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Salicylic Acid's Role in Reducing the Impact of Salinity on Plant Calendula (*Calendula officinalis* L.)

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EXOGENOUSLY applied salicylic acid (SA) as a growth regulator has been increased owing to tolerance to different stresses for example salt stress. The experiment was shown to conclude the influence of SA application on vegetative and flower treats of *Calendula officinalis* L. grown under salt stress and greenhouse conditions. For this purpose, a split-plot design was led with four concentrations of SA (250, 500, and 750 ppm) addition the control under three levels of salinity water irrigation, tap water (EC_1= 0.6 ds·m^{-1}), mixture water (1:1, tap water: well water) (EC_2= 2.3 ds·m^{-1}), and well water (EC_3= 4.1 ds·m^{-1}) with three replications. The results displayed that salinity diminished the time flowering and leaf pigments values. The spray applications of SA resulted in growth parameters (plant height, no. of leaves, leaf area, shoot and root dry masses), and flowering parameters (no. of inflorescences, a diameter of inflorescences and dry inflorescences mass) of calendula plants under salt stress. The highest leaf pigments values were obtained from 250 ppm SA application under well water treatments. The gas exchange system was determined at the flowering stage. Well water with SA increased proline, Ca, Na, and Cl; but they reduced K and N under salinity stress. Plants treated with 250 ppm SA had the best vegetative and flowering growth parameters at well water. These results recommend that 250 ppm SA might be used as a phytohormone to improve calendula plants which they irrigated by well water.

Keywords: Chemical components, Gas exchange, Growth parameters, Leaf pigments.

Introduction

Salinity is becoming additional prevalent because of the intensity of agriculture growths and may be a limiting environmental factor for plant production. In the world land, more than 6% of arable land is negatively suffering from increase salt levels, which decrease crop growth (Ghassemi et al., 1995). Ebert et al. (2002) mention that the main reasons for the risky ion accumulation within the soil solution are unsuitable irrigation water, therefore, the usage of salty irrigation water. In dries and warms zones, the salt level increases within the higher soil layer thanks to high evaporation water losses that surpass rainfall. Expansion of approaches to induce salt pressure resistance in the plant is significant and obtains substantial attention. The methods studied to produce salinity stress-tolerant plants comprise environmental and genetic enhancements (Gunes et al., 2007).

The current Calendula (Family: Asteraceae; *Calendula officinalis* L.), mostly referred to as pot marigold or English marigold, is an annual species widely used around the world as medicinal or ornamental plant and as cut flowers. The marigold inflorescences included essential oils, flavonoids, pigments, oleanolic acid, phenolic acids, glycosides, and saponins, amongst other potentially active chemical constituents. Though, flavonoids have a more vital role within the pharmacological activity of flowers and are represented, in the greatest cases, for the constituent's rutin and quercetin, which are also symbols to assess the standard of the staple (Pacheco et al., 2013).

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Salicylic acid (SA) may be a plant development stimulating referred to as an endogenous phytohormone of phenolic nature, which is involved in several physiological procedures in plants, like equipment of plant tolerance and resistance to abiotic and biotic stresses, growth regulation, elements uptake, plant water relationships, stomatal conductivity to H_2O, senescence respiration, and the stimulation of protection systems against diverse pathogens is well known (Raskin, 1992, Popova et al., 1997; Grant & Lamb, 2006 and Hayat et al., 2010) and enhancement in photosynthesis rate under salt stress (Noreen and Ashraf, 2008). Furthermore, SA might serve such as seed germination, inhibition of fruit maturing and glycolysis (Cult and Klessig, 1992), Srivastava and Dwivedi, 2000) a regulator of gravitropism (Medvedev and Markova, 1991), and other vital processes in the plant (Shakirova et al., 2003).

By the synthesis of signaling molecules, the plants respond to any stress condition. These trigger a variety of signal transductions pathways. Numerous, such as signaling molecules are identified in plants like SA, ethylene (E), jasmonic acid (JA), and calcium (Ca). Ganesan and Thomas (2001) reported that SA is found to play a key role as a protection signal well established in plants. Raskin (1992) stated that Salicylic acid has qualified such as a phytohormone owing to its vital biological and physiological roles in plants. Klessig and Malamy (1994) reported that under stress conditions, a SA has been optional like a messenger or signal transducer. Though, the role of salicylic acid below salt-stress on the element›s uptake, plant pigments and membrane penetrability of the plant is lacking information or not still clear. Extensive searches are taken on to clarify the biology of SA induced systemic acquired resistance (Raskin, 1992; Levin et al., 1994). Nevertheless, this phenomenon is the biochemical and physiological basis owing to therefore the mechanism of signal regulation of plant resistance induced through SA aren›t still documented (Shakirova et al., 2003 and Gunes et al., 2007).

