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INFLUENCE OF FOLIAR APPLICATION OF SALICYLIC ACID ON GROWTH AND FLOWERING OF *CALENDULA OFFICINALIS* L. UNDER LEVELS OF SALINITY

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ABSTRACT: Global climatic changes limited the fresh water supply, which led to increasing the need for irrigation water. For this reason, the main objective of this study was to investigate the influence of diluted seawater irrigation (0, 10, 20 and 30 % seawater) and trying to counteract the adverse effect of seawater salinity by spraying with salicylic acid (SA)(0, 150, and 300 ppm). Additionally, during the two successive seasons of 2017 and 2108 seasons an experimental pots research in Sakha horticultural research station at Kafr El-Sheik Governorate, Egypt, was performed to study the effects of irrigation with diluted seawater combined with foliar application of salicylic acid at on growth, flowering and survival percentage parameters, along with the chemical composition of *Calendula officinalis L.* plants. The results exposed that irrigation with high levels of diluted seawater at 20 and 30 % decreased growth, root and flowering parameters, total green color, carotene contents in flowers and leaf N, P, K, Ca contents and survival % meanwhile, Na, Cl, Na/K ratio and proline were increased. All tested foliar applications of SA especially at 300 ppm increased all vegetative growth, roots and flowering traits. The most effective treatments which enhance growth, flowering parameters, mineral contents, and survival% were found to be application of 0 or 10 % seawater in combination with spraying SA at 150 and 300 ppm. Thus, it could be recommended to irrigate Calendula plant with diluted seawater at 10 % with spraying salicylic acid at 300 ppm to give the best results in terms of growth, flowering and survival %.

Key words: *Calendula officinalis L.,* seawater, salicylic acid, growth, flowering parameters

INTRODUCTION

***Calendula officinalis* L. (Family, *Asteraceae*) known as Calendula or pot marigold is an annual specie widely used around the world as a medicinal, gardens and landscape plant. It grows in sun or partial shade and is easy to grow requiring little cultivation (Dole and Wilkins, 2004). There are numerous varieties of this species, differing primarily in flower shape and color (Hamburger *et al.,* 2003).**

In abiotic stresses, drought and salinity are two environmental factors, which are responsible for the huge loss in agricultural demand and productivity all over the world (Gul and Ahmad, 2004). Water scarcities were expected to lead to loss of crop production globally up to 30 % by 2025 year compared to the current yield. Salinity of agricultural soil is becoming a serious issue due to increasing shortage of fresh water and subsequent necessity to utilize saline water or low-quality water for irrigation (Semiz and Suarez, 2015). Therefore, at the current situation of intention for improving agricultural production under limited water resources, it is required to grow crops that are capable of tolerating drought and water stress (Yao *et al.,* 2016). It would be the most economical approach to improve productivity based on limited water resources. Agricultural

production consumes more fresh water than any other human activity. To cope with the scarcity of fresh water for the sustainable development of agriculture, there is increasing awareness among agricultural scientists and planners in the utilization of seawater (at least diluted) for irrigation of crops (Jin *et al.,* 1999 and Liu *et al.,* 2003). To increase the sustainable agriculture as much as the use of saline water, there are two traits have to be considered: select the plants tolerant to salinity and treating the plants to enhance their cope to the salinity (Cassaniti *et al*., 2012). At the latest two decades, the global climate relatively had changed which already affected the environment in many ways. The most impacts of global climate change are climate drought and a shortage of irrigation water, especially in arid and semiarid regions. Likely, Egypt will be suffering the plight of water shortage in next decades. Therefore, the search for other sources of irrigation water should be a priority. One of another sources of water irrigation is the use of diluted seawater to compensate for the shortage of irrigation water. The high concentration of ionic elements in seawater is the main restricting factor in the utilization of seawater for irrigation (Xiao-Hua *et al.,* 2009). There are several disadvantages when irrigation with saline water induced abiotic stress and toxic effects on plants which lead to gradual decline in photosynthesis and respiration rates and deterioration in, proteins and nucleic acids ((Munns *et al.,* 1995). Salicylic acid (2-hydroxy benzoic acid) is an endogenous growth regulator of the phenol nature, which participates in the regulation of physiological processes in plants. It plays an important role in the plant response to adverse environmental conditions such as salinity (Baby *et al.,* 2010). Salicylic acid biosynthetic pathway in plants has two distinct pathways, the isochorismate (IC) pathway and the phenylalanine ammonialyase (PAL) pathway. Moreover, salicylic acid plays exclusive role in plant growth, thermo genesis, flower induction and uptake of ions and has diverse effects on tolerance to abiotic stress (Van Breusegem *et al.,* 2001). It affects ethylene biosynthesis, stomatal movement and reverses the effects of ABA on leaf abscission (Yusuf *et al.,* 2013). Therefore, the objective of this study was to evaluate the effects of diluted seawater irrigation and salicylic acid treatments on growth and flowering parameters of calendula plant.

