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Effect of NaCl concentrations in irrigation water on growth and antioxidant enzymes activities of *Atriplex canescens*

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ABSTRACT

Atriplex canescens (fourwing saltbush), is an attractive plant for erosion control and reclamation of marginal lands due to its excellent adaptability. A greenhouse pot experiment was carried out at the greenhouse of Environment and Bio-agriculture Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt during 2015/2016 seasons, to study the effect of NaCl concentrations in irrigation water on growth, cations, anions and antioxidant enzymes activities of *A. canescens*. Three-months-old, uniform sized seedlings of *A. canescens* were irrigated with solution containing 0, 150, 300, 450 and 600 mM NaCl for 3 months. The results showed that, the addition of 150mM NaCl significantly increased fresh weight of *A. canescens* plants compared to control plants. Furthermore, the addition of both 300,450 and 600mM NaCl significantly reduced fresh weight of plant, compared with the control. The higher concentrations of NaCl in irrigation water reduced the dry weight. Additionally, the calcium (Ca2+), magnesium (Mg2+), and phosphorus (P3+), concentrations were decreased with increasing NaCl level. However, sodium (Na+) and chloride (Cl−) concentrations, and antioxidant enzymes activities were increased by increasing NaCl concentrations in irrigation water.

Keywords: ***Atriplex canescens*, NaCl, cations, anions, antioxidant enzymes activities**

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INTRODUCTION

Global water use has increased by a factor of six over the past 100 years and continues to grow steadily at a rate of about 1% per year as a result of increasing population, economic development and shifting consumption patterns (WWDR, 2020). Global water demand is expected to continue increasing at a similar rate until 2050, accounting for an increase of 20 to 30% above the current level of water use, mainly due to rising demand in the industrial and domestic sectors. Over 2 billion people live in countries experiencing high water stress, and about 4 billion people experience severe water scarcity during at least one month of the year (WWDR, 2019). Egypt has reached a state where the quantity of water available is imposing limits on its national economic development (Omnya *et al.*, 2018). As indication of scarcity in absolute terms, often the threshold value of 1000 m^3/capita/year is used. Egypt has passed that threshold already in nineties. As a threshold of absolute scarcity 500 m^3/ca/year is used, this will be evident with population predictions for 2025 which will bring Egypt down to 500 m^3/ca/year (MWRI, 2014). Rapid increase in population growth will threaten a severe shortage of drinking water supplies in nearest future and rapid deterioration is occurring in surface and groundwater quality (Shepl *et al.*, 2017). Globally, 70% of the fresh water is used for agricultural irrigation (Chen et al., 2017). Agriculture consumes the largest amount of the available water in Egypt, with its share exceeding 85% of the total demand for water (MWRI, 2014). It is important to look for alternative water resources that can be used for irrigation. Saline water is a common alternative to freshwater for agricultural production (Feng *et al.*, 2017). However, most conventional crops cannot tolerate very saline environments and their production under these conditions may be economically unsustainable. It has been estimated that global salt induced land degradation and resulting production losses in irrigated areas could be as high as US$27.3 billion per year (Qadir *et al.*, 2014). Salinity tolerance is a complex trait which is an outcome of several physiological and biochemical interactions (Ahanger *et al.*, 2017). However, salinity induces excessive production of reactive oxygen species (ROS), which results in oxidative injury to vital cell constituents such as nucleic acids, membrane lipids and proteins (Hameed *et al.*, 2014; Demidchik, 2015). To protect the plant from oxidative damage, however, plants produce antioxidant enzymes, which quench excessive ROS. These enzymes include superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) (Habib *et al.*, 2016). There are increasing number of halophytes which have been tested and used for food, fodder, fuel production purposes and landscaping purpose (Pessarakli, 2015; Ventura *et al.*, 2015 and).
Atriplex canescens (Pursh) Nutt. (fourwing saltbush), a C4 perennial shrub native to saline and xeric deserts in North America, belongs to family *Chenopodiaceae* with prominent resistance to salinity, drought, and cold (Hao *et al.*, 2013). This species is an attractive fodder shrub for most livestock and large animal’s due to its high palatability as well as rich nutrition (Kong, 2013). Moreover, it is especially useful for erosion control and reclamation of marginal lands due to its extensive root system and excellent adaptability (Benzarti *et al.*, 2013; Kong, 2013). In 1989, *A. canescens* was also used for soil and water conservation, sand-fixing and saline land restoration in north China (Kong, 2013). Thus, the aim of this study was to evaluate the effect of salt stress on antioxidant enzymes activity and cations, anions content of *A. canescens.*

