

Chitosan and Soluble Calcium Effects on Plant Systems under Salinity Stress

Elsaka, M. S.; H. M. Aboelsoud and T. H. Khalifa

Soils, Water & Environment Research Institute, Agric. Res. Centre (ARC), Giza, Egypt



ABSTRACT

Soil salinity is one of the important environmental factors that impair the agricultural production, while the application of some amendments such as soluble calcium alleviates the adverse effect of salinity on plant growth. The objective of the present study was to evaluate the interactive effect of soil application of soluble calcium (sol. Ca) (2 or 4L/fed) and foliar application of chitosan (CHT) (0.5 or 1L/fed) on maize productivity, NPK absorption and some soil properties under salt stress (8.27 dS/m for soil and 2.9 dS/m for irrigation water). The experiment was carried out at Sakha Agric. Res. St. Lysimeter, Kafrelsheikh governorate, Egypt, during summer season (2018). The obtained results proved that these amendments individually or in combination markedly improved the growth and yield of maize (plant height, grain filling, and cobs and straw yields), enhanced N, P and K concentration (in grain and straw) and ameliorated some chemical properties of salt affected soils (ECe, SARe and ESP). It was, therefore, found that the application of higher levels of sol. Ca and CHT in combination significantly affected the plant growth and caused the highest increases in plant height (65.1%), 100- grain weight (112.2%), cobs and straw yields (168.4 and 111.6%, respectively). In addition, this combination achieved the highest NPK contents in grain (3.51, 0.33 and 1.08 %, respectively) and straw (1.73, 0.14 and 1.62 %, respectively). Also, the application of sol. Ca and CHT as well as in combination slightly decreased soil ECe, SARe and ESP values. In general, soil application of sol. Ca and foliar application of CHT ameliorated the adverse effect of salinity on maize productivity, and the combined application of them was more positive effective on plant growth under salt stress.

INTRODUCTION

Application of new technologies have resulted in rapid advances in the agriculture, and made it possible to achieve good crop production. However, for continuation of advances in agricultural productivity, more environmental friendly production technologies must be followed. Some environmental-friendly products that have been widely used in the agricultural applications are for stimulation of plant defenses (Yu and Meuhlbauser 2001). Therefore, Katiyar, *et al* (2015) concluded that chitosan (CHT) enhances the efficacy of plants to reduce the deleterious effect of unfavorable biotic and abiotic stresses conditions, and enhances plant's productivity through defense mechanisms involving various enzymes.

CHT is obtained by de-acetylation of chitin, the most abundant natural amino polysaccharide, its molecular weight ranges between 5×10^4 and 2×10^6 Da and it is available commercially with molecular weight ranging from 10,000 to 1,000,000 Da (Chawla, *et al*, 2014).

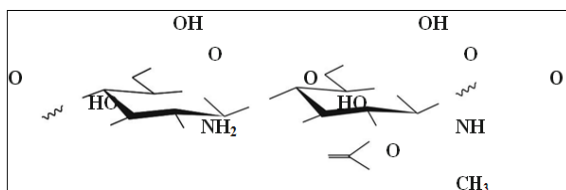


Fig. 1. Chemical structure of chitosan

Also, CHT can be extracted from insects, yeast, mushroom, cell wall of fungi, and marine shellfish such as crab, lobster, krill, cuttlefish, shrimp and squid pens (Kurita K (2006). Malerba and Cerana (2016) added that CHT, the natural, safe, and cheap product of chitin deacetylation, has been proven to stimulate plant growth and induce abiotic and biotic stress tolerance due to its effect on different enzyme activities. In addition, CHT has a potent protective effect on plants such as grain crops, fruits, vegetables and trees (Zhao *et al*. 2009). Also, foliar application of CHT enhanced fruit weight and productivity of tomato (Sathiyabama, *et al*, 2014), fruit yield, plant height, and leaf number of okra (Mondal, *et al*, 2012) and spike number and grains per spike of wheat (Wang, *et al*, 2015). CHT extracted from natural chitin has been used in agriculture as plant growth promoter (Katiyar *et al*, 2014). On the other hand, Ngo, *et al* (2015) reported that

the chitosan is a natural nontoxic biopolymer produced by alkaline de-acetylation of chitin; it is insoluble in water as well as most organic solvents and it possessed diverse biological activities, including antioxidant.

Salt stress is one of the most brutal environmental factors and the abiotic stresses; it is a major factor which reduces crop productivity and nano chitosan has unique physicochemical properties (Zayed, *et al.*, 2017). So, CHT increased root and shoot length, shoot dry weight, relative water content of ajowan under salinity stress (Mahdavi and Rahimi, 2013). Also, Ma *et al.* (2011) reported that wheat seeds which treated with CHT showed higher growth than control under salinity stress. Farouk, *et al* (2011) reported that CHT as soil addition increased plant growth and yield of radish through improving some physiological processes. Moreover, application of chitosan assisted in conserving water in the plants by closing the stomata and decreasing transpiration, hence increasing relative water content in the leaves (Bitteli *et al.*, 2001). Also, Khan, *et al* (2002) observed that foliar application of chitosan pentamer (CH5) caused an increase in maize photosynthetic rate due to increases in stomatal conductance and transpiration rate, while it did not affect maize height, root length, leaf area, shoot or root dry mass. Foliar application of CHT enhanced fruit weight and productivity of tomato (Sathiyabama, *et al.*, 2014), plant growth of Greek Oregano (Yin, *et al*, 2012) and biomass accumulation of ocimum (Mathew and Sankar, 2012). Also, the soil addition of CHT increased height, canopy diameter, and leaf area of capsicum (Chookhongkha, *et al*, 2012).

At present, the plants cannot absorb about 80-90% of P, 40-70% of N, and 50% -70% of K contained in fertilizers, leading to waste of money and severe environmental pollution (Sun, *et al*, 2012). Consequently, CHT-based polymers may be the solution (Davidson and Gu, 2012). In this context, Agbodjato, *et al* (2016) found that the combination of chitosan and plant growth promoting rhizobacteria can be used as biological fertilizers to increase production and nitrogen content of maize. Nanoparticles composed by an inner coating of CHT, an outer coating of urea and a core of water-soluble granular NPK fertilizers showed slow-controlled release of the nutrients without any detrimental impact on the soil (Wu, *et al*, 2008).

