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INFLUENCE OF NANO-SILICON AND NANO-CHITOSAN ON GROWTH, ION CONTENT, AND ANTIOXIDANT DEFENSE ENZYME OF TWO CITRUS ROOTSTOCKS UNDER SALINITY CONDITIONS

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ABSTRACT

Nanoparticle foliar spray is a new and effective approach for improving seedling growth and survival under adverse conditions such as salt stress. The current study was conducted to investigate the impact of commercial silicon dioxide (SiO_2), SiO_2 nanoparticles (SiO_2 NPs), commercial chitosan (CS), CS nanoparticles (CS NPs) on growth, proline, antioxidant defense enzyme, and ions content in One-year-old for sour orange (*Citrus aurantium, L.*) and Volkamer lemon (*Citrus volkameriana*) rootstocks grown under salinity stress. Foliar spray of SiO_2, SiO_2 NPs, Chitosan, and Chitosan NPs with a concentration of 50 ppm was applied at two NaCl concentrations (0, 50 mM). Vegetative growth, including plant height, stem diameter, leaf area, roots, and total fresh and dry weights, were determined. The findings demonstrated that salinity adversely affected plant growth. Salt-stressed plant leaves exhibited a greater activity of peroxidase (POD) and proline content when compared to the control treatment. Na^+ and Cl^- ions accumulated in leaves of salinized plants. Nano-Silicon dioxide and Nano-chitosan achieved a significantly increased of full vegetative growth parameters and antioxidant defense enzyme. Nano treatments decreased Na^+ and Cl^- ions content in the leave tissue. As a result, both SiO_2 NPs and CS NPs are employed as part of a combined approach to increase the growth indices of citrus plants, especially achieving a significant impact in alleviating salinity stress.

INTRODUCTION

Among all abiotic stress criteria, salinity stress is an emerging environmental problem that endangers global food security by stunting plant growth, altering ion ratios, degrading chlorophyll, and disrupting stomatal function [1,2]. In addition, plant metabolisms may be severely disrupted by oxidative damage to lipids and proteins when cultivated in saline conditions [3]. There is evidence that cells under salinity stress produce more internal reactive oxygen species (ROS), namely hydroxyl and superoxide radicals. Plants have evolved various defensive mechanisms to combat this oxidative stress, including antioxidant enzymes that remove harmful reactive oxygen species.[4,5]

Citrus is one of the most significant economic crops. Fifty different nations now cultivate it for profit, making it one of the world's most widely produced fruit crops. Different types of citrus trees and rootstocks have varying tolerance to salty conditions. Rootstocks' salt resistance was measured by their ability to exclude chloride and sodium .[6]

Numerous strategies have been devised to alleviate the detrimental impacts of salt on plants including chemical primers, plant breeding and microorganisms. Most recently, natural polymers and metal oxides-based nanostructured materials such as nano chitosan [7], ZnO nanoparticles [8], SiO2 nanoparticles [9], TiO2 nanoparticles [10] have been developed as safe and efficient materials for improving salt stress tolerance to counteract the effects of abiotic stressors like salinity .[11,12]

Chitosan is a naturally occurring cationic polysaccharide that has attracted significant attention as a bio-stimulant for its potential to increase the growth and yield of agricultural crops [13,14,15,16]. Chitosan has been introduced to boost germination rate, vegetative development, shoot height, and seedling vigor, in addition to having antifungal, antibacterial [17], and antiviral activities [18]. Consequently, chitosan substantially impacts many crops species' germination, maturation, and blooming [13,19,20]. In addition to chitosan's inherent qualities, nano-chitosan has traits such as surface and interface effect, tiny size, and great diffusion impact [21]. The ionic gelation process is the most common approach to making nano-chitosan, and hence it is the one that is utilized here. The procedure's ease and the fact that little or no shear power is required to accomplish the desired result contribute to the method's widespread acceptance. In compared to liquid priming, solid matrix priming is a novel, effective, and considerably more favorable technique.

One of the many promising Nanomaterials with potential use in contemporary farming is nano-silicon dioxide (SiO2 NPs). Although it is not regarded as a vital nutrient, silicon dioxide is involved in many metabolic processes that help plants better endure environmental challenges, including drought and salinity stress. Moreover, silicon's benefits include reducing the stress caused by nutritional deficiencies and increasing the growth, development, and production of a wide range of plant species [22,23]. Tolerance to cold, freezing, salt and drought increases, cell membranes are protected from metal toxicity, oxidative and phenolic browning are avoided, and organogenesis and embryogenesis are facilitated [24,25,26,27]. In addition, applying SiO2 to other crops improved photosynthesis, boosted vegetative growth and total dry output, decreased sodium (Na^+) and chloride (Cl^-) buildup, and boosted potassium content [28,29,30]. Several studies found that the presence of Nano silicon or silicon dioxide reduced the negative effects of salt stress and dramatically boosted photosynthetic contents, including chlorophyll a, b and total chlorophyll .[31]

Herein, chitosan, nano chitosan, silica and nanosilica have been utilized as eco-friendly and effective materials for high-performance Sour orange and Volkamer lemon under harsh conditions such as salinity stress. The nano-silica and nano-chitosan demonstrated an effective and robust approach for mitigating nutrient imbalance stress and enhancing tolerance to salinity. Therefore, this research examined whether nanosized, monodisperse silicon dioxide and chitosan particles may mitigate the negative effects of high sodium chloride (NaCl) on two citrus rootstocks.

MATERIALS AND METHODS

Plant materials and growth conditions

In this experiment, one-year-old rootstocks of sour orange (Citrus aurantium, L.) and Volkamer lemon (Citrus volkameriana) were utilized. These seedlings were headed in the glasshouse of The City of Scientific Research and Technological Applications in Borg El-Arab region. The experimental plants were singly planted in black polyethylene bags, filled with about two kilograms of mixed sandy and clay loam soil. Before the commencement of different salinity and compound treatments, tap water irrigated plants twice a week. Forty citrus seedlings from two experimental rootstocks, as uniform as possible in size and length, were used. The experimental plants were arranged in a Split-Split- Plot Design, with four replicates for each treatment. Thus, 80 plants (2 rootstocks x 2 salinity levels x 5 compounds treatments x 4 replicates) were used in this investigation. The selected seedlings were divided into two groups; each group of 40 plants received one of either salinity treatment: 0 (Tap water; control) and 50 mM NaCl. Salinity treatments were achieved by irrigating each seedling twice a week with 500 ml/ seedling for three months. Treatments as a foliar spray: (i) Control, (ii) Silicon in normal size, (iii) Silicon in nanoparticles size (Nano-Si), (iv) Chitosan in normal size and (v) Chitosan in nanoparticles (Nano-chi) size .