MATERIAL AND METHODS

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Soil analysis: Soil was washed three times with distilled water then, the soil was put into plastic pots (20 cm diameter x 23 cm length) at a rate of 5 kg dry soil/pot. The physical analysis was determined according to Klute, (1986) whereas pH was determined using a WTW pH 526-meter (Eutech instruments, Singapore), soluble anions and soluble cations were determined according to Black, (1965). The chemical and physical properties are presented in Table (1).

Fertilization: The plastic pots were divided into two groups (15 pots / group). The first group was fertilized with compost fertilizer at a rate of 50 g/pots based on compost nitrogen content. Compost was kindly provided by Elsalhia Elgadeda Company, Ismailia, Egypt. The chemical and physical properties of compost are shown in Table (2). The second group was fertilized with the recommended dose of NPK fertilizers at a rate of 70 kg/ha according to Fageer and Assubaie, (2006).

Seedlings: Seedlings of *A. canescens* (80 days old) were kindly provided by Gene Bank, Desert Research Center, Cairo, Egypt. The seedlings were transplanted and grown under greenhouse conditions at the Environment and Bio-agriculture Department, Faculty of Agriculture, Al Azhar University, Cairo, Egypt.

Transplanting: Thirty uniform seedlings were selected and transplanted, under two groups of plastic pots (15 pots/group) where irrigated with 300ml distilled water for two weeks. The seedlings were watered 3 times a week. The growth conditions were 30±2ºC/25±2ºC (day/night), 15 hr. light/9 hr. dark photoperiod and 40-60% relative humidity.

Salinity treatments: Following transplanting, each of the two groups was divided into 5 NaCl treatments (3 pots/treatment). The NaCl concentrations tested were 0 as a control, 150, 300, 450 and 600Mm. The experiment was extended to three months and the pots were watered with 250 ml of each NaCl concentration 3 times/week. Control treatment (0 NaCl) was irrigated with distilled water.

Growth parameters: Shoot fresh and dry plant weights were recorded after 90 days of transplanting.

Cations and Anions analysis: **Cations:** sodium (Na+), Mg^{++}, Ca^{++} and P^{+++} concentrations were determined according to Trinder, (1951); Grindler and King, (1971); Grindler and King, (1972) and El- Merzabani *et al.*, (1977), respectively. **Anion:** chloride (Cl−) concentration was determined according to Schoenfeld and Lewellen, (1964).

Antioxidant enzymes activities assays: Superoxide dismutase (SOD); catalase (CTA) and glutathione peroxidase (GP) were determined according to Zhao *et al.*, (2001); Deisseroth and Dounce, (1970) and Wendel, (1980).

Statistical analysis: The results were statistically analyzed following analysis of variance techniques as outlined by Gomez & Gomez, (1984). The mean values were compared at 5% level of significance using least significant differences (L.S.D) test, using the GENSTATE software.

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RESULTS AND DISCUSSION

Effect of NaCl concentrations in irrigation water on shoot fresh and dry weight (g): In general, plants grown under organic fertilization conditions were heavier in fresh weight than those grown using chemical fertilization, where the fresh weight was 25.77g of plants grown under conditions of organic and 21.85 of plants grown under conditions of chemical fertilization **(Table 3)**. On the other hand, the fresh weight of *A. canescens* plants increased by increasing the salinity concentrations from 0 to 150 mM Nacl, and then decreased by increasing the salinity levels, as the 150 mM Nacl treatment recorded the highest fresh weight for the plant (38.05g), while the 600 mM Nacl treatment recorded the lowest fresh weight for the plant (6.47g). The dry weight of the plant recorded an opposite trend for the fresh weight, as the plants growing under the conditions of chemical fertilization were heavier than those growing under the conditions of organic fertilization. However, there was also a decrease in the dry weight of plants with an increase in salinity levels. The control treatment recorded the highest dry weights (12.97g), while 600 mM Nacl treatment recorded the lowest dry weights (3.04g).Similar results were mentioned by Ya-Qing *et al*., (2016) who stated that, the addition of 100 mM NaCl significantly increased fresh weight of *A. canescens* seedlings by 13%, compared to control plants. Furthermore, the addition of either 200 or 400 mM NaCl significantly reduced fresh weight of plant, compared with the control.

