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Interactive effects of water salinity stress and chitosan foliar-spray application on vegetative and flowering growth aspects and chemical constituents of pot marigold (*Calendula officinalis* L.) plant.

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ABSTRACT

A pot experiment was conducted during the two successive seasons of 2018/2019 and 2019/2020 at west of Somosta, Beni-Suef governorate, Egypt, to explore the effect of both saline water and foliar application of chitosan treatments on growth parameters for vegetative and flowering and on some chemical constituents of *Calendula officinalis* L.

In contrast to control plants which were irrigated with tap water, the plants were irrigated with saline water containing NaCl at concentrations of 1000, 2000, 3000, 4000 and 5000 ppm. They were treated also by chitosan as foliar spray application at 100, 200 and 400 ppm, as well as, the interaction between them were involved. The obtained results revealed that the higher salinity levels (4000 and 5000 ppm NaCl) caused significant decreases in all vegetative and flowering growth measurements of pot marigold plants compared to control. Maximum reduction was observed at 5000 ppm NaCl which showed higher increase of the free proline content, sodium and chloride percentages. Meanwhile, the increase in the concentration of salt in the irrigation water resulted in a decrease in the total chlorophylls in leaves and carotene content in flowers. Foliar application of chitosan at concentrations 200 and 400 ppm alleviated the adverse effect of salinity condition thereby vegetative, flowering characters and also chemical constituents were improved. The best level of foliar application of chitosan was at 400 ppm. Whereas, non-significant effects were found on the vegetative and floral parameters and some chemical components as a result of the interaction between both aspects studied in comparison with untreated treatment, in most cases, in the two experimental seasons (2018/2019 and 2019/2020).

KEYWORDS: Water salinity, *Calendula officinalis*, Chitosan, Vegetative and flowering growth parameters, Chemical constituents, Foliar-spray application .

1. Introduction

Calendula officinalis L. (Marigold) is an annual plant that belongs to the Asteraceae family. It is an important ornamental and medicinal plant. The native region for marigold extends between the regions of the Mediterranean Sea, Egypt and Europe (Nofal *et al*., 2015). Calendulas plants uses as cut flowers and potted flowering plants (Hamrick, 2003). Marigold play a significant role in human health and has antioxidant functions (Meda *et al.*, 2005).

As one of the abiotic stresses, salinity has become a serious problem affecting the growth and productivity of many plants due to the lack of fresh water supplies, in arid or semi-arid areas, in particular. Salinity in irrigation water and soils induces changes in plant metabolic activities such as hormone modification, photosynthesis and respiration balance, mineral uptake and enzymatic activate inhibition (Mazher *et al*., 2007). It is a significant factor that decreases the growth and productivity of plants; it affects the total land area of the world and is the main environmental factor limiting the growth and productivity of plants. Ion cytotoxicity and osmotic stress could be responsible for the adverse effects of salinity on plant growth (Hussain *et al*., 2008). Oxidative stress may also resulted from Imbalances of metabolism triggered by ion toxicity, osmotic stress and deficiency of nutrients under saline conditions (Zhu, 2002).

Chitosan is a natural biopolymer modified from chitin that serves as a potential bio-stimulant and elicitor in agriculture. It is biocompatible, biodegradable and non-toxic, facilitating widespread use theoretically. This enhances physiological

reaction and decreases the negative effects of abiotic stress by secondary messenger(s) via a stress transduction mechanism. Chitosan treatment enhances the closure of stomata through ABA synthesis and photosynthesis, enhances antioxidant enzymes by nitric oxide and hydrogen peroxide signaling pathways and induces the production of sugars organic acids, amino acids and other metabolites needed for osmotic stress-related adaptation, signaling of stress, and metabolism of energy (Hidangmayum *et al*., 2019).

This study was designed to investigate the effect of saline water irrigation containing NaCl and foliar application of chitosan treatments, as well as, their interaction in terms of different vegetative and flowering growth parameters and some chemical constituents of *Calendula officinalis* L.

2. Materials and Methods:

Pot experiments were conducted throughout two successive seasons (2018/2019 and 2019/2020) in a private farm at west of Somosta, Beni-Suef governorate, Egypt.

2.1. Experimental procedure:

Local *Calendula officinalis* seeds L. obtained from the Dept. of Medicinal and Aromatic Plants. Hort. Res. Inst., Agric. Res. Center, Egypt. Seeds were sown in the nursery on the first week of September in both growing seasons. Uniform seedlings 45 days old and nearly 15 cm in height were transplanted into 30 cm diameter plastic pot, filled beforehand with 12 kg of field sandy soil and each contained one seedling. According to Jackson (1973) and Cottenie *et al.* (1982), as shown in Table (1), physical and chemical properties of soil samples have been determined. Black polyethylene was placed under the pots in order to prevent penetration of roots to the ground. After two weeks from transplanting, in addition to the control plants irrigated with tap water, other plants were irrigated with saline water containing NaCl at 1000, 2000, 3000, 4000 and 5000 ppm. In both seasons, irrigation was applied in two day intervals (250 ml/pot).