During the two seasons, the following parameters were measured: The different parameters describing the vegetative growth of the plants, Leaf proline content, Leaf mineral composition (N, P, K, Ca, Mg, Na, Cl) and activity of antioxidant defense enzymes. Statistical analysis will be carried out as a split-split plot design.

Nanomaterials preparations

Synthesis of Nano-chitosan

Chitosan nanoparticles (CS NPs) were synthesized by an ionic gelation technique involving sodium tripolyphosphate (TPP). Typically, 200 mg of Chitosan powder was dissolved in 400 mL deionized water and 2 mL of acetic acid at room temperature using vigorous magnetic stirring. With constant stirring, 100 mL of sodium tripolyphosphate solution with a concentration of (0.75 mg mL-1) was added dropwise to the chitosan solution. The obtained nano-chitosan suspension was centrifuged for 10 minutes at 8000 rpm [32]. The precipitated nano-chitosan was frozen for 24 hours at 40 °C to solidify thoroughly before being vacuum freeze-dried for 48 hours.

Synthesis of Nano-Silica

The sol-gel technique was used to create silica nanoparticles (SiO_2 NPs) using tetraethyl orthosilicate (TEOS). In particular, 20 mL of TEOS was dropped into a combination of 75 mL of ethanol and 10 mL of distilled water, followed by 2 mL of ammonium hydroxide, which was gently dropped into the solution and agitated for 3 hours at room temperature [33]. The ultrafine white precipitate was recovered by centrifuging it at 8000 rpm for 10 minutes, washing it multiple times with ethanol/water to remove unreacted organic compounds, and drying it overnight at 80 oC in a vacuum oven.

Growth parameters

Total plant growth was calculated when the experiment was completed. Stem diameter was measured and the Leaf surface area was calculated. The roots and total fresh and dry weights were measured. In order to determine the root and total dry weights, the plants were separated, their leaves, stems, and roots meticulously split, and then dried in an oven at 80 oC for 2 days.

Activity of antioxidant defense enzyme and Leaf proline content

Based on [34], the spectrophotometer has been utilized to determine the proline 0.5 g of the plant sample was extracted in 5% sulfosalicylic acid and centrifuge at 12,000 rpm for 10 min. Mixture solution of 2 mL of ninhydrin, and 2 mL of glacial acetic acid added to the diluted supernatants and heated for 1h at 100 oC then cooled to room temperature .

The mixture was subjected to 4 mL of toluene, and the spectrophotometric reading of the supernatant aqueous phase was taken at 520 nm .

Fresh leaf samples were tested for peroxidase activity using the following procedure. Typically, 3 mL of the mixture solution containing 25 mmol L-1 phosphate buffer (pH 6.8), 10 mL enzyme extract, 20 mmol L-1 guaiacol, and 40 mmol L-1 H2O2. After adding H2O2 the reaction was monitored by measuring the absorbance at 470 nm for 2 minutes.

Leaf mineral

According to the method described by [35], 0.1 gm of digested dried powdered material from each plant's leaf tissue with H2SO4 and H2O2, we were able to determine the concentrations of the individual mineral elements. The modified Kjeldahl technique was used to predict the total N content of the leaves (Peach and Tracy, 1955). According to the methods given by [36] using a spectrophotometer (Unico, Model UV2150, Dayton, NJ, USA), total P was calculated in this digested solution. The K and Na levels in plants were measured with a flame photometer (Systonic, Model S-935, Haryana, India) [36], and the Ca and Mg levels were measured with a Perkin Elmer atomic absorption spectrophotometer. Cl levels were evaluated using Jackson and Brown's method .[37]

Statistical analyses

For this experiment, we employed a split-split-plot layout, with the rootstocks serving as the main plots, the salt levels serving as the subplots, and the treatments serving as the sub-subplots. Using SPSS, V.18 PASW, we performed an analysis of variance (ANOVA) on the data we collected from the various salinity levels and treatments in both 2020 and 2021. Treatment differences were determined using the Least Significant Difference (LSD) at the 0.05 level of significance.

RESULTS & DISCUSSION

Fourier Transform Infrared (FTIR) Spectra analysis

FT-IR spectrum in Fig. 1 (a) shows the typical characteristics bands for both bulk commercial silica and SiO2 NPs. In case of commercial SiO2 bulk structure, four distinct bands at 474 cm−1 corresponding stretching vibration peak Si-O-Si, Si-

OH band at 951 cm−1, Si-O-Si bending at 1102 cm−1 and broad band of O-H hydroxyl group at 3420 cm−1. For the fabricated SiO2 NPs, the FTIR plot reflects identical characteristics bands commercial SiO2 confirming successful fabrication SiO2 NPs furthermore the lower intensity may be attribute to ultrafine amorphous structure of silica nanoparticles [33,38]. As shown in Fig (b), FTIR plot for the commercial chitosan and the fabricated nanochitosan. Five distinct bands can be observed for the commercial bulk chitosan such as C-O-C at 1095 cm−1, C-N stretching vibrations band located at 1426 cm−1, band at 1426 cm−1 corresponds to (bending vibrations N-H), the band at 2876 cm−1 related to (C-H stretching) and broad bands for overlapping two functional groups of O-H and NH at 3457 cm−1 [7,39]. For the prepared Chitosan nanoparticles, the FTIR demonstrated typical bands which indicating successful fabrication process of pure chitosan nanoparticles without impurities [40,41]. The high intensity bands of the fabricated chitosan nanoparticles reflecting better crystalline structure of nanoparticles compared to commercial polymer of chitosan.