MATERIALS AND METHODS

A pot experiment was carried out during the two successive seasons of 2017/2018 and 2018/2019. Trials took place in Sakha horticultural research station at Kafr El-Sheikh Governorate, Egypt to investigate the influence of diluted seawater irrigation (0, 10, 20, and 30 %) on growth, flowering and chemical composition of pot marigold plant (*Calendula officinalis* L.). Furthermore, trying to counteract the adverse effect of seawater salinity by spraying with salicylic acid at (0, 150 and 300 ppm). In the Sakha Horticultural Research Station, local seeds of pot marigold (*Calendula officinalis* L.) were sown in polyethylene plug trays filled with a mixture of peat moss and vermiculite (2:1 v / v) on October 1St in both growing seasons. The seedlings were transplanted in the pots after 30 days from sowing; the seedlings were planted individually in black plastic pots (20 cm diameter) packed with 7 kg sandy clay soil (2 clay: 1 sand v/v) and it was stayed in open field. Soil samples were taken before the establishment of the study for of the chemical properties (Table, 1) according to Jackson (1967).

Seawater for irrigation was taken from the sea in Baltim town. Its salinity was approximately 42500 ppm in the first and second seasons, respectively. Before irrigation, seawater was diluted with tap

water in a plastic tank to the needed dilution 0, 10, 20 and 30 % seawater. The diluted seawater at various concentrations was used for irrigation after transplanting 20 days through the course of the experiment. The chemical properties of seawater and diluted seawater used for irrigation are shown in Tables (2 and 3).

Three concentrations of salicylic acid were used 0, 150 and 300 ppm as foliar spraying. Salicylic acid was dissolved in an absolute ethanol and then added to water (ethanol: water, 1: 1000 v/v) as explained by Williams *et al*., (2003). Spraying of SA was done after approximately one month from transplanting (November 1st) and was repeated every 15 days for 3 times. Foliar sprayings were applied using a hand-held sprayer. To prevent interferences with different moisture levels, the same quantity of distilled water was sprayed to the control plants at the same time.

The experiment layout was set in a completely randomized design in factorial with two factors (4 seawater irrigation levels x 3 salicylic acid concentrations). Each treatment was replicated three times with nine seedlings for each replicate (3 replicates x 9 seedlings).

Table (1): Physical and chemical properties of the used soil

Ec (dSm^{-1})	O.M (%)	pH	Soluble cations (meq/l)				Soluble anions (meq/l)		
			Ca^{++}	Mg^{++}	K^{+}	Na^{+}	So_4^{--}	HCO_3^{-}	Cl^{-}
1.36	1.24	7.79	3.5	2.98	0.18	6.93	4.03	7.90	1.66
Particle size divisions (%)			Textural class	Available nutrients (ppm)					
Sand	Silt	Clay		N		P		K	
36.37	21.50	42.13	Clayey Sand	233.75		1.31		440.15	

Table (2): Chemical analysis of seawater (mean of two seasons)

Ions Sample	N %	P ppm	K ppm	Ca ppm	Mg ppm	Na ppm	Cl Meq/l	pH	EC dsm^1
Seawater	1.45	116.14	252	32	175	13805	1.6	8.33	57.4

Table (3): pH and EC values of the various diluted seawater treatments at the starting of the experiment

Seawater (%)	pH		EC (dsm^{-1})	
	2017	2018	2017	2018
0	8.5	8.31	0.49	0.46
10	7.81	8.11	5.10	7.77
20	7.99	8.12	8.56	8.88
30	7.78	8.00	17.32	18.60

Data recorded:

Vegetative and root growth measurements and survival % :

Vegetative and root growth parameters were recorded after 90 days from transplanting and included: plant height (cm), number of shoots plant^{-1} number of leaves plant^{-1}, total leaf area plant^{-1} (cm^2), fresh and dry weight (g) of vegetative and root parts as well as root length (cm). and the percentage of survival plants was calculated.

Flowering measurements:

When the flowers were full open, number of flowers plant^{-1}, diameter of flower (cm), fresh and dry weights of flowersplant^{-1} were determined.

Nutritional status:

Twenty mature leaves plant^{-1} (5th fresh leaf) were sampled in both seasons, washed, dried at 70°C to a constant weight, ground and digested for determination leaf minerals (nitrogen, phosphorus, potassium, calcium, sodium, and chloride %). Total nitrogen was determined by micro-Kjeldahle method as summarized by Chapman and Pratt (1978). Phosphorus was determined using spectrophotometer according to Murphy and Riely (1962). Potassium and sodium were determined by flame photometer as described by Jackson (1967), Brown and Lilleland (1946). Chloride was determined according to Kraemer and Stamm (1924). Calcium was determined as described by Cheng and Bray (1951). Carotene contents in flowers were determined as mentioned by Nagata and Yamashita (1992). Proline was assessed colorimetric ally in fresh samples as illustrated by Bates *et al.,* (1973). Total green color was determined in mature leaves by using MINOLTA CHLOROPHYLL METER SPAD-502 (Minolta camera. Co, Ltd Japan) (Wood *et al.,* 1993).

Statistical Analysis:

The mean and ANOVA were analyzed using MSTATC computer software program (Bricker, 1991). The obtained data were subjected to analysis of variance according Snedecor and Cochran (1990). Duncan's multiple range test (Duncan, 1955) for significance was determined at $p < 0.05$.