Table 1. The physical and chemical properties of the experimental soil.

O.M.	CaCO3%	Sand%	Silt %	Clay%	Texture class	pH	ECe (ds/m)
0.8	12.40	81.70	11.80	6.50	sandy loam	7.8	3.2
Soluble ions (meq/L)							
HCO3-	2.5	Mg 2+	2.02	Total N % 0.049			
Cl-	9.3	Na+	4.55	Available P 6.40 ppm			
Fe	1.7	Zn	0.32	Exchange K 1.4 mg/ 1000g (soil)			
Ca2+	6.9	Mn	0.55				

Chitosan (2-amino-2-deoxy-ˇ-d-glucosamine), namely Chito-Care® with a deacetylation degree of 85%, was used as a purified commercial product. To get the desired concentrations of 100, 200 and 400 ppm, chitosan was dissolved in 1 percent acetic acid. Using sodium hydroxide, the pH of the solution was adjusted to 6.5. The foliage of the plants was sprayed to the point of running off. Plants were sprayed with chitosan concentrations weekly until the beginning of inflorescences harvest.

2.2. Experimental design:

The experiment was arranged with 3 replicates in RCBD design in split plot for each treatment (each replicate has 5 plants/treatment). Six water salinity concentrations were used in the main plots (A), while the sub-plots were occupied by four chitosan concentration treatments (B). (A×B) 24 treatments were the interaction treatments.

2.3. Experimental treatments:

Main-plots (A):
1- Control (tab water).
2- Salinity at 1000 ppm (Sal. 1000ppm).
3- Salinity at 2000 ppm (Sal. 2000ppm).
4- Salinity at 3000 ppm (Sal. 3000ppm).
5- Salinity at 4000 ppm (Sal. 4000ppm).
6- Salinity at 5000 ppm (Sal. 5000ppm).

The sub-plots (B):
1- Control (spray with tab water).
2- Chitosan at 100 ppm (Ch.1).
3- Chitosan at 200 ppm (Ch.2).
4- Chitosan at 400 ppm (Ch.3).

2.4. Data recorded:

2.4.1. Vegetative growth parameters:

1- Plant height (cm).
2- Leaf area (cm^2).
3- Number of main branches/plant.
4- Herb dry weight/plant (g).

2.4.2. Floral parameters:

1- Number of inflorescences/plant.
2- Diameter of inflorescences/plant.
3- Inflorescences dry weight (g).

2.4.3. Chemical constituents:

1- Free proline content in dry herb was detected by an acid-ninhydrin method as outlined by Bates *et al*. (1973).
2- Total chlorophylls (mgg^{-1}) were determined in fresh leaves samples according to Welburnand and Lichtenthaler (1984).
3- Carotene content (mg/g) was determined in fresh flowers samples according to Nagata and Yamashita (1992).
4- Sodium percentage was determined in accordance with the method defined by Cottenie *et al*. (1982).
5- Chloride percentage was determined according to the method described by Brown and Jackson (1955).

2.5. Statistical analysis:

Data collected from the both seasons were tabulated and statistically analyzed in accordance with MSTAT-C (1986) and as defined by Mead *et al.* (1993), the mean of the observed data was compared using the (L.S.D.) test at the 5 %.

3. RESULTS AND DISCUSSION

3.1. Vegetative parameters

Data presented in Table (2) concluded that plant height (cm), leaf area (cm^2), number of main branches/plant and dry weight of herb (g) of marigold plants were significantly decreased due to salinity of irrigation water at 4000 and 5000 ppm in the both seasons tried . While, 1000, 2000 and 3000 ppm had non deleterious effect on vegetative growth parameters as the means of these parameters were closely near to those of control with no significant variation in most cases of the two seasons curried out. The only exception was on leaf area in the two seasons The greatest reduction of vegetative growth parameters was obtained under the highest concentration of NaCl (5000 ppm) in both seasons. It produced the highest reduction of plant height (36.03 and 34.88 cm), leaf area (22.16 and 21.69 cm^2), number of main branches/plant (10.48 and 11.08) and herb dry weight/plant (53.65 and 51.45 g) compared to untreated plants in the first and second seasons, respectively. The reducing effect of salinity treatments on vegetative growth parameters obtained in this investigation was also pointed out earlier by Ejaz *et al*. (2015) on calendula plant, Mazhar *et al*. (2012) on *Chrysanthemum indicum* and El-Attar (2017) on *Antirrhinum majus*. The reduction in the growth characteristics of plants as a result of salinity might be attributed to the accumulation of the salts in the soil, which increased the osmotic pressure of tissue cells and decreased the water absorption and/or redistribution of minerals and their utilization (Mazher *et al*., 2006). Likewise, Pessarakli and Touchane (2006) illustrated that the salt mechanism can lead to inhibitory of cell division, thereby reducing the rate of plant growth. however, Jou *et al.* (2006) declared that ATPase is involved in the protein sorting machinery regulated by the endoplasmic reticulum Golgi for both housekeeping function and compartmentalization of excess Na^+ under high salinity which could be a limiting factor and serve as an explanation to the obtained result herewith.