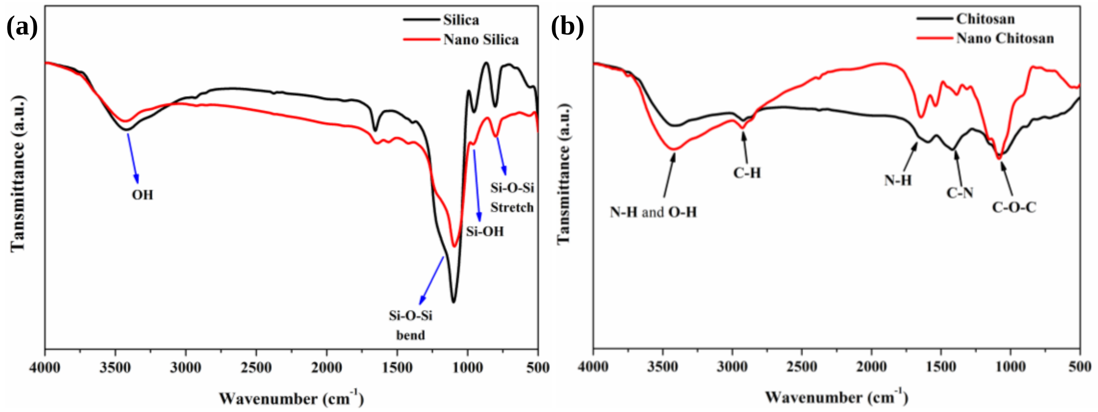

Figure (1): (a, b) FTIR for the commercial and synthesized nanomaterials.

Morphological Performance

Our analysis revealed that salinity stress using NaCl concentration of 50 mM, and (SiO2, SiO2 NPs, chitosan and CS NPs) materials treatments significantly affected the morphological of citrus rootstocks (Tables 1 and 2). Irrigation with 50 mM NaCl significantly decreased all morphological parameters including; plant length, leaf area, stem diameter, Lateral branches number, root and plant fresh weight and root and plant dry weight in both seasons. Among all fabricated materials, SiO2 NPs have remarkable impact for alleviating salinity stress which achieved the highest leaf area, stem diameter and fresh and dry weight of plant (28.91, 10.75 70.00, 46.25 respectively) in sour orange rootstock in 2020 season whereas, Volkamer lemon rootstock (17.73, 7.28 ,40.00, 25.00 respectively) under 50 mM NaCl salinity stress. In addition, NaCl treatment drastically inhibited root growth (Fig. 4). Both the fresh and dry root weights dropped dramatically after irrigation with 50 mM NaCl. Whereas, the highest plant length, fresh and dry weight of roots were observed with nano chitosan (95.38, 27.50, 16.75, respectively) in sour orange rootstock in 2020 season whereas, Volkamer lemon rootstock (94.13, 30.00, 15.00, respectively). However, the sour orange rootstock revealed significant increase in all treatments with nanomaterials.

Table (1): Means of the growth parameters of sour orange and Volkamer lemon during 2020 and 2021 seasons in response to foliar normal, nano-sized silicon, normal and nano-sized chitosan treatments under salinity stress conditions.

Rootstock	Salinity Levels (mM)	Treatments	Leaf area(cm2)		Plant length(cm)		Stem diameter (mm)		growth number	
			2020	2021	2020	2021	2020	2021	2020	2021
sour orange	0	Control	26.100a	21.125a	76.450d	76.900d	9.950a	6.730a	5.500a	2.400b
	50		18.275a	18.425a	74.925d	77.675d	9.587a	7.425a	6.750a	2.850b
	0	Silicon	26.325a	26.912a	84.475c	84.000c	10.650a	8.786a	6.000a	4.350b
	50		19.300a	28.120a	85.012c	79.000c	9.850a	7.983a	7.625a	3.700b
	0	Nano-Si	31.300a	30.000a	89.462b	88.060b	11.462a	9.676a	8.125a	5.550ab
	50		28.912a	20.775a	85.377b	88.985b	10.750a	7.993a	8.375a	4.550ab
	0	Chitosan	28.275a	25.500a	86.525b	92.640a	10.200a	8.950a	8.250a	7.250a
	50		20.000a	26.715a	87.550b	92.070a	9.975a	8.910a	9.250a	5.300a
	0	Nano-chi	28.375a	21.750a	92.825a	93.645a	10.300a	8.216a	9.625a	7.150a
	50		22.825a	24.950a	95.382a	93.660a	10.362a	9.478a	8.875a	5.700a
Volkamer lemon	0	Control	15.650a	22.100a	69.223d	75.575b	6.987a	7.008a	6.375a	3.450b
	50		14.750a	19.300a	71.745d	73.655b	7.037a	7.635a	6.250a	2.900b
	0	Silicon	20.425a	26.850a	84.162c	86.935c	7.812a	8.565a	7.750a	4.600b
	50		14.300a	24.175a	83.455c	83.575c	7.450a	8.448a	7.125a	3.200b
	0	Nano-Si	18.222a	24.575a	85.205b	92.225b	7.200a	7.634a	6.375a	4.500b
	50		17.725a	24.175a	92.662b	84.985b	7.275a	8.911a	7.250a	3.800b
	0	Chitosan	18.875a	25.650a	86.517b	92.435a	7.437a	8.171a	9.000a	3.500a
	50		16.800a	24.325a	93.017b	93.635a	7.487a	8.605a	7.750a	4.500a
	0	Nano-chi	22.050a	26.100a	89.830a	92.705a	7.225a	9.395a	8.250a	6.500a
	50		17.025a	29.400a	94.127a	96.530a	7.975a	8.172a	6.625a	5.050a
LSD			0.056	0.138	.074	0.168	0.320	0.128	0.052	0.121

Means with the same letter are not significantly different according to the least significant difference LSD at 0.05 levels.

Table (2): Means of the growth parameters of sour orange and Volkamer lemon during 2020 and 2021 seasons in response to foliar normal, nano-sized silicon, normal and nano-sized chitosan treatments under salinity stress conditions.