There are investigational results indicating the contribution of SA caused by the ability of SA to induce a defensive influence on plants under abiotic and biotic stresses. Conclusive results have been got on the SA-encouraged increase in the resistance of Hollyhock to water deficit (Oraee et al., 2019) and of Hibiscus plants to salinity (Sakhanokho and Kelley, 2009), of Ornamental pepper plants to low and warmth temperature (Zhang et al., 2020) of Zinnia and Iras plants to Cu and Cadmium toxicity, respectively (Afrousheh et al., 2015 and Han et al., 2015) as well as on the damaging action of heavy metals on Rosmarinus plants (Qiu et al., 2012). The data have been found foliar spraying the SA-induced increasing quality of *Zinnia elegans* L. plants (Elbohy et al., 2018).

Below oxidative stress conditions like drought, low or warmth temperature and salinity, plants release active oxygen types, which are damaging to plant development owing to their negative influences on the metabolism and subcellular constituents of the plant, resulting in the oxidative obliteration of cells plant. Shim et al. (2003) mention that it's normally putative that oxygen it'd be transformed to peroxide (H_2O_2) then metabolized to H_2O through enzyme ascorbate peroxidase then glutathione reductase in plant. Mishra and Choudhuri (1999) found that underactive O_2 types reason deteriorating of membrane fats, resulting in the increased leak of solutes from membranes of cells. The upkeep of membrane integrity to improved stress-tolerance is worth it. This is often why the key role of Salicylic acid on elements uptake, H_2O_2 production, lipid peroxidation, and membrane leak of plants under saline conditions is of large importance (Gunes et al., 2007).

The goal of this study is to get the best vegetative growth, flowering parameters, and chemical components of Calendula plants by applying diverse levels of salicylic acids under different salinity irrigation water conditions.

Materials and Methods

Plant materials

Calendula seeds (*Calendula officinalis* L.) were germinated in peatmoss in plastic germination trays 50 X 60 cm under greenhouse conditions of the College of Food and Agricultural Sciences, K.S.U. Saudi Arabia, during two seasons of 2017 and 2018. Seeds of Calendula were sown in a nursery on November 5th and 9th in both seasons, respectively. Constant size seedlings (6-8 cm) were transplanted to plastic pots (16 cm upper diameter 2.6 Kg soil) and filled with combination of loam: sandy soil (v:v, 1:1). The soil of the study field was sandy loamy with pH 7.7, consisted of loamy (9.9%), sand (81.8%) and silt (8.3%); contains total N (194 ppm), total P (8.9 ppm), and total K (115 ppm) with an EC of 0.13 ds.m^{-1} (average two years). The plants were grown in an obviously floodlit greenhouse with day/night steady temperatures of 24/17 °C.

Growth conditions and treatments

A split-plot design was conducted with four concentrations of SA (0, 250, 500, and 750 ppm) under three levels of salinity water irrigation water tap water (EC_1= 0.6 ds.m^{-1}), mixture water (1:1, tap water: well water) (EC_2= 2.3 ds.m^{-1}), and well water (EC_3= 4.1 ds.m^{-1}) with three replications. First, in absolute ethanol was dissolved Salicylic acid after that additional drop to water (1/1000, v/v: Ethanol/Water). We were used exogenously foliar applied SA of solutions two times at intervals of seven days. Thirty-five days after transplanting, SA was first spray applied on the foliar of Calendula plants, than the second spray applied after one week from the first one with a hand pump pressure sprayer. Forty-two days after planting, salinity irrigation applications of various sources were applied to the Calendula plants using a manual irrigation water system that carried out the water to the soil plants until the end of the experiment. Through the experiment study, the plants were carefully irrigated every three days to keep soil moisture near to the FC (84%, v/w).

Data recorded

Data of vegetative and flowering growth parameters were collected at the end of each season separately, while chemical components were determined at the second season only. The obtained results were as follows:

Vegetative growth parameters

Plant height (cm), stem diameter (mm), branches and leaves number, leaf area (cm^2), with the LI-COR-3000 Model system (*LI-COR, Inc., Germany*), dry weights of shoots and roots parts (g), and root length (cm).