Obtained data in Table (2) illustrated that chitosan treatments at 200 and 400 ppm caused considerable and significant augmentation in all vegetative parameters except leaf area only in the two seasons, in comparison with control treatment. The chitosan treatment (400 ppm) caused higher increasing of plant height by 13.46% and 12.85%, leaf area by 7.47% and 9.84%, number of main branches/plant by 15.66% and 20.12% and herb dry weight/plant by 16.26% and 16.52% compared to control in the both experimental seasons, respectively. It might be interesting to mention that no significant differences existed between chitosan at 100 ppm and control. These results are in a harmony with those obtained by Mondal *et al*. (2013) on *Vigna radiate*, El-Attar (2017) on *Antirrhinum majus*, Masjedi *et al*. (2017) on *Triticum aestivum* and Ananthaselvi *et al*. (2019) on *Tagetes erecta*. Chitosan contains nitrogen in its chemical structure, which is recognized as one of the most important nutrients for plants and soil. When nitrogen is dissolved in chitosan, it gradually penetrates and remains in the soil for longer periods of time and can be effective in this regard. The substantial promotion of chitosan for plant growth may be attributed to an improvement in the main enzyme activities of nitrogen metabolism (glutamine synthetase, nitrate reductase and protease) and increased photosynthesis that enhanced the plant growth (Gornik *et al.*, 2008).

Regarding the interaction between saline water irrigation and foliar chitosan treatments on plant height (cm), leaf area (cm^2), number of branches/plant and dry weight of herb/plant (g), it was clear that there were insignificant differences on all vegetative growth parameters compared with control plants in both seasons as shown in Table (2). These results mean that chitosan treatments gave positive effect on growth parameters, they reduced the harmful effect of salinity.

Table 2. Effect of water salinity and chitosan foliar-spray application on plant height (cm), leaf area (cm^2), number of main branches/plant and herb dry weight/plant (g) of *Calendula officinalis* L. during 2018/2019 and 2019/2020 seasons.

Salinity Treatments (A)	Chitosan (B)									
	Control (water)	Ch.1	Ch.2	Ch.3	Mean (A)	Control (water)	Ch.1	Ch.2	Ch.3	Mean (A)
	First season(2018/2019)					Second season(2019/2020)				
	Plant height (cm)									
Control(water)	46.8	47.2	48.5	49.8	**48.08**	44.8	45.4	46.8	47.2	**46.05**
Sal. 1000 ppm	44.3	45.5	49.3	50.2	**47.33**	42.1	43.8	46.5	46.5	**44.73**
Sal. 2000 ppm	41.5	43.7	45.2	47.6	**44.50**	40.5	44.1	45.3	46.3	**44.05**
Sal. 3000 ppm	39.6	41.5	44.3	47.2	**43.15**	37.2	40.6	42.1	43.6	**40.88**
Sal. 4000 ppm	36.5	38.3	42.6	43.3	**40.18**	34.6	36.5	40.3	42.7	**38.53**
Sal. 5000 ppm	34.3	35.7	36.5	37.6	**36.03**	33.3	35.3	34.8	36.1	**34.88**
Mean (B)	**40.5**	**41.98**	**44.4**	**45.95**		**38.75**	**40.95**	**42.63**	**43.73**	
L.S.D. at 5%	**A: 5.43**		**B: 2.31**		**AB: 5.63**	**A: 5.35**		**B: 2.51**		**AB: 6.12**
	Leaf area (cm^2)									
Control(water)	41.55	42.05	42.32	43.28	**42.30**	39.12	39.65	41.08	43.15	**40.75**
Sal. 1000 ppm	38.67	39.33	40.45	41.30	**39.94**	36.45	37.67	38.33	39.75	**38.05**
Sal. 2000 ppm	35.41	34.27	36.18	37.85	**35.93**	35.85	36.71	37.42	37.65	**36.91**
Sal. 3000 ppm	34.22	33.95	35.64	37.65	**35.37**	33.12	34.15	35.77	36.79	**34.96**
Sal. 4000 ppm	25.05	24.85	26.33	26.75	**25.75**	26.44	24.35	25.42	27.50	**25.93**
Sal. 5000 ppm	21.88	18.67	23.42	24.65	**22.16**	19.75	18.95	23.42	24.65	**21.69**
Mean (B)	**32.80**	**32.19**	**34.06**	**35.25**		**31.79**	**31.91**	**33.57**	**34.92**	
L.S.D. at 5%	**A: 3.63**		**B: 2.31**		**AB: 5.64**	**A: 2.92**		**B: 1.83**		**AB: 4.46**
	Number of main branches									
Control(water)	14.1	13.7	14.3	15.6	**14.43**	14.4	14.6	15.2	14.9	**14.78**
Sal. 1000 ppm	13.4	14.2	13.9	14.7	**14.05**	13.5	14.2	14.6	14.6	**14.23**
Sal. 2000 ppm	14.3	12.8	13.5	14.8	**13.85**	12.8	14.3	13.6	15.1	**13.95**
Sal. 3000 ppm	12.3	13.7	14.4	14.5	**13.73**	11.3	13.1	12.8	14.5	**12.93**
Sal. 4000 ppm	11.1	12.3	12.7	13.2	**12.33**	9.8	12.3	12.1	14.3	**12.13**
Sal. 5000 ppm	8.4	10.4	10.8	12.3	**10.48**	10.4	9.2	11.4	13.3	**11.08**
Mean (B)	**12.26**	**12.85**	**13.27**	**14.18**		**12.03**	**12.95**	**13.28**	**14.45**	
L.S.D. at 5%	**A: 1.35**		**B: 0.88**		**AB: 2.15**	**A: 1.92**		**B: 1.18**		**AB: 2.88**
	Herb dry weight/ plant (g)									
Control(water)	67.3	70.7	73.4	74.8	**71.55**	65.5	66.3	70.4	74.8	**69.25**
Sal. 1000 ppm	64.8	68.5	73.7	75.3	**70.58**	64.1	67.2	69.5	72.4	**68.30**
Sal. 2000 ppm	61.2	62.7	70.4	72.6	**66.73**	62.5	62.7	65.5	71.7	**65.60**
Sal. 3000 ppm	55.3	60.3	63.2	66.2	**61.25**	55.6	60.4	64.4	64.9	**61.33**
Sal. 4000 ppm	53.6	54.8	58.6	61.3	**57.08**	52.3	54.6	57.4	63.8	**57.03**
Sal. 5000 ppm	48.6	52.1	56.2	57.7	**53.65**	46.2	48.5	55.3	55.8	**51.45**
Mean (B)	**58.47**	**61.52**	**65.91**	**67.98**		**57.70**	**59.95**	**63.75**	**67.23**	
L.S.D. at 5%	**A: 4.96**		**B: 3.44**		**AB: 8.39**	**A: 3.85**		**B: 2.68**		**AB: 6.54**