Soil salinity is one of the most serious environmental threats for plant survival and crop yield and it covers 19.5%

of irrigated land and 2.1% dry land agriculture across the globe (FAO, 2000). Considerable interest has been focused on calcium (Ca) due to its ability to induce a protective effect on plants under adverse environmental conditions. Ca is an essential plant nutrient that determines the structure of cell wall and membranes, and plays as a role regulator of plant growth and development (Hepler, 2005). Ca plays a vital role in salt stress tolerance (Khan *et al.* 2010) and also induces antioxidant enzyme activities and reduces lipid peroxidation of cell membranes under abiotic stress (Jiang and Huang 2001). Ca also stabilizes cell membrane surfaces, influences the pH of cells and prevents solute leakage from a cytoplasm (Hirschi 2004). It is a counteraction for inorganic and organic anions in the vacuole, and the cytosolic Ca concentration is an obligate intracellular messenger coordinating responses to numerous developmental cues and environmental challenges (White and Broadley 2003).

The external Ca may interfere with cellular Ca and affect osmotic adjustment of cell under stress conditions. It is, also, required for various structural roles in the cell wall and membranes. However, the application of Ca markedly improved plant growth, photo-synthetic pigments and activities of antioxidant enzymes peroxidase under salt stress (Al-Whaibi, *et al.* 2012). Oh, *et al.* (2014) found that the increase in the exogenous calcium levels enhanced soybean root elongation and suppressed the cell death in root tip under flooding stress. They suggest that calcium might affect the cell wall/hormone metabolisms, protein degradation/synthesis, and DNA synthesis in roots under flooding stress. Also, the exogenous calcium alleviated the suppression of plant growth, maintain it and modulate cellular function by relieving gene repression under stresses of salt (Henriksson and Nordin Henriksson, 2005). Also, calcium improves tolerance to short-term hypoxia by calcium-mediated reduction of polyamine degradation, elevation of nitrate uptake, and accelerated synthesis of heat-stable proteins and polyamines was reported in muskmelon roots (Gao *et al.*, 2011). In addition, calcium has functions in protecting the integrity of cell membranes, reducing membrane permeability and preventing ionic leakage caused by biotic and abiotic stresses (Lin *et al.*, 2008). Khan, *et al.* (2010) showed that application of CaCl₂ and gibberellic acid alone as well as in combination mitigated the adverse effect of salinity, but combined application of these treatments proved more effective in alleviating the adverse effects of salinity stress because they appear to confer greater osmoprotection. Navarro, *et al.* (2000) found that application of Ca²⁺ ameliorated tomato plants grown in saline culture due to its effect on root hydraulic conductivity and increase of NH₄⁺ incorporation into the amino acid synthesis which reduce the negative effect of salinity. Gulser, *et al.* (2010) observed that the applications of calcium nitrate (50 mg kg⁻¹) significantly affected pepper growth and increased its fresh and dry root weight, stem diameter, root length and shoot length in saline soils. Kaya, *et al.* (2002) reported that Ca as CaCl₂ supplied in nutrient solution ameliorated the negative effects of salinity

on growth and fruit yield of strawberry. Also, calcium ameliorated stress-induced damage in some crop species (He *et al.*, 2012). Ca²⁺ sustains K⁺ transport and K⁺/Na⁺ selectivity in Na⁺ challenged plants, while the interaction of Na⁺ and Ca²⁺ on plant growth is well established (Rengel, 1992). This study aims to evaluate the effect of sol. Ca and CHT on maize irrigated by saline water under salt-affected clay soil.

MATERIALS AND METHODS

1. The experimental treatments: The experiments was carried out at Sakha Agric. Res. St. Lysimeter, Kafrelsheikh governorate, Egypt, during summer growing season (2018) to study the effect of soluble calcium (sol. Ca) and chitosan (CHT) on maize irrigated by saline water under salt-affected clay soil (8.27 dS/m) and irrigation by well water (2.9 dS/m). the experiments was located at 31°05'26.4" N latitude and 30°55'30.7" E longitude, with an elevation of about 6 meters above the sea level. The experiment was designed in a split plots with three replicates. Sol. Ca allocated to the main plots and CHT occupied the sub main plots. Soil application of sol. Ca (CaO, 14%) was applied 4 times with irrigation at the rates of zero, 2 and 4 L/fed, while foliar application of CHT (chitosan 5.5% and 15% N) was done three times at 25, 40 and 55 days after sowing at the rate of zero, 0.5 and 1.0 L/fed. Maize (variety hybrid 10) was planted on May, 06th, 2018 and harvested on Aug., 15th, 2018. All recommended agronomic practices were applied uniformly to all plots. The experiment consists of the following treatments:

No	Symbol	Treatments
1	Ca ₀ CHT ₀	zero Calcium + zero Chitosan
2	Ca ₀ CHT ₁	zero Calcium +0.5 L Chitosan/fed
3	Ca ₀ CHT ₂	zero Calcium +1.0 L Chitosan/fed
4	Ca ₁ CHT ₀	2.0 L Calcium/fed+ zero Chitosan
5	Ca ₁ CHT ₁	2.0 L Calcium/fed +0.5 L Chitosan/fed
6	Ca ₁ CHT ₂	2.0 L Calcium/fed+1.0 L Chitosan/fed
7	Ca ₂ CHT ₀	4.0 L Calcium/fed+ zero Chitosan
8	Ca ₂ CHT ₁	4.0 L Calcium/fed+0.5 L Chitosan/fed
9	Ca ₂ CHT ₂	4.0 L Calcium/fed+1.0 L Chitosan/fed

Plant height was measured; 100-grain weight, cobs and straw yields were determined at maturity stage. NPK contents in maize grain and straw were determined using wet digestion method (H₂SO₄+ H₂O₂) according to Peterburgski (1968) and Jackson (1973). Available N in soil was determined using K₂SO₄ (1N) according to Jackson (1973). Also, available P and K in soil were extracted by ammonium bicarbonate- DTPA and determined according to Soltanpour (1985). The obtained data were analyzed statistically using analysis of variance according to Gomez and Gomez (1984). Treatment means were compared using the least significant difference test (LSD) at 0.05 and 0.01 levels. Representative soil samples before planting from 0-15, 15-30 and 30-45 cm depths were subjected to chemical and physical analysis according to Black *et al.*, (1983) as shown in Tables (1-3).

