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MITIGATING THE EFFECTS OF SALINITY BY FOLIAR APPLICATION OF NITROPHENOLATE BASED BIOSTIMULANT ON CALENDULA PLANT

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Abstract

This investigation was conducted at the Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University, Giza, Egypt during years 2015/2016 and 2016/2017 aims to study the response floral characters and chemical composition of *Calendula officinalis* plants to the treatments of salinity at the levels (0, 1000, 2000 and 3000 ppm) and Atonik at the rate of 0, 1 and 2 ml/L. The results showed that raising the salt concentration in the irrigation water caused a significant reduction of inflorescence parameters. Atonik at level 2 ml/L gave the highest number of ray florets and inflorescence diameter. Applying atonic to stressed plants by salinity had a positive significant effect on the number of ray florets, inflorescence diameter and total flavonoids. Salinity at the concentration of 3000 ppm resulted in 46.2 and 113.5 ray florets/inflorescence while reaching 172.5 and 156.5 when spraying the stressed plants with Atonik 2 ml /L in the first and second seasons, respectively. The highest dose of Atonik (2 ml/L) gave the highest value of N, P and K % in both seasons. Control plants gave the best values of Carotenoids. The highest activity of catalase enzyme obtained from plants irrigated by 3000 ppm saline water and sprayed with 2 ml/L Atoink (0.196 U/g) compared to control plants (0.123 U/g). It could be concluded that, Atonik helped the plants to tolerate the salt stress and promoted flowering growth by increasing the endogenous protective catalase enzyme.

Key words : Calendula Plant, Atonik, inflorescence parameters, flavonoids.

Introduction

Marigold (*Calendula officinalis*) is an annual plant belongs to family Asteraceae. It is an important medicinal and ornamental plant. The native range of marigold is between the Mediterranean Sea regions, Egypt and Europe (Nofal *et al.,* 2015). It is an erect herbaceous and grows up to 80 cm in height. Marigold has a composite flower (Muley, *et al.,* 2009). The traditional uses of marigold flower are used as a stimulant and antispasmodic (Khavarinejad and Lucia, 2004). Calendula is used internally for the alleviation of gastrointestinal disorders and externally, as an ointment or lotion, for the treatment of minor wounds and rashes. Flavonoids, triterpenoids, essential oil, and polysaccharides are the principal constituents of calendula flowers. Calendula flowers have not less than 0.4% of flavonoid content (Evans *et al*., 2002).

Abiotic stresses such as drought, excessive watering, temperatures, and salinity effect negatively on plant growth. Salt stress is one of the major environmental factors limiting crop production. The salinity problem became of great importance for agriculture production. Salinity causes inhibition in the growth of plants is the result of osmotic and ionic effects (Munns, 2002).

Another important cultural practice that markedly affects plant growth is the application of Atonik. Atonik is improving plant growth and rooting (Arora *et al*., 1981).

The objective of this study was to investigate the effect of irrigation water salinity on the flowering and chemical composition of *Calendula officinalis* plants and to assess the possibility of using Atonik treatments to overcome the adverse effects of the water salinity treatments.

Materials and Methods

This study was carried out at the Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University, Giza, Egypt, during the two successive seasons of 2015/2016 and 2016/2017. The aim of this study to investigate the response floral characters of *Calendula*

officinalis plants to irrigation water salinity and Atonik treatments.

Experimental Procedures

On 1st October (2015/2016 and 2016/2017), the seedlings of double-flowered marigold obtained from El Fayoum governorate, Egypt. At the experimental field, the seedlings were planted in a plastic pot (30 cm) which filled with a mixture of clay and sand (2:1) with the following characteristics: sand 70.30 %, silt 10.15 %, clay 19. 25 %, pH 7.80, EC 2.40 dS/m, available N 7.30 ppm, available P 4.40 ppm, available K 0.83 ppm, and $CaCO_3$ 0.26%.

At the end of October (in both seasons), the saline irrigation water was initiated at levels (0, 1000, 2000 and 3000 ppm) according to methods described by Kester *et al.,* (1967). Starting from 1 December, the plants were sprayed monthly with Atonik at rates 0, 1 and 2 ml/L until 1stFebruary. Control plants were sprayed with tap water.

Experimental design

The layout of the experiment was a randomized complete block design with two factors of salt stress (4 levels) and Atonik treatments (3 doses).