3.2. Flowering parameters

As a flowering ornamental plant, flowers are a significant asset of calendula, so it is not preferred to have reduced flower numbers and quality in containers or landscapes. From the recorded data in Table (3), both treating marigold plants with irrigation water salinity at 4000 and 5000 ppm led to a significant decrease in number of inflorescences, diameter of inflorescences and inflorescences dry weight compared with control plants, in two seasons. The high salt concentration (5000 ppm) produced the highest reduction of number of inflorescences (26.60 and 26.20), diameter of inflorescences (4.49 and 4.36 cm) and inflorescences dry weight (0.22 and 0.22 g) compared to untreated plants in the both seasons, respectively. Many researchers came to similar conclusions, such as Nofal *et al*. (2015), Swaefy and El-Ziat (2017) and Adamipour *et al*.

Table 3. Effect of water salinity and chitosan foliar-spray application on number of inflorescences, diameter of inflorescences (cm) and inflorescences dry weight (g) of *Calendula officinalis* L. during 2018/2019 and 2019/2020 seasons.

Salinity Treatments (A)	Chitosan (B)									
	Control (water)	Ch.1	Ch.2	Ch.3	Mean (A)	Control (water)	Ch.1	Ch.2	Ch.3	Mean (A)
	First season(2018/2019)					Second season(2019/2020)				
	Number of inflorescences									
Control(water)	34.0	34.4	35.4	35.6	**34.85**	32.4	33.2	36.0	35.6	**34.30**
Sal. 1000 ppm	30.8	33.6	34.2	35.4	**33.50**	28.8	29.6	33.6	34.8	**31.70**
Sal. 2000 ppm	29.8	29.4	31.6	33.4	**31.05**	30.2	31.4	32.2	33.0	**31.65**
Sal. 3000 ppm	28.8	29.4	30.8	32.8	**30.45**	29.8	30.2	32.8	31.6	**31.10**
Sal. 4000 ppm	26.6	27.8	28.2	29.6	**28.05**	27.4	27.4	29.2	29.8	**27.85**
Sal. 5000 ppm	24.8	26.4	27.4	27.8	**26.60**	26.2	24.8	26.8	27.0	**26.20**
Mean (B)	**29.13**	**30.17**	**31.27**	**32.43**		**29.13**	**29.43**	**31.77**	**31.97**	
L.S.D. at 5%	**A: 4.55**		**B: 1.92**	**AB: 4.68**		**A: 3.68**		**B: 1.33**	**AB: 3.23**	
	Diameter of inflorescences (cm)									
Control(water)	5.21	5.18	5.35	5.50	**5.31**	4.77	4.95	5.26	5.44	**5.11**
Sal. 1000 ppm	5.11	5.32	5.38	5.46	**5.32**	4.82	4.72	4.90	5.15	**4.90**
Sal. 2000 ppm	5.05	5.15	5.27	5.38	**5.21**	4.65	4.76	4.84	5.11	**4.84**
Sal. 3000 ppm	4.88	4.95	5.07	5.25	**5.04**	4.48	4.63	4.66	4.83	**4.65**
Sal. 4000 ppm	4.50	4.75	4.88	5.05	**4.80**	4.22	4.47	4.50	4.67	**4.47**
Sal. 5000 ppm	4.25	4.42	4.60	4.72	**4.49**	4.18	4.33	4.45	4.48	**4.36**
Mean (B)	**4.83**	**4.96**	**5.09**	**5.23**		**4.52**	**4.64**	**4.77**	**4.95**	
L.S.D. at 5%	**A: 0.32**		**B: 0.18**	**AB: 0.44**		**A: 0.38**		**B: 0.11**	**AB: 0.27**	
	Inflorescences dry weight (g)									
Control(water)	0.26	0.27	0.27	0.29	**0.27**	0.28	0.29	0.29	0.33	**0.30**
Sal. 1000 ppm	0.26	0.25	0.26	0.28	**0.26**	0.28	0.28	0.30	0.31	**0.29**
Sal. 2000 ppm	0.24	0.23	0.25	0.28	**0.25**	0.26	0.27	0.31	0.30	**0.29**
Sal. 3000 ppm	0.25	0.22	0.27	0.27	**0.25**	0.25	0.27	0.29	0.29	**0.28**
Sal. 4000 ppm	0.23	0.25	0.26	0.24	**0.24**	0.23	0.25	0.26	0.28	**0.26**
Sal. 5000 ppm	0.19	0.23	0.22	0.22	**0.22**	0.20	0.22	0.22	0.25	**0.22**
Mean (B)	**0.24**	**0.24**	**0.26**	**0.26**		**0.25**	**0.26**	**0.28**	**0.29**	
L.S.D. at 5%	**A: 0.02**		**B: 0.01**	**AB: 0.02**		**A: 0.01**		**B: 0.02**	**AB: 0.05**	