Table 1. Some physical and soil moisture characteristics of soil before experiment

Soil depth (cm)	Mechanical analysis (%)			Texture grade	OM %	Total CaCO ₃ (%)	C.E.C. meq/100 g	Field capacity (%)	Wilting point (%)	Available water (%)	Bulk density (g/cm ³)
	Sand	Silt	Clay								
0-15	19.00	33.00	48	clayey	1.25	2.42	40.9	41.9	20.1	21.8	1.20
15-30	18.59	31.41	50	clayey	1.16	2.31	38.2	39.0	21.2	17.8	1.28
30-45	18.52	30.48	51	clayey	1.04	2.24	36.7	37.0	22.7	14.2	1.29
Mean	18.70	31.63	49.67	clayey	1.15	2.32	38.6	39.3	21.3	17.9	1.26

Table 2. Chemical analysis of soil paste extract before experiment.

Depth (cm)	pH	EC _e dS m ⁻¹	SAR	Soluble cations (meq L ⁻¹)				Soluble anions (meq L ⁻¹)			
				Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	CO ₃ ^{-z}	HCO ₃ ⁻	Cl ⁻	SO ₄ ^{-z}
0-15	8.27	10.02	15.7	64.8	1.2	22.0	12.0	0.0	5.5	55.0	39.5
15-30	8.49	7.51	14.4	52.2	0.7	15.9	10.4	0.0	3.5	54.8	20.9
30-45	8.38	7.29	14.3	50.2	1.1	16.0	8.8	0.0	4.5	43.1	28.5
Mean		8.27	14.8	55.7	1.0	18.0	10.4	0.0	4.5	50.9	29.6

Table 3. Available NPK in soil before experiment.

Depth (cm)	Available (mg kg ⁻¹)		
	N	P	K
0-15	19.9	8.9	289.6
15-30	17.8	9.2	264.1
30-45	15.4	9.4	259.6
Average	17.7	9.2	271.1

RESULTS AND DISCUSSION

1. Effect of soluble calcium and chitozan on maize growth and productivity:

Plant height and 100-grain weight:

The statistical analysis of data shown in Tables (4 and 5) revealed that the presence of both of soluble calcium (sol. Ca) and chitosan (CHT) in the growth medium significantly increased plant height and 100-grain weight when compared with the control. Where, the application of sol. Ca and/or CHT had a clear stimulating effect on plant height and grain filling. The plant height was increased from 184 cm in the control plots to 222.7 cm or 240 cm (49.4 or 61.1 % increase) as a result of application of 2 or 4 L sol. Ca/fed, respectively. Also, the weight of 100 grains was increased from 25.1 g in check plots to 37.1 or 40.4 g (47.9 or 61.1 % increase) with application of 2 or 4 L sol. Ca/fed, respectively. These results are in somewhat harmony with those obtained by Gulser, *et al* (2010) who observed that the applications of calcium nitrate significantly affected root and shoot length of pepper under the saline soil condition.

Table 4. The increase of plant height and 100-grain weight as response to sol. Ca and CHT.

Parameters	Plant height (cm)			100-grain weight (g)				
	Treatments Chitosan, B (L fed ⁻¹)			Treatments Chitosan, B (L fed ⁻¹)				
Sol. Ca (A)	Without	0.5	1.0	Mean	Without	0.5	1.0	Mean
Without	32.9	37.6			25.0	42.0		
2 L fed ⁻¹	41.6	53.0	53.7	49.4	64.4	87.8	91.2	47.9
4 L fed ⁻¹	55.0	63.1	65.1	61.1	77.1	101.5	112.2	61.1
Mean	49.7	52.1			16.4	23.4		

Regarding to the effect of the CHT on both parameters, the results indicated that the plant height clearly increased from 197 cm in the untreated plants cm to 223 or 226.7 cm (49.7 or 52.1 % increase) with the treated plants by 0.5 or 1.0 L CHT/fed, respectively. On the other hand, the weight of 100 grains was increased from 30.2 g to 35.1 or 37.3 g (16.4 or 23.4 % increase) as a response to the application of 0.5 or 1.0 L CHT/fed, respectively. The increase of plant growth might be due to stability of cell walls and enhanced activity of some enzymes with CHT application. These results are in agreement with findings of Mondal, *et al* (2012), Chookhongkha, *et al* (2012) and Mahdavi and Rahimi (2013) who reported that the addition of CHT increased the plant height and leaf area.

The results showed also positive significant effects on the vegetative growth and grain filling due to the interaction between soil. Ca and CHT. The application of sol. Ca combined with CHT alleviated the adverse effect of

salinity on plant height and the grain filling when compared with the individual application of them. Therefore, the application of 4 L sol. Ca in addition to 1.0 L CHT /fed achieved the tallest maize plants (246 cm) and the most filled grains (43.5 g/100 grains), while the shortest maize plants (149 cm) and the lowest 100-grain weight (20.5 g) were recorded with the untreated plants.