Table (1): Soil chemical and physical characteristics

pH	T.D.S.	Cations Meq/L				Anions Meq/L					Physical properties			
		Ca	Mg	Na	P	CO_3	HCO_3	Cl	SO_4	$CaCO_3$ (%)	Sand (%)	Silt (%)	Clay (%)	Texture
7.7	118.0	50.0	46	7.6	35	2.0	3.8	8.9	185	49.4	94.2	3.7	1.9	Sandy

Table (2): chemical analysis of compost

Water Content (%)	EC Ds/m (1:10)	pH (1:10)	Total N (%)	NH_4 (ppm)	No_3 (ppm)	OM (%)	OC (%)	Ash (%)	K/N ratio	Total K (%)	Total P (%)
14	4.06	6.55	1.06	81	357	24.34	14.17	75.75	1: 13.4	2	0.96

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Table (3): Effect of Effect of NaCl concentrations in irrigation water on shoot fresh and dry weight (g) of *A. canescens*

Treatments		Fresh Weight (g)	Dry Weight (g)
Fertilizers (F)	**Salinity (L)**		
Chemical	**Zero**	33.09	13.78
	150	34.56	12.13
	300	25.70	9.27
	450	8.90	3.62
	600	6.98	2.57
Mean		**21.85**	**8.27**
Organic	**Zero**	41.18	12.16
	150	41.55	11.90
	300	24.28	8.42
	450	15.87	4.42
	600	5.97	3.52
Mean		**25.77**	**8.09**
Mean Salinity	**Zero**	37.14	12.97
	150	38.05	12.02
	300	24.99	8.84
	450	12.39	4.02
	600	6.47	3.04
LSD at 0.5	**Fertilizer (F)**	**1.33**	**0.96**
	Levels (L)	**2.11**	**1.52**
	F X L	**2.99**	**2.15**

Effect of NaCl concentrations in irrigation water on cations and anions contents: As expected, the values of sodium ($Na+$) and chloride ($Cl-$) concentrations were significantly increased with increasing NaCl levels in irrigation water under organic and chemical fertilization. When plants treated with 600 mM NaCl, the Na+ concentration in plant shoot recorded 21 and 24-fold higher than those in control plants under chemical and organic fertilization, respectively. The chloride ($Cl-$) followed the same trend, where the $Cl-$ concentration in plant shoot recorded 23 and 21-fold higher than those in control plants under chemical and organic fertilization, respectively. These results are consistent with Bueno *et al.*, (2020) who found a significant increase in the sodium concentration with increasing salinity, *A. prostrata* at 300 mM NaCl, Na^+ concentration reached 25-fold that of the control. $Cl-$ accumulation in leaves also was associated with intensification of salinity treatments, values being 50-fold over the control. Also, Pan *et al.*, (2016) stated that under saline conditions, *A. canescens* accumulated more Na^+ which findings exhibited significant increase in different tissues of *A. canescens* seedlings. In addition, Glenn and Brown, (1998) found that Na^+ in the shoots of *A. canescens* var. *grandidentatum* increased sharply across salt levels and *A. canescens* var. *linearis* had 25% higher levels across salinities. Moreover, Silveira *et al.*, (2009) found upon treating *A. nummularia* with NaCl concentrations ranged from 0 to 600 mM, Na^+ concentrations gradually increased. Khan *et al.*, (2000) who showed that *A. griffithii* var. *stocksii* grown in pots with varying concentrations of NaCl had high Na^+ and Cl^- content in plant parts. Also, the same trend was reported by Hussin *et al.*, (2012) who showed that Cl^- concentrations