Recorded data

On 15th March 2015/2016 and 2016/2017, the plants were harvested and data were recorded on the following characteristics:

Flowering growth

The following characters were recorded in both seasons:

Inflorescence fresh and dry weights(g)

Number of ray florets/ Inflorescence

Inflorescence diameter (cm)

Fresh weight of ray florets / Inflorescence

Chemical composition

Nitrogen contents (N %) were determined in both seasons in a known weight (0.25 gm) of the dried herb. Total nitrogen content was determined using the micro-Kjeldahl method, as described by (Pregl, 1945).

Phosphorus (P) content (%) was estimated in both seasons using the method described by King (1951).

Potassium (K) content (%) in both seasons according to the methods described by Cottenie *et al*., (1982).

Catalase U/g in fresh herb in the second season. Catalase reacts with a known quantity of H_2O_2. The reaction is stopped after exactly one minute with catalase inhibitor. In presence of peroxidase (HRP), remaining H_2O_2 reacts with 3, 5-dichloro-2-hydroxybenzen sulphonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with color intensity inversely proportional to the amount of catalase in the original sample. Which briefly required the incubation of a test tube containing 0.5 ml of hydrogen peroxide and 0.1 ml of sample (honey dissolved in ice-cold phosphate buffer pH= 7.3) and 0.5 ml of phosphate buffer pH 7. After incubation at 25°C for 60 sec, then adding 0.5 ml of Chromogen-inhibitor. Incubated 10 min at 37°C, sample absorbance (A sample) against sample blank and standard (A standard) against standard blank at 510 nm using UV/ Vis Spectrophotometer, Jenway, England, (Beyer and Fridovich, 1987).

Total carotenoids (mg/g D.W.) in the second season were determined in dried ray florets according to Britton *et al.* 1995.

Total flavonoids (mg/g D.W.) in the second season were determined in dried ray florets according to Gouveia and Castilho, 2011.

Statistical analysis

The data was analyzed using the Least Significant Difference (L.S.D.) to find out the significance of the difference between salt stress and Atonik treatments (Little and Hills, 1987).

Results and Discussion

Inflorescence fresh and dry weights (g)

The data in table 1 showed that raising the salt concentration in the irrigation water caused a significant reduction of inflorescence fresh and dry weights(g) of *Calendula officinalis* plants. The highest value of inflorescence weight fresh (9.514 and 8.604 g) and inflorescence dry weight (1.372 and 1.256g) in the first and second seasons, respectively obtained from plants irrigated with tap water. On the other hand, plants irrigated with salt concentration at 3000 ppm gave the highest reduction of inflorescence fresh and dry weights of *Calendula officinalis* plants table 1.

The effect of Atonik treatments on inflorescence fresh and dry weights(g) of *Calendula officinalis* plants was non-significant in both seasons. The data in the same Table also showed that the plants spraying with the highest dose of Atonik (2 ml/L) gave the best value of inflorescence fresh and dry weights. These results agree with Heikal (2017) on *Salvia farinacea* plant.

Regarding the interaction between salt stress and Atonik treatments was non-significant on inflorescence fresh and dry weight. The data in table 1 indicated that the highest value of inflorescence fresh and dry weights was found in plants irrigated with tap water and sprayed

Table 1: Effect of salinity and Atonik application on inflorescence fresh and dry weights (g) of *Calendula officinalis* L.

	First season							
	Inflorescence fresh weight(g)				Inflorescence dry weight (g)			
	Control	A1	A2	Mean(S)	Control	A1	A2	Mean(S)
Control	8.127	9.673	10.74	9.514	1.163	1.377	1.577	1.372
S1	8.160	8.367	9.477	8.668	1.160	1.193	1.420	1.258
S2	5.750	5.520	7.547	6.272	0.883	0.903	1.067	0.951
S3	4.267	4.633	6.300	5.067	0.640	0.727	0.980	0.782
Mean(A)	6.576	7.048	8.517		0.962	1.050	1.261	
Lsd at .05								
S		N.S.				0.24		
A		0.91				0.14		
S*A		N.S.				N.S.		
Second season								
Control	7.773	8.503	9.537	8.604	1.123	1.207	1.437	1.256
S1	6.863	7.603	8.033	7.500	0.973	1.107	1.190	1.090
S2	5.217	4.983	6.033	5.411	0.777	0.773	0.963	0.838
S3	3.910	5.01	4.903	4.608	0.543	0.767	0.817	0.709
Mean(A)	5.941	6.525	7.127		0.854	0.963	1.102	
Lsd at .05								
S		N.S.				N.S.		
A		0.98				0.14		
S*A		N.S.				N.S.		

