2SiO_3 NPs in improving plant metabolism, protecting

Table 3. Influence of K_2SiO_3 NPs in antioxidant enzymes effectiveness of King Mandarin Saplings leaves under salt stress

Salts levels	K_2SiO_3 NPs ml L^{-1}	CAT (unit mol^{-1})	POD (unit mol^{-1})	SOD (unit mol^{-1})
0 mM	0	9.06	13.47	17.53
	2	7.53	11.58	15.34
	4	5.69	7.77	12.95
40 mM	0	10.09	13.55	25.02
	2	7.77	11.04	22.08
	4	6.16	8.62	20.39
80 mM	0	9.89	13.23	33.99
	2	8.46	12.10	30.66
	4	6.75	8.91	29.69
LSD. at p< 0.05		0.83	1.66	3.186
Salts levels mM	0	7.43	10.94	15.15
	40	8.01	11.07	22.50
	80	8.37	11.41	31.45
LSD. at p< 0.05		0.48	0.96	2.107
K_2SiO_3 NPs ml l^{-l}	0	9.68	13.41	24.85
	2	7.92	11.57	22.72
	4	6.14	8.43	21.01
LSD. at p< 0.05		0.48	0.96	2.107

cell membranes from photo-oxidation, increasing the efficiency of enzymatic activity, increasing the absorption of beneficial nutrients (Fe, Mg, K, P, N and Ca) and prevent the accumulation of toxic ions as reported earlier (Ashraf,2009; Zhu. *et al.*, 2019). It has been shown from previous studies that potassium silicate helps the plant to tolerate salt stress conditions (Laane, 2018).

Conclusion

The present study concluded that irrigation of King Mandarin Saplings at a salinity level of 80 mM had a negative influence on the chlorophyll, carbohydrates, N, P, K, K/Na, Fe, Ca and Mg in leaves and symptoms of nutrient deficiency appeared, leading to the accumulation of Si, Pro., Cl, Na, CAT, POD and SOD in leaves as a defensive means to resist oxidative stress and the intensity of the effect increases with increasing salinity level. So, these indicators can be used as an index of the tolerance of mandarin seedlings to salinity in future studies. Foliar applications by K_2SiO_3NPs at 4 ml L^{-1} achieved a positive influence on the absorption of ions and bio-chemicals indicators (chlorophyll, Carbohydrates, N, P, K, K/Na, Fe, Ca, Mg and Si in leaves), leading to reduced accumulation of Si, Pro., Cl, Na, CAT, POD and SOD in saplings leaves under the conditions of the current study. It can be concluded from the findings that the use of K_2SiO_3NPs effectively contributed to reducing the negative influence of sodium chloride toxicity in irrigation water.

Conflict of interest

The authors declare that they have no conflict of interest.

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