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showed a similar tendency to that of Na^+ gradually incremented in all plant organs as water salinity rose. The results clearly showed also that the Ca++, Mg++, and P+++ concentrations were significantly decreased with increasing NaCl levels in irrigation water under organic and chemical fertilization (Table 4). Waldron *et al.*, (2019) demonstrated increases in salinity resulted in decreases in Mg, and Ca contents. These effects were previously reported for *A. canescens* and *A. nummularia* (Uchiyama, 1987), Khan *et al*., (2000) who reported that, increased treatment levels of NaCl induced decreases in Ca++ and Mg+ in plants. Marschner, (1995) found that, Mg++ is essential for chlorophyll and protein synthesis. It plays an important role in the activation of some key enzyme in plants like Rubisco and ATP synthase (Marschner, 1995; Koyro, 2000) and carbohydrate synthesis (Greger and Linberg, 1987). Our *results showed* that untreated *A. canescens* plants had markedly higher P+++ concentrations (on average 8.66 µg/g dry weight). Increasing NaCl levels in irrigation water led to decrease of P+++ contents of all plant's organs, with minimum concentrations being 2.8µg/g dry weight at the highest salinity treatment. In the study conducted by Taiz and Zeiger, (2006) they indicated that, P+++ is an essential macronutrient for plants, because it is required for several key compounds, including the sugar-Pi intermediates of respiration and photosynthesis, the phospholipids of the plasma membrane, and nucleic acids, also. NaCl induces a high ionic strength in the soil, which reduces the activity of P. The uptake of P+++ into plants under salt stress may be required for the maintenance of vacuolar membrane integrity, leading to facilitating the compartmentalization of Na^+ ions within vacuoles. This compartmentalization is an important process to prevent the effect of Na^+ ions on metabolic pathways in the cytosol (Cantrell and Linderman, 2001).

Table (4): Effect of Effect of NaCl concentrations in irrigation water on Na^+, Mg^{++}, Ca^{++}, P^{++} and Cl^- content of *A. canescens*.

Treatments		Na^+ mg/g DW	Ca^{++} µg /g DW	Mg^{++} µg /g DW	P^{++} µg /g DW	Cl^- mg/g DW
Fertilizers (F)	**Salinity levels (L)**					
Chemical	**Zero**	2.11	10.52	14.26	8.24	1.48
	150	25.71	5.15	8.45	5.51	16.71
	300	30.49	4.23	7.06	4.54	20.49
	450	39.60	3.39	6.56	3.69	30.60
	600	44.34	2.79	5.71	3.00	35.01
Mean		**28.45**	**5.22**	**8.41**	**5.00**	**20.86**
Organic	**Zero**	1.84	10.35	18.30	9.08	1.50
	150	30.79	4.99	7.19	4.51	15.46
	300	30.46	4.16	6.49	3.84	18.46
	450	39.39	3.46	6.43	4.57	28.72
	600	44.65	2.47	6.27	2.60	32.65
Mean		**29.43**	**5.09**	**8.94**	**4.92**	**19.36**
Mean Salinity	**Zero**	1.98	10.44	16.28	8.66	1.49
	150	28.25	5.07	7.82	5.01	16.08
	300	30.48	4.19	6.77	4.19	19.48
	450	39.50	3.43	6.50	4.13	29.66
	600	44.50	2.63	5.99	2.80	33.83
LSD at 0.5	**Fertilizer (F)**	**2.81**	**0.25**	**0.3**	**0.41**	**0.78**
	Salinity Levels (L)	**4.45**	**0.4**	**0.48**	**0.66**	**1.23**
	F X L	**6.33**	**0.56**	**0.69**	**0.93**	**1.74**

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Effect of NaCl concentrations in irrigation water on antioxidant enzymes activities: It has been noted that there was an increase in activity of SOD, CAT and GP enzymes especially when plants were stressed at 450 and 600mM NaCl (Table 5). This profile is in accordance with Parveza *et al*., (2020) who declare that, the activities of antioxidant enzymes; SOD, CAT and POD increased in both genotypes of quinoa with increasing levels of salinity and as in the growth medium. Also, our results were compatible with Boughalleb *et al*., (2010) who studied the effect of salinity on antioxidants in two halophytes species and stated that the activity of SOD was raised significantly with the increase of salinity in *A. halimus* L. They stated that, the leaves treated with 100 and 400mM NaCl showed 57.1 and 66.3% increase in SOD activity, respectively compared with control (29.8%) plants.