Flowering growth parameters

Flowering date (as number of days from transplanting till flowering appearance of the first flower), inflorescences number heads/plant, inflorescences head diameter (cm), and inflorescences heads dry weight (g). To get the dry weight, the samples were kept in a thermoventilated oven at 70C (until the weight is fixed); following, weight was directly recorded.

Leaf pigments content

Extraction of photosynthetic pigments in Calendula plant A, B, and Total chlorophyll content (mg/g F.W) were carried out using N, N- dimethylformamide (DMF) method determined in leaves Chl A = 13.43 $A^{663.8}$ – 3.47 $A^{646.8}$; Chl B = 22.90 $A^{663.8}$ – 5.38 $A^{646.8}$ and Chl total = 19.43 $A^{663.8}$ – 8.05 $A^{646.8}$ were estimated as described by Porra et al. (1989). Anthocyanin= A^{530} − 0.25 A^{657} (Mancinelli, 1994) and Carotenoid= (1000 A^{470} − 0.89 (Chl A) − 52.02 (Chl B) / 245 (Vicaş et al., 2010).

Chemical components

Proline concentration was measured calorimetrically in fresh Calendula plant samples as described by Bates et al. (1973). The potassium percentage (K%) was estimated by method a flame photometer according to Brown and Lilleland (1946), while, leaf nitrogen percentage was determined by Kjeldahl method (Nelson and Sommers, 1973). Calcium percentage (Ca%) was determined by the Versenate (EDTA) method according to Cheng and Bray (1951). Chloride percentage (Cl%) was estimated by Mohr's method as described by A.O.A.C. (1992). Sodium percentage (Na%) was estimated by flame photometer according to Brown and Lilleland (1946).

Gas exchange

Intercellular CO_2 concentration (C_i), transpiration rate (E), stomatal conductance (g_s), and net photosynthesis rate (P_N) of leaves were resolved somewhere in the range of 11:15 and 1:00 am from completely expanded fourth edges, utilizing a convenient open stream gas trade framework LI-6400 (LI-COR, Inc., Lincoln, NE) at light immersing force on a bright daytime when photosynthetically dynamic radiation was ~ 755 µmol m^{-2}s^{-1}, relative moistness was ~ 54% in addition midair temperature was ~ 25°C on a completely expanded topmost leaf of the fundamental pivot of the plants. Estimations were rehashed multiple times for edges pot^{-1}, and the midpoints were recorded.

Statistical analysis

The data was exposed to analysis of variance (ANOVA) rendering to a split-plot in a completely randomized block design (RCBD), with three replicates per treatment, succeeding the process outlined by Steel et al. (1997). The data were carried out using Statistical Analysis System (SAS) software V. 9.1, (SAS Institute, Cary, NC, USA). Therefore, means of treatments were compared based at least significant difference (L.S.D) so that assess the differences between SA concentrations under of salinity irrigation water, and the level of significance was usual at $P \leq 0.05$. The three levels of applied irrigation water tap water, mixture water, and well water were set in the main plots, and the four exogenously foliar applied SA of solutions containing control 0, 250, 500, and 750 ppm treatment concentrations were randomly assigned to the sub-plots. As resulting in a total of 144 plants [3 salinity irrigation water (tap water, mixture water, and well water) × 4 SA concentrations (0, 250, 500, and 750 ppm) × 3 replicates × 4 plants].

Results

Salicylic acid is known as an endogenous growth regulator in plants, it was important to study the effect of exogenous salicylic on growth parameters in calendula plant.

Vegetative growth parameters

A positive relationship was noticed between vegetative growth parameters and the increasing both salicylic acid and salt levels in water of irrigation. Table (1) the interaction of salicylic acid and salinity water of irrigation on growth parameters of Calendula plants was demonstrated. Plant height, number of branches, number and area of leaves were significantly affected by the interaction. Alike responses were noticed in four previous growth parameters plants in which salicylic acid treatment increased all characters under well water of irrigation 4.1 ds m^{-1} salinity level as well as the control plants. The highest values were recorded in the vegetative growth parameters salt water of irrigation with the highest salinity 4.1 ds m^{-1} in well water under 250 ppm levels of salicylic acid. Furthermore, salicylic acid foliar application increased plant height (30.00 and 29.86 cm), number of branches (5.66 and 4.73), number and area of leaves (32.66, 37.06, 128.35 cm^2, and 127.12 cm^2) of the plant compared to control plants in the first and second seasons, respectively.

In Table 2 different kinds of water and levels of applied SA treatments were significantly influenced stem diameter and root length. Well water irrigation with 250 ppm of SA obtained the high value of stem diameter were (7.15 and 7.39 mm) and root length (36.83 and 39.24 cm) in the first and second seasons, respectively.