Rootstock	Salinity Levels (mM)	Treatments	Root fresh weight (g/plant)		Root dry weight (g/plant)		Plant fresh weight (g/plant)		Plant dry weight (g/plant)	
			2020	2021	2020	2021	2020	2021	2020	2021
sour orange	0	Control	20.000$_{b}$	16.725$_{ab}$	9.000$_{b}$	5.200$_{ab}$	51.250$_{d}$	33.850$_{ab}$	40.000$_{b}$	14.500$_{ab}$
	50		22.500$_{b}$	15.075$_{ab}$	12.500$_{b}$	6.625$_{ab}$	45.000$_{d}$	35.275$_{ab}$	30.000$_{b}$	15.850$_{ab}$
	0	Silicon	21.500$_{b}$	19.025$_{ab}$	10.000$_{b}$	11.100$_{ab}$	66.250$_{bc}$	48.275$_{b}$	51.250$_{a}$	21.700$_{ab}$
	50		25.000$_{b}$	25.732$_{ab}$	12.500$_{b}$	7.625$_{ab}$	60.000$_{bc}$	35.925$_{b}$	40.000$_{a}$	16.575$_{ab}$
	0	Nano-Si	24.500$_{b}$	25.825$_{b}$	10.000$_{b}$	10.425$_{ab}$	80.000$_{a}$	51.550$_{b}$	57.500$_{a}$	23.950$_{ab}$
	50		27.500$_{b}$	34.725$_{b}$	13.750$_{b}$	7.600$_{ab}$	70.000$_{a}$	42.725$_{b}$	46.250$_{a}$	19.700$_{ab}$
	0	Chitosan	20.750$_{b}$	27.300$_{a}$	11.000$_{b}$	10.175$_{a}$	61.250$_{c}$	62.500$_{a}$	45.000$_{a}$	25.250$_{a}$
	50		25.000$_{b}$	29.875$_{a}$	14.250$_{b}$	12.575$_{a}$	60.000$_{c}$	50.925$_{a}$	42.500$_{a}$	23.400$_{a}$
	0	Nano-chi	28.750$_{a}$	19.175$_{b}$	14.000$_{a}$	8.925$_{b}$	65.000$_{ab}$	46.600$_{b}$	43.750$_{a}$	21.100$_{b}$
	50		27.500$_{a}$	15.650$_{b}$	16.750$_{a}$	15.650$_{b}$	72.500$_{ab}$	65.625$_{b}$	47.250$_{a}$	32.050$_{b}$
Volkamer lemon	0	Control	20.000$_{b}$	9.825$_{ab}$	11.250$_{b}$	5.000$_{ab}$	35.000$_{d}$	26.575$_{b}$	21.000$_{b}$	13.275$_{ab}$
	50		16.250$_{b}$	12.775$_{ab}$	9.000$_{b}$	5.325$_{ab}$	30.000$_{d}$	31.700$_{ab}$	18.000$_{b}$	15.075$_{ab}$
	0	Silicon	28.750$_{b}$	17.775$_{b}$	15.250$_{b}$	6.950$_{ab}$	40.000$_{bc}$	40.475$_{b}$	26.250$_{a}$	17.900$_{ab}$
	50		17.500$_{b}$	17.100$_{b}$	12.500$_{b}$	7.375$_{ab}$	40.000$_{bc}$	38.700$_{b}$	23.250$_{a}$	18.000$_{ab}$
	0	Nano-Si	25.000$_{b}$	16.325$_{b}$	13.000$_{b}$	7.000$_{ab}$	43.750$_{a}$	44.475$_{ab}$	22.500$_{a}$	21.000$_{ab}$
	50		20.000$_{b}$	18.200$_{b}$	9.500$_{b}$	8.475$_{ab}$	40.000$_{a}$	42.650$_{b}$	25.000$_{a}$	21.125$_{ab}$
	0	Chitosan	20.000$_{b}$	12.200$_{a}$	12.750$_{b}$	6.275$_{a}$	52.500$_{c}$	31.200$_{a}$	33.000$_{a}$	16.200$_{a}$
	50		22.500$_{b}$	27.125$_{a}$	10.000$_{b}$	10.700$_{a}$	30.000$_{c}$	57.525$_{a}$	18.500$_{a}$	25.600$_{a}$
	0	Nano-chi	20.000$_{a}$	13.525$_{b}$	12.000$_{a}$	6.400	53.750$_{ab}$	36.800$_{b}$	33.500$_{a}$	17.500$_{b}$
	50		30.000$_{a}$	16.175$_{b}$	15.000$_{a}$	6.650	33.750$_{ab}$	34.900$_{b}$	26.500$_{a}$	15.625$_{b}$

Means with the same letter are not significantly different according to the least significant difference LSD at 0.05 levels.

Mineral composition

The nutrient uptake by both rootstocks was analysed in the leaf. Under salinity conditions, although there were no significant differences, the leaf content including N, P, K, Ca and Mg element demonstrated a decrease in sour orange rootstock in both season (Table 3). On the other hand, the leaf content of N, P, K, Ca and Mg in Volkamer lemon rootstock revealed slight increase. Exceptional improvements in nitrogen contents under salt stress was observed with SiO2-NP treatment for both rootstocks in the two seasons. Whereas, the highest P, K, Ca and Mg under the 50 mM NaCl were depicted with nano-chitosan. Generally, The Na+ and Cl- concentrations in the leaves rose dramatically as salt stress intensified (Tables 4). The highest Na+ content significantly decreased in the leaves following nano-silicon treatment under salinity stress. On the other hand, when leaves were treated with nano silicon, Cl- content dropped dramatically over both growing seasons.

Proline and peroxidase

Citrus rootstocks raised under NaCl stress have a higher proline content, as shown in (Fig. 2). The rootstocks treated with nano chitosan showed the greatest proline content in both growing seasons. When subjected to salt stress, Volkamer lemon rootstock fared better than sour orange rootstock. Nevertheless, the Volkamer lemon rootstock showed substantial growth after exposure to any nanomaterial treatments.

Table (3): Means of leaf nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) of sour orange and Volkamer lemon during 2020 and 2021 seasons in response to foliar normal, nano-sized silicon, normal and nano-sized chitosan treatments under salinity stress conditions.