Table 5. Effect of sol. Ca and CHT on plant height and 100-grain weight

Parameters	Plant height (cm)				100 grains weight (g)			
	Treatments Chitosan, B (L/fed)				Treatments Chitosan, B (L/fed)			
Sol. Ca (A)	Without	0.5	1.0	mean	Without	0.5	1.0	mean
Without	149	198	205	184	20.5	25.6	29.1	25.1
2 L fed ⁻¹	211	228	229	222.7	33.7	38.5	39.2	37.1
4 L fed ⁻¹	231	243	246	240	36.3	41.3	43.5	40.4
mean	197	223	226.7		30.2	35.1	37.3	
(A) F-test & LSD	**				**			
	LSD _{0.05} = 3.74				LSD _{0.05} = 0.936			
	LSD _{0.01} = 6.20				LSD _{0.01} = 1.552			
(B) F-test & LSD	**				**			
	LSD _{0.05} = 3.70				LSD _{0.05} = 0.515			
	LSD _{0.01} = 5.18				LSD _{0.01} = 0.854			
(AxB) F-test & LSD	**				**			
	LSD _{0.05} = 3.73				LSD _{0.05} = 0.657			
	LSD _{0.01} = 6.20				LSD _{0.01} = 1.090			

Maize yield:

The obtained results showed that the plants supplemented by the sol. Ca and /or CHT exhibited an enhancement of its growth. The statistical analysis of the data in Tables (6 and 7) proved that the cobs and straw yields were markedly increased as response to the application of sol. Ca and/or CHT comparing to the check plots. The application of 2 or 4 L sol. Ca /fed increased the cobs yield by 56.8 or 72.8 %, respectively over the control. The corresponding increases in straw yield with both Ca application levels were 44.2 and 59.9%, respectively comparing to that produced from the untreated plots. The positive effect of Ca on maize productivity may be related to that it is an essential nutrient for plant that determines the structure of cell wall and membrane, stabilizes cell membrane surface, influences the pH of cells and plays a role in growth development (Hepler, 2005 and Hirschi 2004). Also, Ca improves plant growth through the enhancement of its photo-synthetic pigments and activates of the antioxidant enzymes under salt stress (Al-Whaibi, *et al*, 2012), Gulser, *et al* (2010) and Navarro, *et al* (2000) or it increases the plant tolerance to salinity stress (Khan *et al.*, 2010 and Gao *et al.*, 2011).

On the other hand, the cobs yield showed 16.0 % or 32.7% increases, respectively over the control due to application of 0.5 or 1.0 L CHT/fed, respectively comparing to that produced from the check plots. Also, the straw yield was increased by 14.0 or 32.8 % in the plots treated by 0.5 or 1.0 L CHT/fed, respectively, over that recorded in the untreated plots. These results may be attributed to that CHT causes an enhancement in maize photosynthetic rate through an activates of some enzymes and through some

physiological processes (Khan, *et al*, 2002 and Farouk, *et al*, 2011) or due to the increases in the stomatal conductance and transpiration rate (Katiyar, *et al*, 2015).

Table 6. The increase of cobs and straw yields as response to the sol. Ca and CHT.

Parameters	Cobs yield increase (%)				Straw yield increase (%)			
	Without		1.0		Without		1.0	
Treatments	Chitosan, B (L fed ⁻¹)				Chitosan, B (L fed ⁻¹)			
Sol. Ca (A)	Without	0.5	1.0	Mean	Without	0.5	1.0	Mean
Without		54.4	75.4		17.4	45.3		
2 L fed ⁻¹	98.2	115.8	154.4	56.8	40.7	76.7	103.5	44.2
4 L fed ⁻¹	128.1	140.4	168.4	72.8	81.4	87.2	111.6	59.9
Mean		16.0	32.7		14.0	32.8		

Table 7. Effect of sol. Ca and CHT on maize productivity.

Parameters	Cobs yield (kg plot ⁻¹)				Straw yield (kg plot ⁻¹)			
	Without		1.0		Without		1.0	
Treatments	CHT, B (L fed ⁻¹)				CHT, B (L fed ⁻¹)			
Sol. Ca (A)	Without	0.5	1.0	Mean	Without	0.5	1.0	Mean
Without	0.57	0.88	1.00	0.81	0.86	1.01	1.25	1.04
2 L fed ⁻¹	1.13	1.23	1.45	1.27	1.21	1.52	1.75	1.50
4 L fed ⁻¹	1.30	1.37	1.53	1.40	1.56	1.61	1.82	1.66
Mean	1.00	1.16	1.33		1.21	1.38	1.61	
(A) F-test & LSD	** LSD _{0.05} = 0.120 LSD _{0.01} = 0.182				** LSD _{0.05} = 0.122 LSD _{0.01} = 0.203			
(B) F-test & LSD	** LSD _{0.05} = 0.073 LSD _{0.01} = 0.102				** LSD _{0.05} = 0.094 LSD _{0.01} = 0.132			
(AxB) F-test & LSD	* LSD _{0.05} = 0.120 LSD _{0.01} = 0.182				ns LSD _{0.05} = 0.120 LSD _{0.01} = 0.182			

The plants supplemented by sol. Ca + CHT exhibited a significant enhancement of its productivity compared to that supplemented by one of them alone or the untreated plants. Therefore, the statistical analysis showed that the positive enhancement degree of sol. Ca combined with CHT on maize productivity was greater than one of them alone. So, the highest cobs yield (1.53 kg plot⁻¹ with 168.4 % increase) and straw yield (1.82 kg plot⁻¹ with 111.6 % increase) were

achieved due the application of 4 L sol. Ca with 1.0 L CHT/fed , comparing to that produced from the untreated plots (0.57 and 0.86 kg plot⁻¹, respectively).

Grain and straw quality:

The effect of sol. Ca and CHT on grain and straw quality are presented in Table (8-9). It is evident from the results that the presence of sol. Ca and CHT in the growth medium enhanced the accumulation of N, P and K in maize grain and straw when compared with their respective control plots.

a- NPK contents in the grain:

It is evident from the results (Table 8) that NPK concentrations in grain were significantly increased as a result of application of sol. Ca comparing to that in the untreated plants. The concentration of N was increased from 2.18 % in untreated plants to 3.04 or 3.21 % in plants treated by 2 or 4 L sol. Ca /fed, respectively. Also, both Ca application rates increased P concentration levels in grain from 0.12 % in check plots to 0.26 or 0.29 %, respectively. In the same direction, K concentration was increased in grain from 0.68 % to 0.96 or 1.01 % with both Ca application levels, respectively.

In regard to the effect of CHT, the application of 0.5 or 1 L CHT/fed increased the concentration of N in grain to 2.79 or 3.08%, respectively comparing to that in the untreated plants (2.55%). The corresponding concentrations of P in grain with both CHT application rates were 0.23 or 0.27%, respectively comparing to the control (0.16%). In case of K, its concentrations in grain were 0.91 or 0.97 % with the two application rates of CHT, respectively, while the concentration in the check plots was 0.77%.