Under different levels of SA increased shoot and root dry (dry matter yield) significantly both in non-saline and saline conditions. Though, this influence of SA was extra marked in well water than mixture, and tap water. In irrigation well water, with the foliar treatment SA (250 ppm), dry matter yield augmented up to the high value of shoot dry 5.38 and 5.49 g, and root dry 4.08 and 4.77 g, respectively.

Obtainable data proved from the investigation that salicylic acid improved dry matter under non-saline and saline water of irrigation but act more in plants grown under normal conditions or used slightly saline water of irrigation.

TABLE 1. Effect of salicylic acid (0, 250, 500, and 750 ppm) foliar applications under three levels of salinity water irrigation tap water, mixture water, and well water on the Calendula growth parameters.

Salinity	Salicylic acid (ppm)	Plant height (cm)		Number of branches		Number of leaves		Leaf area per plant (cm^2)	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Tap water 0.6 ds·m^{-1}	0	8.06E	8.40H	1.06E	1.03E	8.40E	10.06G	39.13EF	54.67E
	250	10.50E	9.80GH	1.13E	1.13E	13.73D	11.70FG	49.28DE	64.78E
	500	10.06E	10.23GH	1.16E	1.03E	9.36E	9.06G	59.20CD	84.75D
	750	8.10E	10.73G	1.10E	1.06E	8.70E	9.66G	36.88F	38.01F
Mix water 2.2 ds.m^{-1}	0	17.90D	17.06F	2.40D	2.70CD	16.03D	13.73F	62.71C	60.62E
	250	24.30B	19.96DE	3.73BC	3.40BC	22.70C	22.36D	88.86B	95.46CD
	500	19.33CD	21.33CD	2.70D	2.75CD	24.66BC	25.06CD	92.60B	101.76BC
	750	19.66CD	18.46EF	2.33D	2.03D	15.06D	18.40E	66.19C	85.33D
Well water 4.1 ds.m^{-1}	0	21.13C	20.20DE	3.03CD	4.10AB	26.10B	27.00C	93.33B	103.13BC
	250	30.00A	29.86A	5.66A	4.73A	32.66A	37.06A	128.35A	127.12A
	500	25.50B	25.70B	4.43B	4.03AB	27.00B	33.33B	119.53A	109.19B
	750	24.03B	22.43C	4.36B	3.70B	23.00C	26.33C	83.27B	102.77BC

TABLE 2. Effect of salicylic acid (0, 250, 500, and 750 ppm) foliar applications under three levels of salinity water irrigation tap water, mixture water, and well water on the Calendula growth parameters.

Salinity	Salicylic acid (ppm)	Stem diameter (mm/plant)		Shoot dry weight (g/plant)		Root length (cm/plant)		Dry root weight (g/plant)	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Tap water	0	3.01E	3.80F	3.43F	3.32E	18.43C	20.20GH	1.52DEF	2.00DE
	250	3.64DE	4.09EF	3.72EF	3.67DE	29.22C	19.66H	2.45CD	1.84DE
0.6 ds.m-1	500	2.83E	3.11F	3.45F	3.93CDE	21.76C	29.06CD	1.08EF	1.73EF
	750	3.06E	3.77F	3.28F	3.60E	20.23C	23.20FG	0.38F	1.41EF
Mix water	0	3.34E	3.95EF	4.18DE	3.92CDE	21.66C	22.86FG	1.48DEF	0.85F
	250	4.90C	4.89DE	4.67BCD	4.52BC	28.80B	31.40BC	2.25CDE	1.89DE
2.2 ds.m-1	500	5.16C	5.31CD	4.50BCD	4.61BC	29.91B	29.36CD	3.06ABC	2.81CD
	750	4.50CD	5.24CD	4.33CDE	4.32BCD	29.76B	34.13B	2.37CD	3.53BC
Well water	0	6.07B	6.12BC	4.71BCD	4.89AB	30.93B	27.56DE	3.15ABC	2.83CD
	250	7.15A	7.39A	5.38A	5.49A	36.83A	39.241A	4.08A	4.77A
4.1 ds.m-1	500	6.83AB	6.70AB	5.04ABC	4.86AB	27.90B	30.06CD	3.80AB	3.83B
	750	4.66C	6.54AB	5.10AB	5.36A	17.43C	25.83EF	2.61BCD	3.51BC

Flowering parameters

Foliar application of SA treatments on Calendula plants significantly decreases time flowering per plant (days), on the other side increased inflorescences number per plant, inflorescences diameter (cm), and inflorescences dry weight (g) compared with the control plants treatments (Table 3). Foliar application of SA at 250 ppm under well water gave the highest significant increases in flowering parameters. The result was obtained at 250 ppm under well water (7.73 and 7.13) inflorescences per plant, (5.70 and 5.83 cm) diameter of inflorescences per plant, and (2.49 and 3.58 g) dry inflorescences weight; while the lowest decrease was obtained after at time flowering per plant (117.36 and 109.70 day) in first and second seasons.