Rootstock	Salinity Levels (mM)	Treatment	N%		P%		k%		Ca%		Mg%	
			2020	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**
sour orange	**0**	**Control**	2.063$_{a}$	2.072$_{b}$	0.138$_{a}$	0.138$_{d}$	1.500$_{c}$	1.550$_{c}$	3.675$_{d}$	3.720$_{d}$	0.350$_{d}$	0.355$_{a}$
	50		1.250$_{a}$	1.950$_{b}$	0.131$_{a}$	0.139$_{d}$	1.100$_{c}$	1.400$_{c}$	3.247$_{d}$	3.292$_{d}$	0.340$_{d}$	0.345$_{a}$
	0	**Silicon**	2.195$_{a}$	2.427$_{ab}$	0.142$_{d}$	0.141$_{c}$	1.650$_{b}$	1.700$_{b}$	3.927$_{c}$	3.923$_{c}$	0.372$_{c}$	0.370$_{d}$
	50		1.583$_{a}$	1.900$_{ab}$	0.138$_{d}$	0.140$_{c}$	1.400$_{b}$	1.675$_{b}$	3.427$_{c}$	3.450$_{c}$	0.365$_{c}$	0.365$_{d}$
	0	**Nano-Si**	2.120$_{a}$	2.400$_{a}$	0.142$_{b}$	0.143$_{b}$	1.725$_{a}$	1.775$_{a}$	4.112$_{b}$	4.203$_{b}$	0.385$_{b}$	0.388$_{c}$
	50		2.045$_{a}$	2.375$_{a}$	0.140$_{b}$	0.142$_{b}$	1.550$_{a}$	1.675$_{a}$	4.017$_{b}$	4.047$_{b}$	0.377$_{b}$	0.388$_{c}$
	0	**Chitosan**	1.625$_{a}$	2.125$_{ab}$	0.141$_{c}$	0.142$_{b}$	1.550$_{b}$	1.725$_{a}$	3.902$_{b}$	3.970$_{b}$	0.360$_{b}$	0.363$_{b}$
	50		1.573$_{a}$	1.900$_{ab}$	0.136$_{c}$	0.141$_{b}$	1.225$_{b}$	1.600$_{a}$	4.067$_{b}$	4.105$_{b}$	0.407$_{b}$	0.413$_{b}$
	0	**Nano-chi**	1.700$_{a}$	2.175$_{a}$	0.142$_{a}$	0.142$_{a}$	1.700$_{a}$	1.750$_{a}$	3.985$_{a}$	4.020$_{a}$	0.387$_{a}$	0.390$_{a}$
	50		1.730$_{a}$	1.850$_{a}$	0.142$_{a}$	0.145$_{a}$	1.350$_{a}$	1.400$_{a}$	3.935$_{a}$	3.997$_{a}$	.415$_{a}$	0.417$_{a}$
Volkamer lemon	**0**	**Control**	2.415$_{a}$	2.425$_{b}$	0.146$_{a}$	0.147$_{d}$	1.600$_{c}$	1.575$_{c}$	4.080$_{d}$	4.112$_{d}$	.385$_{d}$	0.388$_{a}$
	50		2.838$_{a}$	2.650$_{b}$	0.148$_{a}$	0.148$_{d}$	1.800$_{c}$	1.850$_{c}$	4.410$_{d}$	4.450$_{d}$	.407$_{d}$	0.410$_{a}$
	0	**Silicon**	2.183$_{a}$	2.425$_{ab}$	0.146$_{d}$	0.147$_{c}$	1.700$_{b}$	1.775$_{b}$	4.282$_{c}$	4.300$_{c}$	.390$_{c}$	0.398$_{d}$
	50		2.718$_{a}$	2.700$_{ab}$	0.145$_{d}$	0.147$_{c}$	1.700$_{b}$	1.750$_{b}$	4.505$_{c}$	4.550$_{c}$	.440$_{c}$	0.450$_{d}$
	0	**Nano-Si**	2.318$_{a}$	2.275$_{a}$	0.150$_{b}$	0.151$_{b}$	1.725$_{a}$	1.825$_{a}$	4.397$_{b}$	4.492$_{b}$	.417$_{b}$	0.420$_{c}$
	50		2.918$_{a}$	2.975$_{a}$	0.152$_{b}$	0.152$_{b}$	1.825$_{a}$	1.950$_{a}$	4.615$_{b}$	4.680$_{b}$	.460$_{b}$	0.465$_{c}$
	0	**Chitosan**	2.700$_{a}$	2.775$_{ab}$	0.148$_{c}$	0.150$_{b}$	1.850$_{b}$	1.925$_{a}$	4.592$_{b}$	4.650$_{b}$	.432$_{b}$	0.448$_{b}$
	50		2.673$_{a}$	2.825$_{ab}$	0.151$_{c}$	0.152$_{b}$	1.925$_{b}$	1.950$_{a}$	4.732$_{b}$	4.762$_{b}$	.462$_{b}$	0.468$_{b}$
	0	**Nano-chi**	2.843$_{a}$	2.875$_{a}$	0.151$_{a}$	0.151$_{a}$	1.925$_{a}$	1.975$_{a}$	4.787$_{a}$	4.805$_{a}$	.440$_{a}$	0.455$_{a}$
	50		2.580$_{a}$	3.100$_{a}$	0.154$_{a}$	0.153$_{a}$	2.000$_{a}$	2.025$_{a}$	4.820$_{a}$	4.875$_{a}$	.480$_{a}$	0.485$_{a}$

Means with the same letter are not statistically different at the 0.05 level, according to the least significant difference LSD.

In both seasons, leaf peroxidase activities rise significantly in response to salt stress (Fig. 3). For both rootstocks, nano chitosan treatment resulted in the maximum peroxidase activity over both growing seasons. Volkamer lemon rootstock performed better than sour orange rootstock when subjected to salt stress. The Volkamer lemon rootstock, however, showed substantial growth after being exposed to any of the nanomaterial treatments.

Table (4): Means of leaf sodium content (Na) leaf chloride content (Cl) of sour orange and Volkamer lemon during 2020 and 2021 seasons in response to foliar normal, nano-sized silicon, normal and nano-sized chitosan treatments under salinity stress conditions.