RESULTS AND DISCUSSION

1- Vegetative and root growth:

a-Vegetative growth:

Results presented in Tables (4 and 5) revealed that irrigation by diluted seawater at 10, 20 and 30% seawater significantly decreased all vegetative growth parameters (plant height, number of shoots plant-1, number of leaves/plant, total leaf area plant-1, plant fresh and dry weight) compared with control (tap water) in both seasons. The highest values were obtained by control followed in descending order to 10, 20 and 30 % seawater except for the parameter of dry weight plant-1 in the second season only which gave the highest values by control and diluted seawater at 10 % treatments without significant differences in between. Reducing vegetative growth parameters by high salinity may be associated with the reducing of turgor pressure and the high energy consumed of massive salt secretion and osmoregulation. Moreover, excess salt decreases the leaf water potential, as in water deficit conditions, reduces water and nutrients uptake by plants, and finally leads to decrease the growth (Xiao-Hua et al., 2009). Also, abiotic stresses counting salt-stress induce accumulation of reactive oxygen species (ROS) that are harmful to cells at high concentrations as they cause the oxidative injury to membrane lipids, proteins, and nucleic acids (Ashraf and Harris, 2004). The obtained results agree with those

reported by Hashish et al., (2015), Nofal et al., (2015) and Abou El-Ftouh et al., (2018) on Calendula plants. In addition, El-Mahrouk et al., (2010) on buttonwood plants, Turhan et al., (2014) on lettuce, Bafeel et al., (2016) on Jojoba plants and El- Sayed et al., (2017), on Duranta plumieri. They indicated that the plant growth was negatively correlated with increasing saline water levels. The values of growth parameters were reduced with increasing salinity levels.

Table (4): Effect of diluted seawater irrigation, salicylic acid spray levels and their interaction on plant height and No. of shoots and leaves plant^{-1} of *Calendula officinalis* L. during 2017/2018 and 2018/2019 seasons

Treatments		Plant height (cm)		No. of shoots plant^{-1}		No. of leaves plant^{-1}	
		1st season	2nd season	1st season	2nd season	1st season	2nd season
Seawater (%)							
Cont.		47.22a	46.44a	10.78a	10.22a	279.00a	275.78a
10 %		43.56b	43.33b	9.22b	8.78b	257.44b	260.78b
20 %		40.33c	40.11c	7.67c	7.22c	169.33c	172.67c
30 %		36.11d	36.00d	6.44d	7.00c	151.22d	152.33d
Salicylic acid (ppm)							
0		38.42c	37.75c	7.50c	7.25b	198.67c	198.67b
150		41.75b	41.00b	8.50b	8.42a	218.50b	220.67a
300		45.25a	45.67a	9.58a	9.25a	225.58a	226.83a
Seawater	SA (ppm)	Interaction					
Cont.	0	45.00bcd	43.67cd	9.67bcd	9.33abcd	259.67b	258.67c
	150	46.33bc	46.00bc	10.67b	10.33ab	285.00a	278.33ab
	300	50.33a	49.67a	12.00a	11.00a	292.33a	290.33a
10 %	0	39.33fg	39.67e	8.00ef	7.33efg	240.67c	242.00d
	150	43.33cde	42.67d	9.33cd	9.00bcde	262.33b	270.33bc
	300	48.00ab	47.67ab	10.33bc	10.00abc	269.33b	270.00bc
20 %	0	37.33g	35.33f	6.67ghi	6.33g	154.67e	158.00g
	150	40.67ef	39.67e	7.67efg	7.00fg	173.00d	176.33ef
	300	43.00de	45.33bc	8.67de	8.33cdef	180.33d	183.67e
30 %	0	32.00h	32.33g	5.67i	6.00g	139.67f	136.00h
	150	36.67g	35.67f	6.33hi	7.33efg	153.67e	157.67g
	300	39.67fg	40.00e	7.33fgh	6.67defg	160.33e	163.33fg

Means within a column having the same letters are not significantly differences according to Duncan's multiple range test (DMRT).

Table (5): Effect of diluted seawater irrigation, salicylic acid spray levels and their interaction on total leaf area and fresh and dry weight plant^{-1} of *Calendula officinalis* L. during 2017/2018 and 2018/2019 seasons

Treatments		Total leaf area (cm^2)		Fresh weight plant^{-1} (gm)		Dry weight plant^{-1} (gm)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season
Seawater (%)							
Cont.		5252a	5292.26a	251.78a	259.79a	33.35a	34.77a
10 %		4667.67b	4642.73b	229.65b	241.34b	32.21b	34.13a
20 %		2791.83c	2892.96c	206.81c	214.80c	30.41c	31.92b
30 %		2342.98d	2337.66d	176.96d	184.15d	27.44d	28.63c
Salicylic acid (ppm)							
0		3265.09c	3320.15c	206.66c	213.81c	28.44c	29.71c
150		3884.44b	3868.17b	213.99b	221.41b	30.74b	31.98b
300		4141.82a	4185.88a	228.26a	239.83a	33.39a	35.39a
Seawater	SA (ppm)	Interaction					
Cont.	0	4639.72d	4837.29c	247.90b	250.50b	31.72cd	32.45d
	150	5329.28b	5270.75b	249.47b	254.27b	33.26b	33.86c
	300	5788.92a	5768.74a	257.98a	274.60a	35.06a	37.98a
10 %	0	4090.17e	4108.47d	220.80e	230.80d	29.92e	31.55d
	150	4900.00cd	4769.75c	228.17d	239.88c	32.40bc	34.18c
	300	5012.84c	5049.97bc	240.00c	253.33b	34.32a	36.65b
20 %	0	2358.20h	2488.94g	195.84g	202.48f	27.67f	28.89e
	150	2870.34fg	2946.70f	206.77f	215.67e	30.82de	32.49d
	300	3146.95f	3243.24e	217.82e	226.24d	32.75b	34.38c
30 %	0	1972.25i	1845.90h	162.11i	171.45g	24.43h	25.95g
	150	2438.12h	2485.49g	171.53h	175.84g	26.46g	27.39f
	300	2618.59gh	2681.58fg	197.25g	205.15f	31.43cd	32.56d

Means within a column having the same letters are not significantly differences according to Duncan's multiple range test (DMRT).