Also, the quality of maize grain significantly affected by the interaction of sol. Ca with CHT treatments according to the statistical analysis. Meanwhile, the highest concentration of N, P and K contents (3.51, 0.33 and 1.08 %, respectively) were achieved in maize grains due to the application of 4 L sol. Ca combined with 1 L CHT/fed. On the other side, the lowest N, P and K contents (1.92, 0.05 and 0.48 %, respectively) were recorded in grains produced from the untreated plants.

Table 8. Effect of soluble Ca and chitosan on N, P and K concentration in maize grain.

Parameters	N %				P %				K %			
	Chitosan, B (L fed ⁻¹)				Chitosan, B (L fed ⁻¹)				Chitosan, B (L fed ⁻¹)			
	without	0.5	1.0	mean	without	0.5	1.0	mean	without	0.5	1.0	mean
Sol. Ca (A)												
without	1.92	2.08	2.53	2.18	0.05	0.12	0.18	0.12	0.48	0.72	0.84	0.68
2 L fed ⁻¹	2.84	3.07	3.20	3.04	0.22	0.26	0.29	0.26	0.94	0.95	1.00	0.96
4 L fed ⁻¹	2.89	3.23	3.51	3.21	0.22	0.32	0.33	0.29	0.89	1.06	1.08	1.01
mean	2.55	2.79	3.08		0.16	0.23	0.27		0.77	0.91	0.97	
(A) F-test & LSD	** LSD _{0.05} = 0.164 LSD _{0.01} = 0.272				** LSD _{0.05} = 0.024 LSD _{0.01} = 0.040				** LSD _{0.05} = 0.046 LSD _{0.01} = 0.076			
(B) F-test & LSD	** LSD _{0.05} = 0.072 LSD _{0.01} = 0.119				** LSD _{0.05} = 0.009 LSD _{0.01} = 0.013				** LSD _{0.05} = 0.047 LSD _{0.01} = 0.078			
(AxB) F-test & LSD	** LSD _{0.05} = 0.164 LSD _{0.01} = 0.272				** LSD _{0.05} = 0.024 LSD _{0.01} = 0.040				** LSD _{0.05} = 0.046 LSD _{0.01} = 0.076			

b- NPK contents in the straw:

The results showed that N, P and K contents in straw (Table 9) significantly increased as a response of supplemented the plants by sol. Ca and/or CHT comparing to the untreated plants. Concerning the impact of sol. Ca, the

application of 2 or 4 L sol. Ca /fed led to increase of N concentration in straw from 0.82 % in untreated plants to 1.52 or 1.63 %, respectively. Also, P concentrations were increased with both sol. Ca application levels from 0.01 % in untreated plots to 0.12 or 0.13%, respectively. In addition, K

concentration was increased from 1.06% in untreated plants to 1.51 or 1.57% with both Ca application levels, respectively. These results may be attributed to that the presence of sol. Ca in growth medium enhances the absorption of different nutrients such as potassium (Rengel, 1992) and nitrogen (Gao *et al.*, 2011).

In regard to the effect of CHT on NPK contents, the application of 0.5 or 1 L CHT/fed increased N concentration in straw to 1.34 or 1.47%, respectively comparing to the untreated plants (1.17%). Also, the concentration of P were increased to 0.09 or 0.10%, respectively with both CHT levels comparing to that in plants without CHT treatment (0.08%). The corresponding K concentrations with both CHT levels were 1.42 or 1.47%, respectively, while its concentration in check plants was 1.24%. This behavior may be related to that CHT increases the fertilizers use efficiencies

by plant (Davidson and Gu, 2012) which are about 40–70% for N, 80-90% for P and 50-70% for K contained in fertilizers (Sun, *et al.*, 2012). Also, CHT can be used as biological fertilizers according to Agbodjato, *et al.* (2016) leading to increase the production and N content in maize.

Also, significant impacts of the interaction between sol. Ca and CHT treatments on the quality of maize straw were found according to the statistical test. The application of sol. Ca combined with CHT clearly enhanced the absorption of N, P and K by maize plant comparing with the application of sol. Ca or CHT alone. Therefore, the highest N, P and K contents in straw (1.73, 0.14 and 1.62%, respectively) were achieved in maize straw from the plots received 4 L sol. Ca with 1 L CHT/fed., while the lowest contents (0.56, 0.01 and 0.85%, respectively) were recorded in straw of plants grown in the control plots.

Table 9. Effect of sol. Ca and CHT on N, P and K concentration in maize straw.

Parameters Treatments Sol.Ca (A)	N %				P %				K %			
	Chitosan, B (L fed ⁻¹)				Chitosan, B (L fed ⁻¹)				Chitosan, B (L fed ⁻¹)			
	without	0.5	1.0	mean	without	0.5	1.0	mean	without	0.5	1.0	mean
without	0.56	0.86	1.05	0.82	0.01	0.01	0.01	0.01	0.85	1.12	1.22	1.06
2 L fed ⁻¹	1.45	1.50	1.62	1.52	0.10	0.12	0.13	0.12	1.41	1.52	1.59	1.51
4 L fed ⁻¹	1.50	1.66	1.73	1.63	0.12	0.13	0.14	0.13	1.47	1.62	1.62	1.57
mean	1.17	1.34	1.47		0.08	0.09	0.10		1.24	1.42	1.47	
(A) F-test & LSD	** LSD _{0.05} = 0.067 LSD _{0.01} = 0.111				** LSD _{0.05} = 0.005 LSD _{0.01} = 0.008				** LSD _{0.05} = 0.088 LSD _{0.01} = 0.146			
(B) F-test & LSD	** LSD _{0.05} = 0.049 LSD _{0.01} = 0.081				** LSD _{0.05} = 0.005 LSD _{0.01} = 0.007				** LSD _{0.05} = 0.048 LSD _{0.01} = 0.080			
(AxB) F-test & LSD	** LSD _{0.05} = 0.067 LSD _{0.01} = 0.111				** LSD _{0.05} = 0.005 LSD _{0.01} = 0.008				** LSD _{0.05} = 0.088 LSD _{0.01} = 0.146			