Rootstock	Salinity Levels (mM)	Treatments	Na%		Cl%	
			2020	**2021**	**2020**	**2021**
sour orange	**0**	**Control**	0.241_a	0.249_a	0.663_a	0.667_a
	50		0.752_a	0.754_a	0.945_a	0.949_a
	0	**Silicon**	0.221_c	0.238_c	0.544_c	0.546_b
	50		0.430_c	0.437_c	0.669_c	0.679_b
	0	**Nano-Si**	0.208_e	0.212_e	0.434_e	0.429_d
	50		0.316_e	0.332_e	0.527_e	0.521_d
	0	**Chitosan**	0.245_b	0.247_b	0.656_b	0.615_b
	50		0.552_b	0.561_b	0.646_b	0.628_b
	0	**Nano-chi**	0.232_d	0.240_d	0.612_d	0.605_c
	50		0.435_d	0.452_d	0.569_d	0.565_c
Volkamer lemon	**0**	**Control**	0.233_a	0.238_a	0.639_a	0.637_a
	50		0.727_a	0.740_a	0.971_a	0.966_a
	0	**Silicon**	0.218_c	0.226_c	0.546_c	0.540_b
	50		0.522_c	0.534_c	0.659_c	0.662_b
	0	**Nano-Si**	0.202_e	0.212_e	0.428_e	0.411_d
	50		0.425_e	0.434_e	0.479_e	0.491_d
	0	**Chitosan**	0.232_b	0.240_b	0.645_b	0.607_b
	50		0.553_b	0.415_b	0.932_b	0.571_b
	0	**Nano-chi**	0.218_d	0.223_d	0.570_d	0.527_c
	50		0.413_d	0.430_d	0.431_d	$.453_c$

Means with the same letter are not significantly different according to the least significant difference LSD at 0.05 levels.

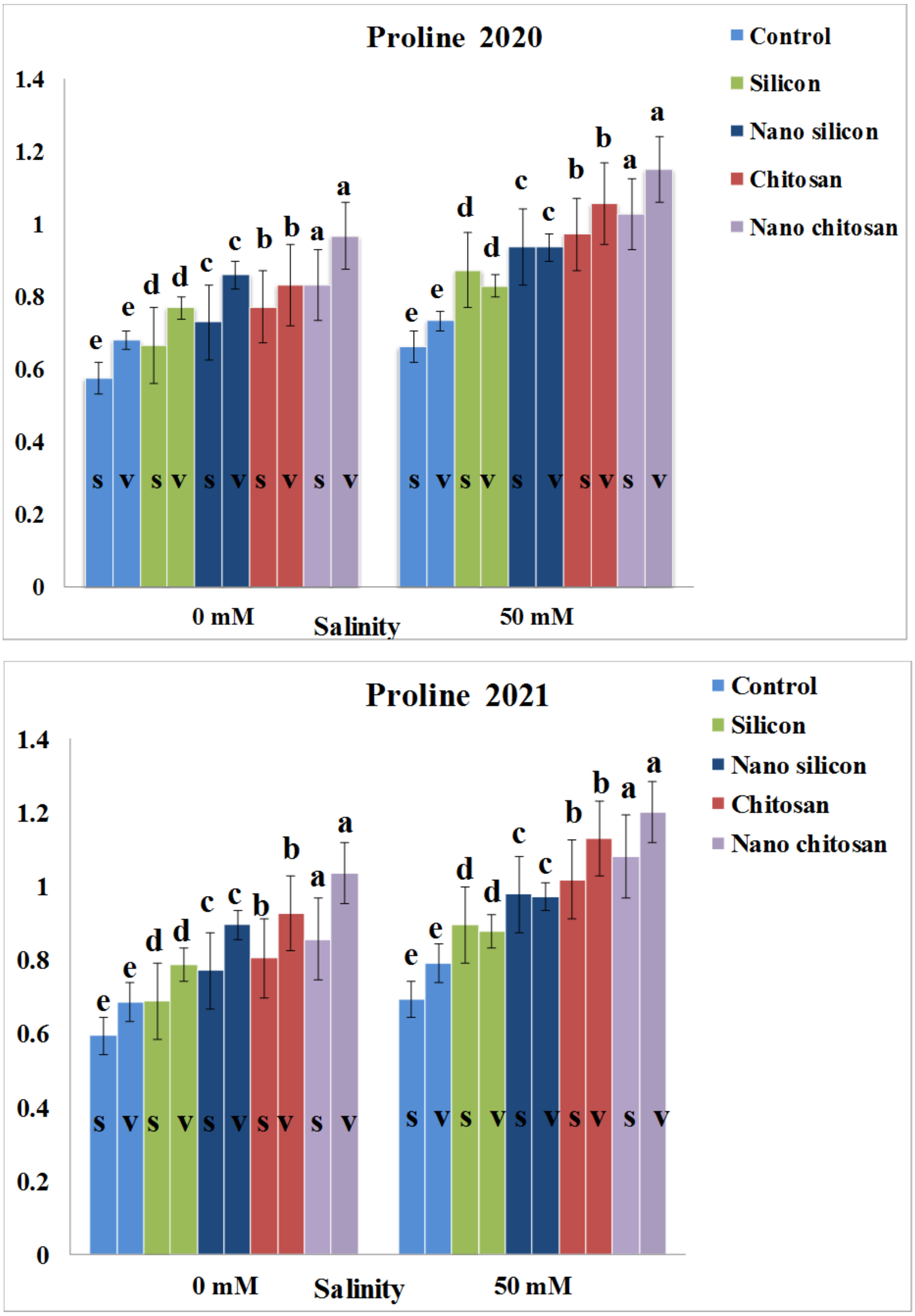

Figure (2): Impact of (CS NPs), SiO2 NPs, foliar normal silicon, and chitosan on proline content of sour orange and Volkamer lemon during 2020 and 2021 seasons under salinity stress conditions. According to the least significant difference LSD, means that share the same letter are not different from one another at the 0.05 level of significance. Vertical bars indicate the mean ± SE.