Regarding salicylic acid (SA) treatments, all tested foliar application significantly increased all vegetative growth parameters (plant height, number of shoots plant-1, number of leaves plant-1, total leaf area plant-1, plant fresh and dry weight) compared with the control treatment in both seasons. Salicylic acid at 300 ppm gave the highest values followed by salicylic at 150ppm. In general, under the same saline water irrigation concentration, spraying salicylic acid alleviated the harmful effects of saline water irrigation on the plants and enhanced the plant growth especially at 300 ppm as compared with control treatment in both seasons. The enhancement in growth parameters by applying SA may be due to enhancing ions absorption and minerals by plant. Moreover, improves plant performance by formation of certain enzymes in plant, hence stimulating chlorophyll synthesis and photosynthetic activities, which improve plant growth (Hayat et al., 2007). In addition, SA plays a main role in plant growth regulation and development is a hormone-like substance (Raskin, 1992) which have defensive effects in contrast to abiotic stress factors such as salinity and heat (Strobel and Kuc, 1995). These results are similar with those of Hashish et al., (2015) on Calendula officinalis and Basit et al., (2018) on Tagetes sp. L.

The interaction of salinity (irrigation by diluted seawater) and salicylic acid on vegetative growth parameters of calendula plants are shown in Tables (4 and 5). All growth parameters were affected by the interaction compared with control plants. Plants watered with tap water (0 % seawater) and spraying by 300 ppm SA followed by plants irrigated with diluted seawater at 10 % and were sprayed with SA at 300 ppm showed increased all growth parameters compared with the other treatments. In this respect, it can be assume that the depressing effects of salinity on plant growth and other relevant physiological activities can be alleviated by spraying plants by the appropriate concentrations of salicylic acid (Afzal et al., 2006). The ameliorative effects of SA have been well documented in inducing salt tolerance in many plants. Moreover, salicylic acid is an endogenous growth regulator of phenol nature, which participates in the regulation of physiological processes in plants. It plays an important role in the plant response environmental conditions such as salinity (Baby et al., 2010). The inhibition effects of salinity on growth parameters might be due to salinity which inhibits of growth through reduced water absorption, reduced metabolic activities due to Na+ and Cl toxicity and nutrient deficiency caused by ionic interference (Delacerda et al.,2003). The obtained results show similarity to findings by Hashish et al., (2015), Nofal et al., (2015) and Abou El-Ftouh et al., (2018) on Calendula plants.

b-Root growth:

Data registered in Table (6) indicated that root growth parameters (root length and fresh and dry weights) were significantly gradually decreased by increasing diluted seawater irrigation levels from 10 to 30 % comparing with the tap water (0 % seawater) in both seasons. The decreasing in growth parameters of roots attributable to salinity might be due to the decline in water and minerals absorption or the reduction in upper ground plant parts (Mazhar et al., 2006). These results are in harmony with those of Nofal et al., (2015) and Abou El-Ftouh et al., (2018) on Calendula plants and El-Mahrouk et al., (2010) on buttonwood plants. With regard the effect of salicylic acid (SA) treatments on Calendula plants, data presented in Table (6) emphasized that foliar application of SA significantly promoted all root growth parameters compared with control. The highest values of root length, fresh and dry weight of roots were recorded by SA treatment at 300 ppm in the first season and SA at 150 and 300 ppm without significant differences in between. As for the interaction between both factors (diluted seawater irrigation x SA) the highest values of root parameters were obtained by the combination treatments of tap water (0 % seawater) x SA at 300 ppm and diluted seawater at 10 % x SA at 300 ppm compared with the other treatments. These results are in harmony with those of Pacheco et al., (2013), Hashish et al., (2015) and Abou El-Ftouh et al., (2018) on Calendula officinalis. They revealed that foliar applications of SA increased root growth characteristics (fresh and dry weights of roots and roots volume).