Soil chemical properties:

The results listed in Table (10) showed that the soil application of sol. Ca and foliar application of CHT slightly affected the soil salinity (ECe) and soil sodicity (SAR and ESP) comparing to the untreated plots.

a- Soil ECe (dS m⁻¹): The ECe value of soil slightly decreased from 7.03 dSm⁻¹ in the check plots to 6.84 or 6.64 dSm⁻¹ in soil treated by sol. Ca at the rate of 2 or 4 L fed⁻¹, respectively. Concerning the effect of chitozan, the ECe value was decreased from 6.93 dSm⁻¹ in untreated soil to 6.80 or 6.79 dSm⁻¹ in plots treated by 0.5 or 1.0 L chitozan fed⁻¹, respectively. On the other hand, the highest ECe value (7.12 dS⁻¹) was recorded in plots that untreated by sol. Ca or chitozan, while the lowest value (6.67 dS⁻¹) was achieved in soil treated by 4 sol. Ca with 1.0 chitozan fed⁻¹.

b- Soil SARE: The SARE values clearly affected by the application of the sol. Ca since it was decreased from 14.44 in untreated soil to 12.22 or 11.94 in soils treated by 2.0 or 4.0 L sol. Ca fed⁻¹, respectively. With chitozan application, SARE values of soil were slightly decreased

from 13.02 in check plots to 12.79 or 12.78 in soils treated by 0.5 or 1.0 L chitozan, respectively. However, the application of 4.0 L sol. Ca with 1.0 L chitozan achieved the lowest SARE value (11.89) comparing to the untreated soil which recorded the highest value (14.53).

c- Soil ESP: The same trend was observed with ESP value, where it slightly decreased from 16.82 in soil without sol. Ca application to 14.54 or 14.25 in plots supplemented by 2.0 or 4.0 L sol Ca fed⁻¹, respectively. Also, ESP value was slightly decreased from 15.37 in untreated plots by CHT to 15.13 or 15.12 as a result of an application of 0.5 or 1.0 L CHT fed⁻¹, respectively. In addition, the combination of sol. Ca and CHT slightly affected the soil ESP, since the application of 4.0 L sol. Ca+1.0 L CHT gave the lowest value (14.20), while the highest value (16.92) was recorded in the untreated plots.

The slight effects of sol. Ca or CHT on some chemical properties of the soil may be attributed to that both materials were applied in very low rates and they in particularly are applied as plant growth regulators.

Table 10. Some soil chemical properties as affected by soluble Ca and chitosan applications.

Parameters Soluble Ca (A)	EC (dS/m)				SARE				ESP			
	Chitosan, B (L fed ⁻¹)				Chitosan, B (L fed ⁻¹)				Chitosan, B (L fed ⁻¹)			
	without	0.5	1.0	mean	without	0.5	1.0	mean	without	0.5	1.0	mean
without	7.12	6.99	6.98	7.03	14.53	14.40	14.39	14.44	16.92	16.78	16.77	16.82
2 L fed ⁻¹	6.93	6.80	6.80	6.84	12.52	12.07	12.07	12.22	14.85	14.39	14.38	14.54
4 L fed ⁻¹	6.73	6.60	6.59	6.64	12.02	11.90	11.89	11.94	14.33	14.21	14.20	14.25
mean	6.93	6.80	6.79	6.84	13.02	12.79	12.78	12.87	15.37	15.13	15.12	15.20

Agri excellent:

Many benefits were achieved by these treatments as follow:-

- A new management with low cost technology and friendly used to decrease the toxic effects caused by high salinity on plant growth and increase the productivity.
- Applications of soluble Ca and/or CHT increase the use efficiency of the fertilizers and consequently decrease severe environmental pollution.
- Development of salt affected soils is one of the processes of soil degradation leading to land desertification and consequently application of CHT and soluble Ca decrease this negative effect.

CONCLUSION

It could be concluded from this study that the soil application of sol. Ca and/or foliar application of CHT alleviated the adverse effect of salinity on maize productivity, and the combined application of them was more effective on maize growth under salt stress. However, there were no obvious decreases in ECe, SARe or ESP for soil treated by the soluble Ca and/or CHT.