*S1= saline water at 1000 ppm, S2= saline water at 2000 ppm, S3= saline water at 3000ppm, A1= 1 ml/L Atonik, A2=2 ml/L Atonik

Table 2: Effect of salinity and Atonik application on number of ray florets/ inflorescence and inflorescence diameter (cm)of *Calendula officinalis* L.

	First season							
	Number of ray florets/Inflorescence				Inflorescence diameter (cm)			
	Control	A1	A2	Mean(S)	Control	A1	A2	Mean(S)
Control	238.5	240.0	338.0	272.2	7.503	8.653	8.850	8.335
S1	231.0	224.5	258.2	237.9	7.503	7.750	8.250	7.834
S2	103.0	120.0	211.4	144.8	7.653	7.350	8.253	7.752
S3	46.20	105.0	172.5	107.9	6.670	7.403	6.750	6.941
Mean(A)	154.7	172.4	245.0		7.332	7.789	8.026	
Lsd at .05								
S		3.38				0.04		
A		3.70				0.06		
S*A		6.40				0.12		
Second season								
Control	271.0	298.8	315.8	295.2	7.303	7.453	8.353	7.703
S1	163.0	240.0	230.5	211.2	8.003	8.000	7.553	7.852
S2	135.5	121.0	168.0	141.5	7.453	7.003	7.653	7.370
S3	113.5	108.5	156.5	126.2	5.400	6.953	7.450	6.601
Mean(A)	170.8	192.1	217.7		7.040	7.352	7.752	
Lsd at .05								
S		0.19				0.12		
A		0.49				0.11		
S*A		0.86				0.19		

S1= saline water at 1000 ppm, S2= saline water at 2000 ppm, S3= saline water at 3000ppm, A1= 1 ml/L Atonik, A2=2 ml/L Atonik

by 2 ml/L Atonik. On the other hand, the lowest value of inflorescence fresh and dry weights was obtained from plants irrigated with high salt concentration (3000 ppm) in both seasons.

The reduction in floral characters is in harmony with Nofal *et al*., (2015) and Bayat *et al*., (2012) on *Calendula officinalis* plants.

3.2 Number of ray florets/ inflorescence and inflorescence diameter (cm)

Data in table 2 in both seasons showed that increasing salinity levels from 1000 to 3000 ppm led to steadily decreased the mean value of the number of ray florets and inflorescence diameter (cm) compared to control plants. The data in the same table also showed that spraying plants with Atonik at level 2 ml/L gave the highest number of ray florets and inflorescence diameter (245.0, 217.7 and 8.026,7.752) in the first and second seasons respectively.

As regards the effect of the interaction of salt stress and Atonik treatments on marigold plants, it has a significant effect on the number of ray florets and inflorescence diameter. The data in table 2 indicated that irrigated plants by tap water and sprayed with Atonik at level 2 ml/L gave the highest mean value of the number of ray florets and inflorescence diameter (338, 315.8 and 8.850, 8.353) in the first and second seasons respectively. On the other hand, plants irrigated with high salt concentration at 3000 ppm and sprayed with zero Atonik gave the lowest number of ray florets and inflorescence diameter in both seasons. High salinity

causes an increase in reactive oxygen species and metabolic toxicity in the absence of any protective mechanism, this may explain the decrease in the flowering parameters with the salinity treatment as shown data in tables 1and 2. Similar results reported by Pacheco *et al*., (2013), Nofal *et al*., (2015) and Bayat *et al*., (2012) on *Calendula officinalis*.