Table (6): Effect of diluted seawater irrigation, salicylic acid spray levels and their interaction on root length, root fresh and dry weight of *Calendula officinalis* L. during 2017/2018 and 2018/2019 seasons

Treatments		Root length (cm)		Root fresh weight (gm)		Root dry weight (gm)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season
Seawater (%)							
Cont.		26.00a	27.11a	22.72a	25.21a	4.59a	5.07a
10 %		21.33b	22.44b	20.02b	21.64b	4.15b	4.48b
20 %		17.11c	18.11c	15.95c	16.70c	3.40c	3.61c
30 %		13.33d	14.00d	12.81d	14.06d	2.84d	3.13d
Salicylic acid (ppm)							
0		18.33b	19.25b	16.29c	17.73c	3.40c	3.69c
150		19.33b	20.42ab	17.80b	19.19b	3.70a	4.00b
300		20.67a	21.58a	19.53a	21.28a	4.14a	4.53a
Seawater	SA (ppm)	Interaction					
Cont.	0	24.67b	25.67b	21.21c	23.49c	4.32c	4.73c
	150	26.00ab	27.33a	22.78b	25.18b	4.59b	5.07b
	300	27.33a	28.33a	24.15a	26.95a	4.87a	5.43a
10 %	0	20.33d	21.33d	19.22d	20.91d	3.96d	4.29d
	150	21.00d	22.33d	19.44d	21.23d	3.99d	4.36d
	300	22.67c	23.67c	21.41c	22.77c	4.50bc	4.79c
20 %	0	16.00fg	17.00f	14.22g	14.53g	2.99f	3.09f
	150	17.33ef	18.33e	16.00f	16.32f	3.37e	3.48e
	300	18.00e	19.00e	17.92e	19.24e	3.85d	4.25d
30 %	0	12.33h	13.00h	10.51i	11.99h	2.31g	2.67g
	150	13.00h	13.67h	12.98h	14.03g	2.85f	3.07f
	300	14.67g	15.33g	14.93fg	16.17f	3.36e	3.64e

Means within a column having the same letters are not significantly differences according to Duncan's multiple range test (DMRT).

2- Flowering measurements:

Data presented in Table (7) showed that diluted seawater irrigation treatments significantly decreased all flowering parameters (number of flowers, diameter, flower fresh and dry weight) compared with the irrigation by tap water treatment (control). The highest values in these parameters number of flowers (21.5 and 20.44), flower diameter (4.50 and 4.61cm), flower fresh weight (28.87 and 28.99) and dry weight (3.74 and 3.95 g) were recorded by the control followed by the treatment of diluted seawater irrigation at 10 % seawater which recorded the second rank in number of flowers (16.22 and 15.44), diameter (4.17 and 4.06 cm), flower fresh weight (16.48 and 16.63 g) and dry weight (2.33 and 2.41 g) , while the lowest values of number of flowers (7.00 and 6.22), flower diameter (3.44 and 3.39 cm), flower fresh weight (5.92 and 5.44 g) and dry weight (0.81 and 0.82 g) were obtained by the treatment of seawater irrigation at 30 % seawater in both seasons, respectively. The reduction in flowering parameters may ensue from the plants inability to adjust somatically, counteraction toxicities or related disruptive phenomena or from the excessive energy demand placed upon the metabolic machinery required by such homeostatic systems (Greenway and Munns, 1980). These results are in harmony with those of Hashish *et al.,* (2015) and Abou El-Ftouh *et al.,* (2018) on *Calendula officinalis.*

Table (7): Effect of diluted seawater irrigation, salicylic acid spray levels and their interaction on No. of flowers plant-1, flower diameter and flower fresh and dry weight of *Calendula officinalis* L. during 2017/2018 and 2018/2019 seasons.

Treatments		No. of flowers plant^{-1}		Flower diameter (cm)		Flower fresh weight (g)		Flower dry weight (g)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Seawater (%)									
Cont.		21.56a	20.44a	4.50a	4.61a	28.87a	28.99a	3.74a	3.95a
10 %		16.22b	15.44b	4.17b	4.06b	16.48b	16.63b	2.33b	2.41b
20 %		10.33c	9.89c	3.78c	3.78b	9.06c	9.15c	1.38c	1.42c
30 %		7.00d	6.22d	3.44d	3.39c	5.92d	5.44d	0.81d	0.82d
Salicylic acid (ppm)									
0		10.92c	10.00c	3.79a	3.79b	11.51c	10.91c	1.49c	1.51c
150		13.58b	12.75b	3.92a	3.88b	14.64b	14.03b	1.96b	2.07b
300		16.83a	16.25a	4.21a	4.21a	19.10a	20.23a	2.74a	2.86a
Seawater	SA (ppm)	Interaction							
Cont.	0	19.00c	17.33c	4.17bc	4.33bc	23.70c	22.72c	2.86c	2.99c
	150	21.67b	20.33b	4.50ab	4.50ab	29.02b	27.74b	3.64b	3.84b
	300	24.00a	23.67a	4.83a	5.00a	33.89a	36.52a	4.72a	5.03a
10 %	0	13.67e	13.33d	4.00bc	4.00bcd	13.48e	13.05d	1.79e	1.89e
	150	16.00d	14.67d	4.17bc	4.00bcd	15.40e	14.20d	2.19d	2.30d
	300	19.00c	18.33c	4.33b	4.17bcd	20.57d	22.64c	3.00c	3.05c
20 %	0	6.67g	5.33fg	3.67cd	3.67de	5.49g	4.60f	0.84g	0.73g
	150	10.00f	10.00e	3.67cd	3.83cd	8.68f	8.98e	1.30f	1.42f
	300	14.33de	14.33d	4.00bc	3.83cd	13.01e	13.87d	2.00de	1.42f
30 %	0	4.33h	4.00g	3.33d	3.17e	3.38g	3.25f	0.48h	0.44g
	150	6.67g	6.00f	3.33d	3.17e	5.45g	5.18f	0.71gh	0.74g
	300	10.00f	8.67e	3.67cd	3.83cd	8.93f	7.90e	1.25f	1.28f

Means within a column having the same letters are not significantly differences according to Duncan's multiple range test (DMRT).