Table (5): Effect of Effect of NaCl concentrations in irrigation water on antioxidant enzymes activities SOD, CAT and GP of *A. canescens*

Treatments		Antioxidant Enzymes Activity		
Fertilizers (F)	Salinity Levels (L)	Superoxide Dismutase (μg^{-1}FW)	Catalase (μg^{-1}FW)	Glutathione Peroxidase (μg^{-1}FW)
Chemical	Zero	6.33	6.43	14.67
	150	14.73	12.83	19.00
	300	16.20	14.57	23.00
	450	19.00	16.43	25.67
	600	21.33	18.53	27.00
Mean		**15.52**	**13.76**	**21.87**
Organic	Zero	7.03	4.67	13.67
	150	15.07	13.13	18.33
	300	16.33	14.20	22.00
	450	19.33	15.83	26.33
	600	21.67	18.83	27.83
Mean		**15.89**	**13.33**	**21.63**
Mean Salinity	Zero	6.68	5.55	14.17
	150	14.90	12.98	18.67
	300	16.27	14.38	22.50
	450	19.17	16.13	26.00
	600	21.50	18.68	27.42
LSD at 0.5	Fertilizer (F)	**N.S**	**N.S**	**N.S**
	Salinity Levels (L)	**1.20**	**1.2**	**1.21**
	F x L	**1.7**	**1.69**	**1.72**

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CONCLUSION

A. canescens is a moderate salt tolerant halophyte; it has the potential to complete its life cycle under high saline matrix. Its growth may be stimulated by the presence of salts in the growth medium. Salinity stress reduces Mg^{++}, Ca^{++}, P^{+++}. In the present study, it seemed that, the accumulation of Na^{+} and Cl^{-} inside *A. canescens* leaves did not exert any toxic effect not only with low concentrations but also with high concentration of 600mM NaCl. Accumulation of antioxidant metabolism resulting in prevention of oxidative damage by reducing the excess ROS accumulation thereby contributing to growth and photosynthetic protection. Salt stress indicated the adaptability of the plant to saline conditions. In addition, it can be cultivated using saline irrigation water since often high-quality irrigation water is not available for crops in arid regions and brackish waters must be used.

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تأثير تركيز كلوريد الصوديوم في مياه الري على النمو ونشاط الإنزيمات المضادة للأكسدة لنبات الأتربلكس

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الملخص العربي

يعتبر نبات الاتربلكس Atriplex canescens (شجيرة الملح ذات الأجنحة الأربعة) ، هي نبات جذاب للتحكم في التعرية واستصلاح الأراضي الهامشية نظرًا لقدرتها الممتازة على التكيف. وقد أجريت هذة التجربة داخل أصص زراعية في صوبة قسم البيئة والزراعة الحيوية ، كلية الزراعة ، جامعة الأزهر ، القاهرة ، مصر خلال مواسم 2016/2015 ، لدراسة تأثير تركيزات كلوريد الصوديوم في مياه الري على النمو ، الكاتيونات ، الأنيونات و نشاط الإنزيمات المضادة للأكسدة . تم ري شتلات نبات الاتربلكس من النوع A. canescens عمرها ثلاثة أشهر بمحلول يحتوي على صفر ، 150 ، 300 ، 450 ، 600 ملي مول كلوريد الصوديوم لمدة 3 أشهر. أظهرت النتائج أن المستويات المنخفضة من الملوحة تسبب في تثبيط طفيف للنمو ، لكن التراكيز الأعلى قللت من طول الساق ووزن النبات الطازج والجاف. بالإضافة إلى ذلك ، انخفضت تركيزات الكالسيوم Ca^{2+} والماغنسيوم Mg^{2+} والفسفور P^{3+} والبوتاسيوم K^{+} مع زيادة مستويات الملوحة ، بينما تمت زيادة تركيزات الصوديوم والكلور وزيادة نشاط الانزيمات المضادات للاكسدة بزيادة مستويات ملوحة مياه الري.