Nitrogen, Phosphorus and Potassium content (%)

The results in table 3 indicated that N, P, and K of *Calendula officinalis* were decreased steadily by raising the salt concentration in irrigation water. According to the highest value of N % (3.21 and 3.53), P% (0.30 and 0.27%) and K% (1.44 and 2.02%) in the first and second seasons, respectively were obtained from control plants (irrigated with tap water). On the other hand, plants irrigated with the highest salt concentration (3000 ppm) had the lowest value of N% (1.67 and 1.84%), P% (0.06 and 0.08%) and K% (0.37 and 0.51%) in the first and second seasons, respectively. This effect may be due to that leaf nitrogen is associated with the photosynthetic apparatus, the plant responds to water shortage by optimizing its use of nitrogen and slow down the leaf expansion and the production of new tissues (Lambers *et al*., 2008). These results are similar to those obtained by Mazhar *et al*., (2012) on *Chrysanthemum indicum* and Nahed *et al*., (2011) on *Matthiola incana.*

Table 3 showed the effect of Atonik treatments on N, P and K%. the data showed that the highest dose of Atonik (2 ml/L) gave the highest value of N% (2.74 and 3.02%), P % (0.19 and 0.20%) and K % (1.12 and 1.36%) in the first and second seasons, respectively. Moreover, the interaction between salt stress and Atonik treatments indicated that irrigation plants with tap water and sprayed by Atonik at 2 ml/L gave the best value of N% (4.25 and 4.68), P% (0.38 and 0.31%) and K% (2.01 and 2.40%) in the first and second seasons, respectively.

Catalase U/g activity

The effect of salinity on the activity of Catalase U/g in *Calendula officinalis* presented in tabled 4. Catalase (CAT) activity has been seen to bedecisive and fateful for the cellular defense against salt stress in leaves (Chaparzadeh *et al.,* 2004). Data in table 4 showed that using saline water at level 2000 ppm increased the activity of catalase enzyme (0.176 U/g) followed by using saline water at level 3000 ppm (0.172 U/g). Moreover, Atonik treatments enhanced the activity of catalase enzyme. Results in Table 4 showed that using Atonik at the dose (1 or 2 ml/L) gave the same effect on the activity of catalase enzyme with the value of 0.164 U/g. Regarding

Table 3: Effect of salinity and Atonik application on Nitrogen, Phosphorus and Potassium contents (%) in dry herb of *Calendula officinalis* L.

	First season											
	Nitrogen %				Phosphorus%				Potassium%			
	Control	A1	A2	Mean (A)	Control	A1	A2	Mean(S)	Control	A1	A2	Mean (S)
Control	2.60	2.78	4.25	3.21	0.21	0.31	0.38	0.30	1.12	1.20	2.01	1.44
S1	2.40	2.41	2.50	2.44	0.14	0.17	0.19	0.17	1.01	1.02	1.10	1.04
S2	1.93	2.02	2.39	2.11	0.10	0.13	0.13	0.12	0.71	0.71	0.83	0.75
S3	1.44	1.73	1.83	1.67	0.04	0.06	0.07	0.06	0.15	0.42	0.53	0.37
Mean(S)	2.09	2.23	2.74		0.12	0.17	0.19		0.75	0.84	1.12	
L.S.D (0.05)												
S	0.11				0.06					0.15		
A	0.04				0.03					0.04		
S* A	0.08				n.s					0.08		
Second season												
Control	2.86	3.05	4.68	3.53	0.25	0.26	0.31	0.27	1.55	2.11	2.40	2.02
S1	2.64	2.65	2.75	2.68	0.19	0.20	0.23	0.21	1.02	1.03	1.40	1.15
S2	2.12	2.22	2.62	2.32	0.12	0.16	0.18	0.15	0.87	0.97	0.99	0.94
S3	1.59	1.91	2.01	1.84	0.06	0.08	0.09	0.08	0.34	0.54	0.65	0.51
Mean(S)	2.30	2.46	3.02		0.16	0.17	0.20		0.95	1.16	1.36	
L.S.D (0.05)												
S	0.22				0.04					0.24		
A	0.07				0.03					0.04		
S* A	0.14				n.s					0.08		

*S1= saline water at 1000 ppm, S2= saline water at 2000 ppm, S3= saline water at 3000ppm, A1= 1 ml/L Atonik, A2=2 ml/L Atonik.

Table 4: Effect of salinity and Atonik application on Catalase content in fresh herb of *Calendula officinalis* L.