Foliar application of salicylic acid treatments on Calendula officinalis plants significantly increased number of flowers plant-1, flower diameter, flower fresh and dry weight compared with the control. SA at 300 ppm gave the highest significant increases in number of flowers per plant (16.83 and 16.25) in both seasons respectively, meanwhile, there were non-significant differences among SA treatments for flower diameter in the first season but in the second one the differences were significant, the highest value (4.21cm) was recorded with SA at 300 ppm followed by SA at 150 and 0 ppm without significant differences between themselves. The highest values of flower fresh weight (19.10 and 20.23 g) and dry weight (2.74 and 2.86 g) were obtained by SA at 300 ppm followed in a descending order by SA at 150 and 0 ppm. The highest values of flowering parameters were obtained by the combination of control (tap water) + 300 ppm SA. Also 10 % seawater + 300 ppm SA. The positive effect of SA was attributed to enhancing CO2 assimilation, chlorophyll concentration, photosynthetic rate and improved mineral uptake by stressed plants which were treated with SA (Karlidage et al., 2009). In the same line, Pacheco et al., (2013), Hashish et al., (2015) and Basit et al., (2018) on Calendula plant, showed that, foliar application of salicylic acid treatments on marigold plants significantly enhanced the flowering compared with untreated plants.

3- Chemical composition:

a- Leaf total chlorophyll, carotene contents in flowers and proline content

Data presented in Table (8) illustrated that all tested diluted seawater treatments significantly decreased leaf total chlorophyll (total green color, SPAD) and carotene contents in flowers, while proline content was increased compared with the tap water treatment (control) in both seasons.

The highest values of total green color and carotene were obtained with control treatment (tap water) followed by diluted seawater irrigation treatment at 10 % compared with the lowest values recorded by diluted seawater treatment at 30 %. The lowest photosynthetic ability under salt stress condition was due to stomata closure, inhibition of chlorophyll synthesis, a decrease of carboxylase and due to high chlorophyllase activity (Batanouny *et al.,* 1988). Similar response was previously observed in other plant as salinity caused a decrease in pigment content of calendula plants (Hashish *et al.,* 2015 and Abou El-Ftouh *et al.,* 2018).

All tested foliar spraying salicylic acid treatments significantly increased total green color, carotene contents in flowers, proline content compared with the control in both seasons. spraying of SA at 150 and 300 ppm gave the highest values of flower carotenes and proline contents. Such findings showed a similar trend, changes in photosynthetic content due to stomatal inhabitation associated with metabolic factors other than photosynthetic pigments (Arfan *et al.,* 2007). The application of SA resulted in alleviating the effects of salt stress, because, SA application reduced stomata conductance, intercellular CO_2 concentration and transpiration. These results coincide with those reported by Hashish *et al.,* (2015) and Basit *et al.,* (2018) on Calendula plant. The interaction of diluted seawater treatment and salicylic acid on total green color, carotene contents in flowers, proline content of Calendula officinalis plants were demonstrated in Table (8). All parameters were affected by the interaction compared with control. The highest significant values of carotene contents in flowers and total green color (SPAD) were obtained by plants irrigated with tap water and sprayed with SA at 150, 300 ppm with in both seasons. However, the highest significant values of proline content in both seasons were obtained by the combination treatment of diluted seawater irrigation treatment at 20 and 30 % seawater x SA at 300 ppm.

Table (8): Effect of diluted seawater irrigation, salicylic acid spray levels and their interaction on leaf total green color , carotene contents in flowers and proline content of *Calendula officinalis* L. during 2017/2018 and 2018/2019 seasons

Treatments		Total green colour (SPAD)		Carotene contents in flowers (mg/100gm)		Proline (µmole/gFW)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season
Seawater (%)							
Cont.		27.76a	29.92a	1.247a	1.252a	607.81a	160.09d
10 %		22.24b	23.72b	0.977b	0.978b	484.06b	254.23c
20 %		20.81c	22.00c	0.892c	0.896c	254.06c	481.50b
30 %		17.54d	18.36d	0.792d	0.794d	159.71d	608.22a
Salicylic acid (ppm)							
0		21.11c	22.34c	0.915c	0.919c	380.55a	381.02a
150		22.22b	23.46b	0.988b	0.990b	389.04a	389.25a
300		22.94a	24.70a	1.028a	1.031a	357.38a	357.77a
Seawater	SA (ppm)	**Interaction**					
Cont.	0	26.77b	28.53b	1.130c	1.131c	153.20g	153.37g
	150	27.27b	29.60b	1.250b	1.253b	160.67fg	161.00fg
	300	29.33a	31.63a	1.360a	1.370a	165.27efg	165.90efg
10 %	0	21.40d	22.43def	0.954f	0.955e	247.00def	247.37def
	150	22.47c	23.87cd	0.988e	0.986d	255.00de	255.33de
	300	22.87c	24.87c	0.990d	0.992d	260.17d	260.00d
20 %	0	19.80e	21.17f	0.866i	0.875g	538.00b	538.67b
	150	21.27d	22.10ef	0.900h	0.904f	540.17b	540.33b
	300	21.37d	22.73de	0.910g	0.910f	365.00c	365.50c
30 %	0	16.47g	17.23h	0.710l	0.714j	584.00ab	584.67ab
	150	17.87f	18.27gh	0.815k	0.816i	600.33ab	600.33ab
	300	18.30f	19.57g	0.851j	0.851h	639.11a	639.67a