(2019) on *Calendula officinalis.* The reduction in plant growth might be due to the reduction of cell division and cell elongation. This may be attributed to the increase of losing water by leaves so it affects reproduction development (Fricke and Peters, 2002). Also, Greenway and Munns (1980) said that reduction in flowering parameters may ensue from the plants inability to adjust osmotically, counteraction toxicities or related disruptive phenomena or from the excessive energy demand placed upon the metabolic machinery required by such homeostatic systems. Also, Abdalla (2011) demonstrated that The quantity of abscisic acid in the roots is increased by water stress, which is transferred to the shoot from the roots, where it serves as a cytokinin and auxin antagonist, which has very important role in cell enlargement and division, respectively. In addition, it inhibits synthesis of DNA.

Regarding the response of number of inflorescences, diameter of inflorescences and inflorescences dry weight to different concentrations of chitosan. Table (3) proved that all flowering parameters were significantly enhanced due to these abovementioned treatments compared to control treatment in the two experimental seasons. The gradual raise in the concentration of chitosan gave gradual augmentation of flowering characters with the higher values being obtained due to chitosan 400 ppm followed by 200 ppm. The increase percentage were 11.33, 8.28 and 8.33 % to number of inflorescences, diameter of inflorescences and inflorescences dry weight in the first season and 9.75, 9.51 and 16.00 % in the second season, respectively, compared to control treatment. Similar findings have been obtained by Wanichpongpan *et al*. (2001) on gerbera, also, Salachna *et al*. (2017) on verbena.

The beneficial effects of chitosan may be attributed to its role in various physiological processes; it act as a free radical scavenger or DNA-protective characteristics, and its structure, which

has large numbers of hydroxyl and amino groups available to react with reactive oxygen species, may be related to the chitosan scavenging mechanism (Salachna and Zawadziñska, 2014). Chitosan also induces endogenous plant hormone synthesis (Uthairatanakij *et al.*, 2007) or induces closure of stomata, which decreases transpiration (Iriti *et al.*, 2009).

Insignificant effects on the flowering aspects were found among the interaction treatments, in most cases, in the two growing seasons. On the whole, foliar application of chitosan alleviated the adverse effect of salinity condition thereby flowering characters were improved.