REFERENCES

- Agbodjato, N.A., Noumavo, P.A., Adjanohoun, A., Agbessi, L. and Baba-Moussa, L. (2016). Synergistic Effects of Plant Growth Promoting Rhizobacteria and Chitosan on *In Vitro* Seeds Germination, Greenhouse Growth, and Nutrient Uptake of Maize (*Zea mays* L.). *Biotechnology Research International* Vol. 2016, Article ID 7830182, 11 pages.
- Al-Wahaibi, M.H., Siddiqui, M.H. and Basalah, M.O. (2012). Salicylic acid and calcium-induced protection of wheat against salinity. *Protoplasma*, 249:769–778. DOI 10.1007/s00709-011-0322-1
- Bittelli, M., Flury, M., Campbell, G.S. and Nichols, E.J. (2001). Reduction of transpiration through foliar application of chitosan. *Agric. and Forest Meteorology*, 107 (3):167-175.
- Black C. (1983). *Methods of soil analysis, Part I and Part II*. Amer Argon.Inc. Publ. Madison, Wisconsin, USA.
- Chawla ,S.P, Kanatt, S. R. and Sharma, A. K. (2014). Chitosan. *Polysaccharides*, DOI 10.1007/978-3-319-03751-6_13-1.
- Chookhongkha, N.; Miyagawa, S.; Jirakiattikul, Y.; Photchanachai, S. (2012). Chili growth and seed productivity as affected by chitosan. In *Proceedings of the International Conference on Agriculture Technology and Food Sciences (ICATFS'2012)*, Manila, Philippines, 17–18 Nov. 2012; pp. 146–149.
- Davidson, D. and Gu, F.X. (2012). Materials for sustained and controlled release of nutrients and molecules to support plant growth. *J. Agric. Food Chem.*, 60, 870–876.
- FAO (2000). *Production year book*. Food and Agriculture Organization of the United Nations, Rome.
- Farouk S., Mosa A.A., Taha A. A., Ibrahim Heba M., El-Gahmery A.M.,(2011). Protective effect of humic acid and chitosan on radish (*Raphanus Sativus*, L. *Var Sativus*) plants subjected to cadmium stress. *J. of Stress Physiology and Biochemistry*, 7 (2): 99-116. ISSN 1997-0838.
- Gao, H., Jia, Y., Guo, S., Lv, G., Wang, T., and Juan, L. (2011). Exogenous calcium affects nitrogen metabolism in root-zone hypoxia-stressed muskmelon roots and enhances short-term hypoxia tolerance. *J. Plant Physiol.*, 168: 1217–1225. doi: 10.1016/j.jplph.2011.01.022
- Gomez, K.A. and A.A. Gomez. (1984). *Statistical Procedures for Agricultural Research*. 2nd, John Wiley and Sons, USA.
- Gulser,F., Sonmez, F. and Boysan, S. (2010). Effects of calcium nitrate and humic acid on pepper seedling growth under saline condition. *Journal of Environmental Biology*, 31(5): 873-876.
- He, L., Lu, X., Tian, J., Yang, Y., Li, B., Li, J., et al. (2012). Proteomic analysis of the effects of exogenous calcium on hypoxic-responsive proteins in cucumber roots. *Proteome Sci.* 10:42. doi: 10.1186/1477-5956-10-42
- Henriksson, E., and Nordin Henriksson, K. (2005). Salt-stress signalling and the role of calcium in the regulation of the Arabidopsis ATHB7 gene. *Plant Cell Environ.* 28, 202–210. doi: 10.1111/j.1365-3040.2004.01263.x.
- Hepler, P. K. (2005). Calcium: a central regulator of plant growth and development. *Plant Cell* 17, 2142–2155. doi: 10.1105/tpc.105.032508.
- Hirschi KD (2004). The calcium conundrum. Both versatile nutrient and specific signal. *Plant Physiol* 136:2438–2442.
- Jackson, M. L. (1973). "Soil Chemical Analysis." 2nd Ed. Prentice Hall of India Private and L. T. D., New Delhi, India.
- Jiang, Y., Huang B (2001). Effect of calcium on antioxidant activities and water relations associated with heat tolerance in two cool-season grasses. *J Exp Bot* 355:341–349.
- Katiyar, D, Hemantaranjan, A. and Singh, B. (2015). Chitosan as a promising natural compound to enhance potential physiological responses in plant: a review. *Ind J Plant Physiol*, 20 (1):1-9. DOI 10.1007/s40502-015-0139-6.
- Katiyar, D.; Hemantaranjan, A.; Bharti, S.and Nishant, B. A. (2014). A future perspective in crop protection: Chitosan and its oligosaccharides. *Advances in Plants and Agriculture Research* 1(1): 1–8.
- Kaya, C., Kirnak, H. Higgs, D. and Saltali, K. (2002). Supplementary calcium enhances plant growth and fruit yield in strawberry cultivars grown at high (NaCl) salinity. *Scientia Horticulturae*, Vol. 93, Issue 1, 28 February 2002: 65-74. [https://doi.org/10.1016/S0304-4238\(01\)00313-2](https://doi.org/10.1016/S0304-4238(01)00313-2)Get rights and content.
- Khan,M.N., Siddiqui,M.H., Mohammad, F., Naeem, M. and Khan, M.M.A. (2010). Calcium chloride and gibberellic acid protect linseed (*Linum usitatissimum* L.) from NaCl stress by inducing antioxidative defence system and osmoprotectant accumulation. *Acta Physiol Plant*, 32:121–132. DOI 10.1007/s11738-009-0387-z.
- Khan,W.M., Prithiviraj, B. and Smith, D.L. (2002). Effect of foliar application of chitin and chitosan oligosaccharides on photosynthesis of maize and soybean. *Photosynthetica*, 40 (4): 621-624.