Means within a column having the same letters are not significantly differences according to Duncan's multiple range test (DMRT).

b- Leaf mineral (N, P, K, Ca, Na and Cl) and survival %:

Data presented in Tables (9 and 10) displayed that all tested diluted seawater irrigation treatments significantly decreased nitrogen, phosphorus, potassium and survival % whereas, sodium and chloride were significantly increased in both seasons. Also, the ratio of Na/K was increased with increasing the percentage of diluted seawater from 0 to 30 % seawater. Salt stress of seawater irrigation led to direct increase in sodium content of aerial parts and the increase in sodium content resulted an increase in Na/K ratio in the aerial parts of buttonwood plants (El-Mahrouk *et al.,* 2010). In the same line were the findings of Hussein *et al.,* (2010) on millet and Turhan *et al.,* (2014) on lettuce plants and Abou El-Ftouh et al., (2018) on calendula plant.

Table (9): Effect of diluted seawater irrigation, salicylic acid spray and their interaction on leaf N, P, K and Ca content of *Calendula officinalis* L. during 2017/2018 and 2018/2019 seasons

Treatments		N %		P %		K %		Ca %	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Seawater (%)									
Cont.		1.49a	1.50a	0.181a	0.181a	1.59a	1.60a	2.29a	2.31a
10 %		1.43b	1.46b	0.177b	0.178b	1.56b	1.57b	2.20b	2.45b
20 %		1.33c	1.34c	0.172b	0.173c	1.49c	1.50c	2.15c	2.20c
30 %		1.25d	1.26d	0.164b	0.164d	1.45d	1.46d	2.11d	2.16d
Salicylic acid (ppm)									
0		1.31c	1.32c	0.158c	0.158c	1.47c	1.48c	2.05b	2.15c
150		1.36b	1.37b	0.171b	0.171b	1.50b	1.51b	2.26a	2.25b
300		1.47a	1.48a	0.193a	0.194a	1.60a	1.62a	2.25a	2.28a
Seawater	SA (ppm)	Interaction							
Cont.	0	1.45c	1.46c	0.166h	0.167h	1.52e	1.53d	2.23bc	2.24c
	150	1.46c	1.46c	0.179e	0.179e	1.55c	1.56c	2.33a	2.37a
	300	1.56a	1.56a	0.198a	0.199a	1.70a	1.71a	2.31a	2.31b
10%	0	1.41e	1.45cd	0.163i	0.164i	1.50f	1.51e	2.10d	2.23c
	150	1.40d	1.42de	0.172f	0.173f	1.50f	1.51e	2.25b	2.26bc
	300	1.49b	1.51b	0.196b	0.197b	1.69b	1.70b	2.23bc	2.24c
20%	0	1.25h	1.26g	0.155k	0.155k	1.45i	1.46g	2.00e	2.13d
	150	1.31g	1.32f	0.170g	0.172g	1.48h	1.49f	2.23bc	2.24c
	300	1.42d	1.44cd	0.190c	0.192c	1.54d	1.55c	2.22bc	2.23c
30%	0	1.12i	1.13h	0.145l	0.146l	1.40j	1.41h	1.90f	2.00e
	150	1.25h	1.26g	0.160j	0.161j	1.47g	1.47g	2.22bc	2.26bc
	300	1.39f	1.40e	0.185d	0.186d	1.48g	1.50f	2.21c	2.24c

Means within a column having the same letters are not significantly differences according to Duncan's multiple range test (DMRT).

Table (10): Effect of diluted seawater irrigation, salicylic acid spray and their interaction on leaf Na, Cl content, Na/K ratio and survival % of Calendula officinalis L. during 2017/2018 and 2018/2019 seasons

Treatments		Na (ppm)		Cl (ppm)		Na/K		Survival %	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Seawater (%)									
Cont.		0.765d	0.766d	0.231d	0.236c	0.486d	0.484d	100.00a	100.00a
10 %		0.805c	0.806c	0.354c	0.344c	0.523c	0.520c	88.90b	87.44b
20 %		0.825b	0.860b	0.843b	0.771b	0.555b	0.575b	83.50c	82.62c
30 %		0.888a	0.893a	0.918a	0.912a	0.615a	0.616a	75.38d	74.04d
Salicylic acid (ppm)									
0		1.013a	1.015a	0.682a	0.667a	0.690a	0.687a	87.54a	87.05a
150		0.864b	0.866b	0.590b	0.592a	0.577b	574b	86.53b	85.00a
300		0.586c	0.614c	0.488c	0.439b	0.368c	0.384c	86.77ab	86.03a
Seawater	SA (ppm)	Interaction							
Cont.	0	0.972c	0.972c	0.236j	0.237e	0.638d	0.634d	100.00a	100.00a
	150	0.784g	0.785f	0.234j	0.244e	0.505h	0.503h	100.00a	100.00a
	300	0.538k	0.539j	0.223k	0.228e	0.316l	0.315l	100.00a	100.00a
10 %	0	0.982b	0.982b	0.431g	0.382de	0.653c	0.649c	94.17b	93.33b
	150	0.881f	0.882e	0.332h	0.332de	0.589g	0.583g	86.17c	84.33c
	300	0.553j	0.555i	0.301i	0.318de	0.328k	0.327k	86.37c	84.67c
20 %	0	0.984b	0.985b	0.986b	0.989ab	0.677b	0.674b	81.83e	81.53e
	150	0.890e	0.895d	0.822d	0.817bc	0.600f	0.599f	83.67d	82.33de
	300	0.600i	0.700g	0.722e	0.506d	0.389j	0.452i	85.00cd	84.00cd
30 %	0	1.113a	1.120a	1.076a	1.059a	0.793a	0.792a	74.17g	73.33g
	150	0.900d	0.900d	0.972c	0.975ab	0.613e	0.611e	76.27f	73.33g
	300	0.651h	0.661h	0.707f	0.703c	0.439i	0.441j	75.70f	75.47f