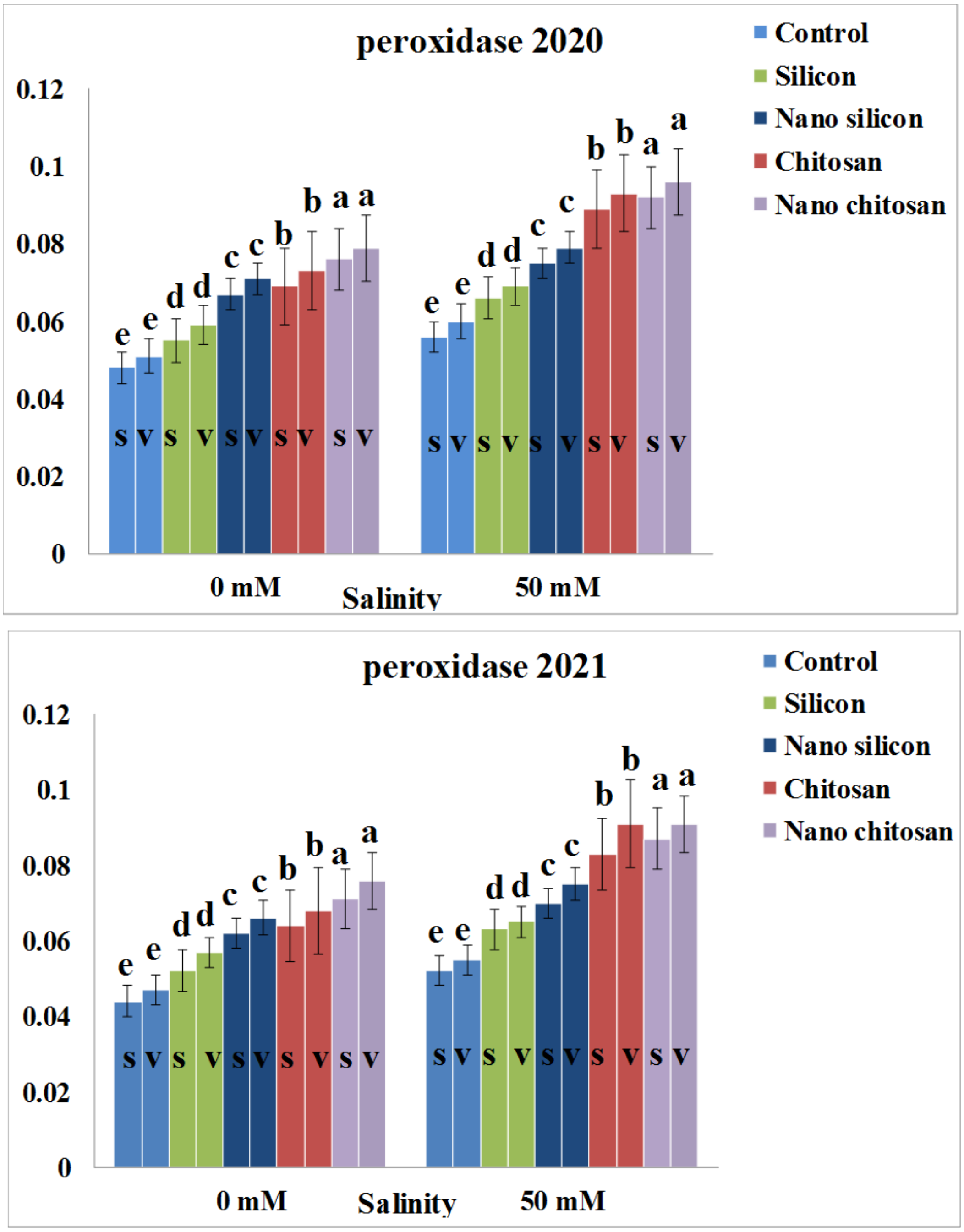

Fig. 3. Effect of CS NPs, SiO2 NPs, foliar normal silicon, and chitosan on peroxidase of sour orange and Volkamer lemon during 2020 and 2021 seasons under salinity stress conditions. The Least Significant Difference (LSD) at the 0.05 level indicates that means sharing a letter do not vary statistically. The vertical bars represent the mean ± SE.

Figure (4): Phenotypic effects (CS NPs), SiO2 NPs, foliar normal silicon and chitosan on sour orange and Volkamer lemon during 2020 season. a. Volkamer lemon with 0 mM NaCl; b. Volkamer lemon with 50 mM NaCl; c. sour orange with 0 mM NaCl; d, sour orange with 50 mM NaCl.

Plants are profoundly affected by salinity stress. While several studies have considered how salinity and silicon (Si) interact in higher plants, few investigations are introduced how Nano-silicon treatment can help reduce salt stress damage in citrus rootstocks. In this analysis, we compared the effectiveness of CS NPs and SiO2 NPs in protecting citrus rootstocks from the harmful effects of salt stress. Growth rate, leaf area, stem diameter, number of growths, root, and plant fresh weight, and root and plant dry weight were all negatively impacted by salinity in the present research (Tables 1 and 2). Zayed et al., 2017 reported that plants with nano-chitosan under salinity stress led to high significant increase in value of salt tolerance index for all growth indices (plant height, leaf area and fresh and dry weight of shoot and root) [42]. It can be attributed to a drop in N, P, and K content with increasing leaf salt content (Tables 3 and 4). Reduced plant growth and morphology are a result of salt stress's influence on photosynthesis, antioxidant responses, proline metabolism, and osmolyte build up [43,44]. Several pathways are triggered in plants in response to saline stress, making them more resistant to the effects of salt .