- Kurita K (2006). Chitin and chitosan: functional biopolymers from marine crustaceans. *Marine Biotechnol*, 8: 203-226.
- Lin, K. H., Chiou, Y. K., Hwang, S. Y., Chen, L. F. O., and Lo, H. F. (2008). Calcium chloride enhances the antioxidative system of sweet potato (*Ipomoea batatas*) under flooding stress. *Ann. Appl. Biol.*, 152:157-168. doi: 10.1111/j.1744-7348. 2007. 00211. x.
- Ma, L.; Li, Y., Yu, C.; Wang, Y.; Li, X.; Li, N.; Chen, Q. and Bu, N. (2011). Alleviation of exogenous oligochitosan on wheat seedlings growth under salt stress. *Protoplasma* 249: 393-399.
- Mahdavi, B. and Rahimi, A. (2013). Seed priming with chitosan improves the germination and growth performance of ajowan (*Carum copticum*) under salt stress. *Eurasia J Biosci* 7: 69-76.
- Malerba, M. and Cerana, R. (2016). Chitosan Effects on Plant Systems. *Int. J. Mol. Sci.*, 17 (7), 996; <https://doi.org/10.3390/ijms17070996>. Impact Factor 3.687.
- Mathew, R.; Sankar, P.D (2012). Effect of methyl jasmonate and chitosan on growth characteristics of *Ocimum basilicum* L., *Ocimum sanctum* L. and *Ocimum gratissimum* L. cell suspension cultures. *Afr. J. Biotechnol.*, 11, 4759-4766.
- Mondal, M.M.A.; Malek, M.A.; Puteh, A.B.; Ismail, M.R.; Ashrafuzzaman, M.; Naher, L. (2012). Effect of foliar application of chitosan on growth and yield in okra. *Aust. J. Crop Sci.*, 6: 918-921.
- Navarro, J.M., Martinez, V.V. and Carvajal, M. (2000). Ammonium, bicarbonate and calcium effects on tomato plants grown under saline conditions. *Plant Science*, Vol. 157, Issue 1, 8 August 2000: 89-96. [https://doi.org/10.1016/S0168-9452\(00\)00272-7](https://doi.org/10.1016/S0168-9452(00)00272-7) Get rights and content.
- Ngo, D., Vo, T., Ngo, D., Kang, K. Je, J., Pham, H. N., Byun, H. and Kim, S. (2015). Biological effects of chitosan and its derivatives. *Food Hydrocolloids*, Vol. 51: 200-216.
- Oh, M., Nanjo, Y. and Komatsu, S. (2014). Gel-free proteomic analysis of soybean root proteins affected by calcium under flooding stress. *Frontiers in Plant Science*, Vol. 5, Article 559.
- Peterburgski, A.V. (1968). "Hand Book of Agronomic Chemistry". Kolos Publishing House, Moscow (in Russian): 29-86.
- Rengel Z (1992). The role of calcium in salt toxicity. *Plant Cell Environ*, 15:625-632.
- Sathiyabama, M.; Akila, G.; Einstein Charles, R. (2014). Chitosan-induced defence responses in tomato plants against early blight disease caused by *Alternaria solani* (Ellis and Martin) Sorauer. *Arch. Phytopathol. Plant Prot.*, 47:1777-1787.
- Soltanpour, N.P. (1985). Use of ammonium bicarbonate DTPA soil test to evaluate elemental availability and toxicity. *Soil Sci. Plant Anal.*, 16 (3): 323- 338.
- Sun, B.; Zhang, L.; Yang, L.; Zhang, F.; Norse, D.; Zhu, Z. (2012). Agricultural non-point source pollution in China: Causes and mitigation measures. *Ambio*, 4, 370-379.
- Wang, M.; Chen, Y.; Zhang, R.; Wang, W.; Zhao, X.; Du, Y.; Yin, H. (2015). Effects of chitosan oligosaccharides on the yield components and production quality of different wheat cultivars (*Triticum aestivum* L.) in Northwest China. *Field Crops Res.*, 172:11-20.
- White PJ, Broadley MR (2003). Calcium in plants. *Ann Bot* 92:487-511.
- Wu, L.; Liu, M.; Liang, R. (2008). Preparation and properties of a double-coated slow-release NPK compound fertilizer with superabsorbent and water-retention. *Bioresour. Technol.*, 99, 547-554.
- Yin, H.; Frette, X.C.; Christensen, L.P.; Grevsen, K. (2012). Chitosan oligosaccharides promote the content of polyphenols in Greek oregano (*Origanum vulgare* ssp. *hirtum*). *J. Agric. Food Chem.*, 60, 136-143.
- Yu, G., & Meuhlbaauer, G. (2001). Benzothiadiazole-induced gene expression in wheat spikes does not provide resistance to *Fusarium* head blight. *Physiological and Molecular Plant Pathology*, 59: 129-139.
- Zayed, M. M.; Elkafafi, S. H.; Zedan, Amina M. G. and Dawoud, Sherifa F. M. (2017). Effect of Nano Chitosan on Growth, Physiological and Biochemical Parameters of *Phaseolus vulgaris* under Salt Stress. *J. Plant Production, Mansoura Univ.*, Vol. 8 (5): 577-585.
- Zhao, Y., Tu, K., Su, J., Tu, S., Hou, Y., et al. (2009). Heat treatment in combination with antagonistic yeast reduces diseases and elicits the active defense responses in harvested cherry tomato fruit. *Journal Agriculture Food Chemistry*, 57: 7565-7570.

تأثير الشيتوزان والكالسيوم الذائب على النظم النباتية محمد سامي السقا ، هشام محمود ابو السعود و تامر حسن خليفة مركز البحوث الزراعية ، الجيزة ، مصر معهد بحوث الاراضى والمياه والبيئة

تعد ملوحة التربة أحد العوامل البيئية المهمة التي تضعف الإنتاج الزراعي ، في حين أن إضافة بعض المحسنات مثل الكالسيوم الذائب (نترات كالسيوم) و/أو الشيتوزان يخفف من التأثير الضار للملحة على نمو النبات. كان الهدف من هذه الدراسة هو تقييم تأثير إضافة الكالسيوم ارضيا بمعدل 2 او 4 لتر/فدان التفاعلي للتطبيق التربة والرش الورقي للشيتوزان بمعدل 0.5 أو 1 لتر /فدان على إنتاجية الذرة ، وامتصاص النبات للنيتروجين والفوسفور والبوتاسيوم وبعض خواص التربة تحت ظروف الاراضى الملحية (8.27 ديسيسمنز) والرى بالمياه الجوفية (2.9 ديسيسمنز). أثبتت النتائج التي تم الحصول عليها أن هذه التعديلات منفردة أو مجتمعة أدت إلى تحسن ملحوظ في نمو الذرة (ارتفاع النبات، تعبئة الحبوب ، محصول الكوز والقش) ، تركيز النيتروجين والفوسفور والبوتاسيوم في الحبوب والقش وتحسين بعض الخواص الفيزيائية والكيميائية للتربة المتأثرة بالأملاح. كذلك ، وجد أن أعلى مستوى إضافة من الكالسيوم والشيتوزان مجتمعة أثر بشكل كبير على نمو النبات وتسببت في أعلى زيادة في ارتفاع النبات (65.1%) ، ووزن 100 حبة (112.2%) ، وإنتاجية الكيزان والقش (168.4 و 111.6 % على التوالي). إضافة ، حققت هذه المعاملة أعلى تركيز للنيتروجين والفوسفور والبوتاسيوم في الحبوب (3.51 ، 0.33 و 1.08 ، على التوالي) والقش (1.73 ، 0.14 و 1.62 ، على التوالي). وبشكل عام ، فإن إضافة إضافة الكالسيوم الذائب و/أو الشيتوزان أدى إلى تخفيف التأثير السلبي للملحة على إنتاجية الذرة ، بينما ثبت أن إضافة كلاهما كان أكثر فاعلية في تحسين نمو ذرة تحت الظروف الملحية. ومع ذلك ، لم يكن هناك انخفاض واضح في قيم الـ ESP ، SAR ، ECE للمعالجة بالكالسيوم والشيتوزان.