	Catalase U/g			
	Control	**A1**	**A2**	**Mean(S)**
Control	0.123	0.130	0.132	0.128
S1	0.150	0.168	0.178	0.165
S2	0.187	0.193	0.148	0.176
S3	0.156	0.163	0.196	0.172
Mean(A)	0.154	0.164	0.164	

*S1= saline water at 1000 ppm, S2= saline water at 2000 ppm, S3= saline water at 3000ppm, A1= 1 ml/L Atonik, A2=2 ml/L Atonik.

the interaction between salt stress and Atonik treatments effect on the activity of catalase U/g in *Calendula officinalis*. The highest activity of catalase enzyme obtained from plants irrigated by 3000 ppm saline water and sprayed with 2 ml/L Atoink (0.196U/g) compared to control plants which gave the lowest activity of catalase enzyme (0.123 U/g). It can be said that Atonik helped the plants to tolerate the salt stress and promoted flowering growth as data shown in tables (1, 2, 3 and 4) by increasing the endogenous protective catalase enzyme. These results agreed with Lacramioara *et al*., (2015) on *Calendula officinalis*.

Flavonoids content

The obtained results in table 5 showed that total flavonoids content reduced steadily by raising the salt concentration in irrigation water. The high concentration (3000 ppm) gave the lowest value of total flavonoids (11.34 mg/g d.w.) compared with control, which gave the highest value of total flavonoids (13.24 mg/g d.w.). The data in the same table also noticed that the highest concentration of Atonik (2ml/L) gave the best value of total flavonoids (12.72 mg/g d.w.). Regarding the interaction between salt stress and Atonik treatment was significant on total flavonoids. Data presented indicated that control plants gave the best values of total flavonoids. On the other hand, plants irrigated with saline water at 3000 ppm and non-sprayed with Atonik gave the lowest values of total flavonoids. These results agreed with Olennikov *et al*., (2017) studied the chemical composition of 23 double-flowered varieties of *Calendula officinalis*. They found that the variation of flavonoids ranged between 10.52 and 46.87 mg/g d.W. Also Gruszczyk and Berbeæ, 2004 studied the effect of the foliar application of Atonik (0.2 percent) on *Chrysanthemum parthenium*. They found that Atonik had a positive effect on plant growth, resulting in a significant increase in active components.

Carotenoids content

Carotenoids are used as food colorants and in nutritional supplements because it has antioxidant property, vitamin A, enhancement of the immune system and reduces the risk of cancer and cardiovascular diseases (Fraser and Bramley, 2004).

Data in table 5 indicated that the high concentration (3000 ppm) gave the lowest value of Carotenoids (26.54 mg/g d.w.) compared with control, which gave the highest value of Carotenoids (40.76 mg/g d.w.). In the same Table also noticed that the effect of Atonik treatments was significant on carotenoids content. The best value of Carotenoids (35.43 mg/g d.w.) obtained from plants sprayed with Atonik at level 1ml/L. Regarding the interaction between salt stress and Atonik treatment was significant on Carotenoids content. Plants irrigated with saline water at 3000 ppm and non-sprayed with Atonik gave the lowest values of Carotenoids (24.80 mg/g d. w.) compared to control plants, which gave the best values of Carotenoids. These results agreed with Grigore and Oprica (2016) and Kozminska *et al*., (2017) on *Calendula officinalis*.

Table 5: Effect of salinity and Atonik application on total carotenoids and flavonoids contents (mg/g) in dry ray florets of *Calendula officinalis* L.

	Second Season							
	Carotenoids mg /g d. w.				Total flavonoids mg/g d. w.			
	Control	**A1**	**A2**	**Mean(S**	**Control**	**A1**	**A2**	**Mean(S)**
Control	41.37	42.2	38.7	40.76	12.97	13.58	13.18	13.24
S1	41.07	33.53	36.33	36.98	13.97	12.93	13.15	13.35
S2	32.2	36.3	35.83	34.78	11.96	12.26	12.81	12.34
S3	24.8	29.67	25.16	26.54	10.94	11.36	11.72	11.34
Mean(A)	34.86	35.43	34.01		12.46	12.53	12.72	
L.S.D (0.05)								
S		1.163				0.315		
A		0.707				0.179		
S*A		2.014				0.545		

*S1= saline water at 1000 ppm, S2= saline water at 2000 ppm, S3= saline water at 3000ppm, A1= 1 ml/L Atonik, A2=2 ml/L Atonik.

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