3.3. Chemical constituents

It was noticed from the obtained data in Table (4) that, increasing the salt concentration in water irrigation from 4000 to 5000 ppm showed a significant increase in free proline content compared with control treatment. The plants irrigated with the highest salt concentration (5000 ppm) had the highest mean values of free proline (4.56 and 4.82 µ mole) in the two seasons, respectively. Conversely, plants which have been irrigated with tap water had the lowest mean values (2.12 and 2.54 µ mole) of free proline content. The increase in the concentration of salt in the irrigation water resulted in a decrease in the total chlorophylls and carotene content in flowers which reached its lowest values of (total chlorophylls 1.35 and 1.41 mg/g) and (carotene content 0.720 and 0.808 mg/g) in the first and second seasons, respectively compared with control plants which gave the highest value. All salt concentrations in the irrigation water treatments caused significant augmentation in the leaves contents of sodium and chloride. The only exception was the treatment of water salinity at 1000 ppm for chloride content in the second season. The increase was parallel to the increase of salinity levels. Therefore, the application of high level of salinity (5000 ppm) gave the highest values of sodium and chloride contents in leaves which reached 2.21 and 2.02 % for sodium and 1.71 and 1.76 % for chloride in both experimental seasons, respectively. Such findings are in line with those obtained by many researches who reported increments in free proline content like, Kozminska *et al.* (2017) on calendula, El-Attar (2017) on snapdragon and Krupa-Malkiewicz and Smolik (2019) on petunia, results of Na and Cl % was found also by Don *et al.* (2010) on *Gerbera jamesonii*, Mahmoud (2016) on *Calendula officinalis*, Koksal *et al.* (2016) on *Tagetes erecta* and El-Attar (2017) on *Antirrhinum majus* due to raising salinity. Decreasing total chlorophylls detected in this investigation was also recorded by Lacramioara *et al.* (2014), (Swaefy and El-Ziat, 2017) and Kozminska *et al.* (2017) on *Calendula officinalis*. However, some other authors pointed out that chlorophyll and carotenoid contents in C. *officinalis* were not affected by salinity (Mirlotfi *et al.*, 2015). This apparent contradiction may be attributed to the use of different experimental growth conditions in these reports.

The rise in proline content is one of calendula plant defensive mechanisms against salinity stress. Greenway and Munns (1980) found that proline can be considered as a stabilizer of osmotic pressure within a cell and can make a contribution to cytoplasmic osmotic adjustment . Azevedo Neto and Silva (2015) suggested that increased activity of enzymes, complexes and membranes of proteins, protein stabilisation, cell redox homeostasis maintenance, the stocks of carbon and nitrogen, regulating cytosolic pH and removing free radicals are the functions attributed to proline accumulation. Inhibition of chlorophyll synthesis, along with activation of its degradation by the enzyme chlorophyllase, caused the reduction of chlorophyll levels in salt-treated plants (Santos, 2004). This is not the only explanation for photosynthesis inhibition in the presence of salt, because NaCl also inhibits main enzymes such as Rubisco and PEP carboxylase that are involved in this process (Soussi *et al.*, 1998).

With respect to the response of the contents of free proline to different concentration of chitosan, data obtained in Table (4) indicated that the content of free proline, was significantly decreased due to chitosan at 200 and 400 ppm in the second season except at 400 ppm in the first season compared to check control treatments. The lowest contents of free proline were achieved due to the following treatments in descending order: chitosan foliar application at 400ppm followed by 200ppm over those of check treatment by 17.45 and 10.65 % in the first season, and by 22.65 and 13.49 % in the second season, respectively. Whereas, no significant differences were recorded between the aforementioned two treatments. These findings align with those published by El-Attar (2017) on *Antirrhinum majus* plants.

With regard to the influence of chitosan concentrations, data given in Table (4) proved that these treatments had a favorable impact on enhancing the accumulation of total chlorophylls and carotene content. The increase of the total chlorophylls and carotene contents in flowers was gradual due to the gradual raise in the examined concentration. The highest contents of total chlorophylls were obtained from the following chitosan concentrations in descending order: chitosan at 400 ppm followed by 200 ppm then 100 ppm. These three treatments increased the total chlorophylls over those of control plants by 29.71, 20.29 and 12.32 % in the first season and by 19.86, 11.64 and 0.68 % in the second one, respectively. While, for carotene content in flowers,

Table 4. Effect of water salinity and chitosan foliar-spray application on free proline, total chlorophylls, carotene content/flowers, sodium and chloride percentages of *Calendula officinalis* L. during 2018/2019 and 2019/2020 seasons.