Means within a column having the same letters are not significantly differences according to Duncan's multiple range test (DMRT).

Foliar spraying of salicylic acid treatments (150 and 300 ppm) significantly increased leaf nitrogen, phosphorus, potassium, calcium %, while sodium and chloride percentages as well as Na/K ratio were decreased as compared with control treatment in both seasons. These results are in harmony with those of Hashish *et al.,* (2015) and Abou El-Ftouh *et al.,* (2018) on Calendula plants.

Regarding the interaction, there were significant differences among combination treatments in both seasons. The highest values of leaf N, P, K and Ca were obtained by the combination treatment of tap water (control) + high SA level, 300 ppm in both seasons while the highest values as for Na, Cl and Na/K ratio were obtained with the combination treatment of diluted seawater irrigation at 20 and 30 % + SA at 0 ppm (control). Furthermore, the highest significant values of survival % were attained by the application of SA (0, 150 and 300) and control (0 % seawater), also were obtained by SA foliar spraying at 150 and 300 ppm with diluted seawater irrigation at 10 % seawater in both seasons. These results agree with those reported by Bayat *et al.,* (2012), and Pacheco *et al.,* (2013).

CONCLUSION

It can be concluded that spraying calendula plant (*Calendula officinalis L.*) with salicylic acid at either 150 or 300 ppm when irrigated with diluted seawater had a positive effect on growth, flowering and increased the nutrition status and survival % of plants and also, it can be achievable to use diluted seawater at 10 % seawater to irrigate it.

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تأثير الرش بحمض السالسيليك على نمو وازهار نبات الاقحوان تحت مستويات مختلفة من ملوحة مياه الري

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قسم بحوث الحدائق النباتية –معهد بحوث البساتين – مركز البحوث الزراعية–الجيزة – مصر

الملخص العربي

التغيرات المناخية أدت الى التقليل من مصادر المياه العذبة مما أدى الى زيادة الحاجة لمياه الرى. ولهذا السبب كان الهدف الرئيسى من البحث هو دراسة تأثير الرى بماء البحر المخفف (٠ و١٠ و ٢٠ و ٣٠ %) بالإضافة الى محاولة تقليل التأثير السلبى للرى بملوحة ماء البحر بواسطة الرش بحمض السالسيليك (٠ و ١٥٠ و ٣٠٠ جزء في المليون) حيث تم إجراء تجربة اصص خلال عامي ٢٠١٧ و ٢٠١٨ في محطة بحوث بساتين بسخا بمحافظة كفر الشيخ ، مصر وذلك لدراسة تأثير الرى بماء البحر المخفف والرش بحمض السلسيلك والتفاعل بينهما على النمو والازهار ونسبة البقاء ، وكذلك التركيب الكيميائي لنباتات الاقحوان. أوضحت النتائج ان هناك انخفاض في قياسات النمو الخضري (ارتفاع النبات ، عدد الافرع والأوراق / النبات ، المساحة الكليه للاوراق لكل نبات والوزن الجاف والطازج للمجموع الخضري والجذرى واللون الأخضر الكلي (SPAD) وقياسات الإزهار (عدد الأزهار / النباتات ، قطر الزهرة والوزن الجاف والطازج) وكذلك تركيز الاوراق من العناصر (النيتروجين و الفوسفور والبوتاسيوم والكالسيوم) في حين كان هناك زيادة معنويه في عنصرى الصوديوم والكلور عند الرى بالمستويات العاليه من ماء البحر المخفف (٢٠ و ٣٠ %) عند المعاملة بحمض السالسيلك مقارنة مع الكنترول. بالإضافة إلى ذلك، وجد ان الرش بحمض السالسيلك بمعدل ١٥٠ أو ٣٠٠ جزء في المليون حسن من معظم الصفات السابقة تحت ظروف الرى بالماء المالح.

وفقًا للنتائج التي تم الحصول عليها، يمكن التوصية برى نباتات الاقحوان بماء البحر المخفف بنسبه ١٠٪ مع الرش بحمض السالسيلك بمعدل ٣٠٠ جزء في المليون حيث سجلت أفضل النتائج من حيث النمو ، والازهار ونسبة البقاء.

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