Increased reactive oxygen species (ROS) scavenging has been identified as one of the most prominent indicators of oxidation caused by saline stress [45,46]. This is facilitated by pathways including the upregulation of certain osmotic substances like proline and the continued upregulation of osmotic pressure and interactions of cells' oxygen-oxidizing [47]. Which agrees with the current investigation results (Table 3 and Figures 2 and 3). Numerous previous studies have examined whether or not SiO2 may mitigate oxidative damage in plants [48,49,50]. Previous works has shown that silicon is essential for maintaining the integrity of the cell wall and reducing cellular damage and toxicity in a variety of crops, including bananas [28,51] and strawberries, sunflowers, maize, and grapes [52,53,54,55,56]. The role of SiO2 in citrus has only been studied by a few researchers thus far. To further understand how SiO2 NPs mitigate salt stress on citrus rootstocks, we conducted the present investigation. After being watered with NaCl solution, citrus rootstocks decreased their mineral content dramatically. Under conditions of osmotic stress, plant water absorption decreases drastically. Various cellular processes, including metabolic rate, cellular development, and stomatal activity, are affected by these shifts in water balance [57]. Additionally, Na+ and Cl- toxicity hinders citrus plants' capacity to absorb carbon dioxide, severely slowing photosynthesis and disturbing the electron transport chain (Piero, 2020). Salt accumulation in the cytoplasm may inhibit glucose-metabolizing enzymes, much as it does in the chloroplasts, where it can slow down photosynthesis. In addition, dehydration of cells may result from salt build up in the apoplast.[58]

Our findings demonstrate that citrus rootstocks' vegetative growth rate, fresh and dry weight for both root and plant, N, P, K, Ca, and Mg are all significantly increased after acquiring a foliar spray containing SiO2 NP and CS NPs, confirming nano-treatment results in reduced salt-induced osmotic stress. Applying the SiO2 NPs influences the activity of antioxidant enzymes [22,27,59] and nonenzymatic antioxidants [60]. Redox homeostasis is also controlled by silica [57]. Our findings reflect that the SiO2 NPs treatment increased the total antioxidant capacity and controlled plants' total soluble protein level. A cascade of physiological, pharmacological, and transcriptional changes occurs in response to Si, which alleviates salt stress's effects. Protein, lipid, and carbohydrate metabolism, as well as the adsorption and transport of metal ions and the creation of cell walls, are all under its control [57]. Under salt stress, proline functions as an osmolyte in plant cells, reducing oxidative damage and promoting growth [61,62].The proline content revealed a significant decreased after the SiO2 NPs treatment compared to the control treatments. This decline may result from SiO2's inductive influence on other antioxidants, which reduces oxidative stress in the plant cell. After subjecting the leaves to Si treatments, we observed a Na+ and Cl- levels reduction. Among the several mechanisms that contribute to salt tolerance, the regulation of cytosolic Na+ concentration is important. The tolerant plants had a reduced rate of Na+ inflow into the cytosol and a higher rate of Na+ sequestration in the vacuole [58]. Root vacuolar Na+ sequestration under NaCl stress regulates cellular water potential and promotes water uptake by hairy roots [63]. Application of CS NPs reflected in more plant growth than that was observed in the control group. Chitosan's ability to activate some hormone signalling pathways, including those for auxin and gibberellins [64], as well as its regulation of several metabolic processes, likely account for its growth-promoting activity. When it comes to protecting themselves from damage caused by reactive oxygen species (ROS), plants have developed many different defensive mechanisms, one of the most notable being the metabolism of antioxidants [65]. In the present investigation, salt boosted the antioxidant enzyme peroxidase activity, and CS NPs treatment boosted it even more (Fig. 3). Enzymatic antioxidants are essential to plant responses against numerous abiotic stressors due to their principal function as scavengers of reactive oxygen species (ROS) [66,67,68]. While peroxidase activity was likely elevated in salinity-stressed plants, this may not have been enough to shield the plants from ROS and prevent the negative consequences of the salt. In this experiment, CS NPs administration also caused significant peroxidase activity.

CONCLUSIONS

The study of nanotechnology, which is still relatively new and novel, has a lot of potential for lessening biotic and abiotic plant stress. In the present study, the beneficial role of nano-silicon and nano-chitosan application, in alleviating the adverse effect of salinity stress on osmotic adjustment and antioxidant systems in citrus rootstock is well not documented. Our research on plants exposed to NaCl stress conditions revealed that nanomaterials have a key role in regulating several physiological functions and morphology. Plants' ability to absorb nitrogen, phosphorus, potassium, calcium, and magnesium from nanomaterials improved dramatically. However, under salt stress, the leaves Na+ and Cl- content increased dramatically. The recent research helped shed light on the precise function of nanomaterials in promoting plant development despite salt stress. This information may be useful in finding ways to enhance citrus plant development when under salt stress. The present research results should be relevant in formulating optimal management strategies to improve citrus' resistance to stress.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

تأثير النانو السيليكون والنانو الشيتوزان على النمو ، ومحتوى الأيونات ، والإنزيم الدفاعي المضاد للأكسدة لاثنين من جذور الموالح تحت ظروف الملوحة

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الخلاصة

ان دراسة تقنية النانو ، والتي لا تزال واعده ولديها الكثير من الإمكانات لتقليل إجهاد النبات الحيوي وغير الحيوي. ، حتى الان لم يتم التطرق الى الدورالمفيد لتطبيق النانو شيتوزان والنانو سيليكا في التخفيف من التأثير الضار لإجهاد الملوحة في اصول الموالح. اظهر بحثنا على النباتات المعرضة لظروف إجهاد كلوريد الصوديوم أن المواد النانوية لها دور رئيسي في تنظيم العديد من الوظائف الفسيولوجية والمورفولوجية. تحسنت قدرة النباتات على امتصاص النيتروجين والفوسفور والبوتاسيوم والكالسيوم والمغنيسيوم من المواد النانوية بشكل كبير. ومع ذلك ، تحت ضغط الملح ، زاد محتوى الأوراق Na و Cl بشكل كبير. ساعد البحث في إلقاء الضوء على الوظيفة الدقيقة للمواد النانوية في تعزيز النبات على

الرغم من إجهاد الملح. قد تكون هذه المعلومات مفيدة في إيجاد طرق لتعزيز نمو نباتات الموالح تحت ضغط الملح. يجب أن تكون نتائج البحث الحالية ذات صلة في صياغة استراتيجيات الإدارة المثلى لتحسين مقاومة الموالح للإجهاد.

الكلمات المفتاحية: النانو سيليكون ،النانو شيتوزان ،النمو ،إجهاد الملوحة ، أصول الموالح

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