Salinity Treatments (A)	Control (water)	Ch.1	Ch.2	Ch.3	Mean (A)	Control (water)	Ch.1	Ch.2	Ch.3	Mean (A)
	First season(2018/2019)					Second season(2019/2020)				
Free proline (µ mole g-1 dry weight)										
Control (water)	2.25	2.23	2.11	1.88	**2.12**	3.05	2.75	2.40	1.95	**2.54**
Sal. 1000 ppm	2.63	2.45	2.34	2.41	**2.46**	2.82	3.10	2.60	2.45	**2.74**
Sal. 2000 ppm	2.55	2.67	2.48	2.25	**2.49**	3.38	3.25	2.78	2.66	**3.02**
Sal. 3000 ppm	3.42	3.25	3.05	3.15	**3.22**	4.62	3.92	3.75	2.88	**3.79**
Sal. 4000 ppm	4.35	3.85	3.40	3.30	**3.72**	4.77	4.34	3.97	3.75	**4.21**
Sal. 5000 ppm	5.10	4.68	4.72	3.75	**4.56**	4.95	4.84	4.92	4.55	**4.82**
Mean (B)	**3.38**	**3.19**	**3.02**	**2.79**		**3.93**	**3.70**	**3.40**	**3.04**	
L.S.D. at 5%	**A: 1.32**	**B: 0.55**		**AB: 1.34**		**A: 1.48**	**B: 0.43**		**AB: 1.05**	
Total chlorophylls (mg/g leaf F.W)										
Control (water)	1.62	1.68	1.85	2.02	**1.79**	1.55	1.58	1.83	1.94	**1.73**
Sal. 1000 ppm	1.55	1.73	1.83	1.96	**1.77**	1.61	1.52	1.75	1.91	**1.70**
Sal. 2000 ppm	1.38	1.60	1.66	1.88	**1.63**	1.48	1.47	1.72	1.86	**1.63**
Sal. 3000 ppm	1.33	1.52	1.61	1.76	**1.56**	1.42	1.50	1.58	1.67	**1.54**
Sal. 4000 ppm	1.28	1.44	1.57	1.63	**1.48**	1.37	1.41	1.45	1.62	**1.46**
Sal. 5000 ppm	1.14	1.32	1.46	1.46	**1.35**	1.32	1.36	1.43	1.51	**1.41**
Mean (B)	**1.38**	**1.55**	**1.66**	**1.79**		**1.46**	**1.47**	**1.63**	**1.75**	
L.S.D. at 5%	**A: 0.28**	**B: 0.09**		**AB: 0.22**		**A: 0.15**	**B: 0.07**		**AB: 0.17**	
Carotene content / flowers (mg/g F.W.)										
Control (water)	0.925	0.948	1.065	1.208	**1.037**	1.045	1.118	1.220	1.285	**1.167**
Sal. 1000 ppm	0.904	0.885	0.955	1.190	**0.984**	1.003	0.988	1.085	1.145	**1.055**
Sal. 2000 ppm	0.835	0.795	0.915	1.084	**0.907**	0.932	0.973	1.102	1.110	**1.029**
Sal. 3000 ppm	0.752	0.770	0.874	0.966	**0.841**	0.875	0.920	0.964	0.976	**0.934**
Sal. 4000 ppm	0.711	0.733	0.810	0.882	**0.784**	0.740	0.866	0.923	0.963	**0.873**
Sal. 5000 ppm	0.645	0.668	0.722	0.845	**0.720**	0.694	0.756	0.875	0.905	**0.808**
Mean (B)	**0.795**	**0.799**	**0.890**	**1.029**		**0.882**	**0.937**	**1.028**	**1.064**	
L.S.D. at 5%	**A: 0.225**	**B: 0.078**		**AB: 0.190**		**A: 0.273**	**B: 0.045**		**AB: 0.110**	
Sodium %										
Control (water)	0.86	0.79	0.75	0.72	**0.78**	0.93	0.91	0.84	0.79	**0.87**
Sal. 1000 ppm	1.32	1.27	1.17	1.22	**1.25**	1.37	1.31	1.25	1.17	**1.28**
Sal. 2000 ppm	1.55	1.49	1.42	1.35	**1.45**	1.48	1.51	1.39	1.33	**1.43**
Sal. 3000 ppm	1.72	1.58	1.45	1.38	**1.53**	1.64	1.62	1.57	1.53	**1.59**
Sal. 4000 ppm	1.95	1.88	1.79	1.73	**1.84**	1.82	1.77	1.84	1.65	**1.77**
Sal. 5000 ppm	2.31	2.24	2.12	2.18	**2.21**	2.15	2.07	1.98	1.89	**2.02**
Mean (B)	**1.62**	**1.54**	**1.45**	**1.43**		**1.56**	**1.53**	**1.48**	**1.39**	
L.S.D. at 5%	**A: 0.33**	**B: N.S 0.21**		**AB: 0.51**		**A: 0.27**	**B: N.S 0.19**		**AB: 0.46**	
Chloride %										
Control (water)	1.18	1.12	1.17	0.95	**1.11**	1.22	1.14	1.15	1.08	**1.15**
Sal. 1000 ppm	1.38	1.32	1.28	1.21	**1.30**	1.29	1.24	1.23	1.19	**1.24**
Sal. 2000 ppm	1.52	1.47	1.45	1.40	**1.46**	1.48	1.42	1.39	1.34	**1.41**
Sal. 3000 ppm	1.62	1.58	1.55	1.49	**1.56**	1.60	1.63	1.54	1.47	**1.56**
Sal. 4000 ppm	1.69	1.72	1.63	1.61	**1.66**	1.71	1.68	1.58	1.55	**1.63**
Sal. 5000 ppm	1.75	1.72	1.70	1.65	**1.71**	1.83	1.79	1.71	1.69	**1.76**
Mean (B)	**1.52**	**1.49**	**1.46**	**1.39**		**1.52**	**1.48**	**1.43**	**1.39**	
L.S.D. at 5%	**A: 0.14**	**B: N.S 0.16**		**AB: 0.39**		**A: 0.11**	**B: N.S 0.18**		**AB: 0.44**	

the increment due to these treatments over check treatment was 29.43, 11.95 and 0.50 % in the first season and 20.63, 16.55 and 6.23 % in the second one, consecutively. Such findings are consistent by Mohamed *et al.* (2018), they found that the application of foliar chitosan was significantly improved leaf chlorophyll content in sour orange seedling at different concentrations, since chitosan probably play a vital role in raising the number of chloroplasts/cell, the size and number of cells/unit area, and in stimulating chlorophyll synthesis. The effects of chitosan, in increasing chlorophylls were

confirmed in cucumber, cowpea and radish (Farouk *et al.*, 2011). These results are in harmony with Khan *et al*. (2002) who illustrated that application of chitosan enhanced photosynthesis in leaves of soybean and maize. By enhancing endogenous levels of cytokinins, which promote chlorophyll synthesis, chitosan can relieve the effect of water stress on photosynthetic pigments. As for the effect of chitosan treatments on Na and Cl percentages in the leaves of calendula plants, obtained data postulated that there was no significance between control check treatment and all chitosan treatments.

With regards to the interaction between salt stress and chitosan concentrations on proline, sodium and chloride contents, data showed that the highest mean values of proline, sodium, and chloride contents were obtained in plants irrigated with saline water 5000 ppm and receiving no chitosan treatment (5.10, 4.95 µ mole for proline, 2.31, 2.15 % for sodium and 1.75, 1.83 % for chloride) in the two seasons, respectively. Conversely, the lowest values of proline , sodium and chloride (1.88 and 1.95 µ mole for proline, 0.72 and 0.79 % for sodium and 0.95 and 1.08 % for chloride) in the two experimental seasons, respectively, were obtained from plants irrigated with tap water and sprayed with chitosan at 400 ppm. Whereas, on the total chlorophylls and carotene contents inside flowers, results indicated that the highest values (2.02 and 1.94 mg/g for total chlorophylls) and (1.208 and 1.285 mg/g for carotene contents) in the first season and in the second one, respectively were found from plants irrigated with tap water and sprayed with chitosan at 400 ppm. While, the lowest values of total chlorophylls and carotene contents (1.14 and 1.32 mg/g for total chlorophylls) and (0.645 and 0.694 mg/g for carotene contents) in the first season and in the second one, respectively, were obtained from plants irrigated with saline water at 5000 ppm without spraying chitosan.

Finally in this study, it could be concluded that to reduce the effect of salinity stress, supplying *Calendula officinalis* L. plants with the high concentration of chitosan foliar spray at 400 ppm, to obtain the best vegetative growth, flowering yield and chemical constituents of pot marigold plants which were irrigated with saline water under Beni-Suef governorate conditions.

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الملخص العربى

التأثيرات التفاعلية لإجهاد ملوحة المياه والرش الورقي للشيتوزان على النمو الخضري والزهري والمكونات الكيماوية لنبات الاقحوان

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تم اجراء تجربة اصص خلال الموسمين المتعاقبين ٢٠١٩/٢٠١٨ و ٢٠٢٠/٢٠١٩ فى غرب سمسطا، محافظة بنى سويف، مصر لدراسة تأثير المعاملات بالمياه المالحة والرش الورقي بالشيتوزان على صفات النمو الخضري والزهري وبعض المكونات الكيميائية لنباتات الاقحوان. تم معاملة النباتات بالمياة المحتوية على كلوريد الصوديوم بتركيزات ١٠٠٠ و ٢٠٠٠ و ٣٠٠٠ و ٤٠٠٠ و ٥٠٠٠ جزء في المليون ، بالإضافة إلى معاملة المقارنة (الكنترول). والرش الورقي لمعاملات الشيتوزان (١٠٠ ، ٢٠٠، ٤٠٠ جزء في المليون) ، وكذلك التداخل بينهما على صفات النمو الخضري والزهري وكذلك بعض المكونات الكيميائية لنبات الاقحوان. أظهرت النتائج المتحصل عليها أن مستويات الملوحة العالية (٤٠٠٠ و ٥٠٠٠ جزء في المليون من كلوريد الصوديوم) أدت إلى انخفاض معنوي في جميع قياسات النمو الخضري والزهري لنبات الاقحوان مقارنةً بالكنترول. لوحظ ان اعلى نقص كان عند مستوى ملوحة ٥٠٠٠ جزء في المليون من كلوريد الصوديوم والذي أظهر زيادة في محتوى البرولين الحر والنسبة المئوية للصوديوم والكلوريد. كما أدت زيادة تركيز ملوحة المياه إلى انخفاض المحتوى الكلى للكلوروفيل في الأوراق ومحتوى الكاروتين في الأزهار. كما ان الرش الورقي بالشيتوزان (٢٠٠ و ٤٠٠ جزء في المليون) ادى الى تقليل التأثير الضار للملوحة وبالتالي تحسين صفات النمو الخضرية والزهرية وبعض المكونات الكيميائية. وكانت أفضل معاملة للشيتوزان هى ٤٠٠ جزء في المليون، حيث انه لا يوجد اختلافات معنوية فى معاملات التداخل فى اغلب الصفات محل الدراسة بالمقارنة بالمعاملة الكنترول.

الكلمات المفتاحية: ملوحة الماء، الاقحوان، الشيتوزان ، صفات النمو الخضري والزهرى ، المكونات الكيميائية ، الرش الورقى.