Leaf pigments content

Leaf photosynthetic pigments content of calendula plant under different saline water of irrigation and SA foliar applications; salt-stress water of irrigation caused in significant in the Chl A, Chl B, Chl A+B (total chlorophyll), anthocyanin, and carotenoid contents (mg/g F.W) compared with the control plants (Fig. 1A and B). Chlorophyll values were increased with the tap water than mixed water and well-water. Though, foliar SA applications used in the investigation caused the elevated chlorophyll values. The concentration of chlorophyll, anthocyanin and carotenoids increased in the first treatment 250 ppm than reduced with increased by SA treatments with the different water of irrigation. The highest values of chlorophyll, anthocyanin and carotenoids were obtained from 250 ppm SA application in well-water treatment.

Chemical components

Fig. 2A depicted the proline content activity in Calendula plant grown under different water of irrigation and SA foliar applications. The content of proline in leaf augmented from tap water, mixed water to well-water in absence, as well as in the attendance of salinity and SA. Compared with the non-SA foliar application plants, the proline content was decreased in the SA applied plants with increased SA treatments under the same kind of irrigation water. The highest value of proline activity was in presence plants of 250 ppm under well-water irrigation as compared to control. Salt stress inhibition of proline activity could contribute to the higher contents of proline as shown in Fig. (2A).

All chemical components were significant with the application of salicylic acid under different water of irrigation. Calcium (Ca) levels augmented with irrigation water salinity, though there was no influence of salicylic acid on Ca level (Fig. 2B). Salt stressed plants gave the highest value of chloride (Cl), and Sodium (Na). Increasing concentrations of treated SA significantly reduced the Na and Cl levels as compared to untreated plants (Figs. 2C and D). Salinity of water irrigation decreased nitrogen (N) concentrations of calendula plants (Fig. 2E). Concentrations of (N) of the plants increased significantly compared with the control under different water of irrigation, while N concentrations decreased with increasing levels of SA. Potassium (K) concentration of plants highly significantly with the applied SA (Fig. 2C). Concentration of K decreased with water salinity (Fig. 2F).

Gas exchange

Fig. 3 portrayed the photosynthetic parameters of Calendula plants with different sources of irrigation and SA treatments. The E, g_s, and P_n were higher significantly. Salt stress inhibited photosynthesis in the non-SA-treated plants under the different irrigation water, with reductions in the net assimilation rate values of SA-treated plants (Fig. 3A). The intercellular CO_2 concentration (C_i), (E), and (g_s) was increased with increasing SA treatment with different irrigation water (Fig. 3B, C, and D). Through the non-SA-treated plants, E, and g_s were increased values with tap water and mixed water than well-water of irrigation.

Discussion

Plants grown in saline soils surface many difficulties such as, elements imbalance as a consequence of dejected uptake, shoot transportation of elements and reduced interior distribution; increased high salt levels in the soil solution (osmotic pressure and "drought stress" congruently decreased soil water potential) and high levels of potentially toxic ions (i.e. Cl^- , Ca^{++}, and Na^+), (Marschner 1995; Greenway and Munns, 1980). So that, salt stress works to limit plant development and morphology structure by harmfully affecting several aspects of physiology in addition biochemistry, for example, pigments contents, osmolyte accumulation, proline activity, ion homeostasis, and photosynthesis (Roussos et al., 2013 and Abd El-Gayed, 2020).

In this investigation, we evaluated the influences of salt irrigation water on some physiological (vegetative and flowers) growth parameters symptomatic for stress: leaf pigments content, gas exchange, and level of salinity-stress on elements nutrition by salicylic acid. Salinity irrigation water reduced Calendula plant growth significantly. Alpaslan and Gunes (2001) reported that growth decrease below saltwater circumstances have been well documented in many plants by several investigators. Commonly, salicylic acid is applied in plant agriculture owing to its ability to regulate the plant tolerance response to diverse environmental stresses, chiefly salt-stress (Baby et al., 2010 and Hayat et al., 2010). Shakirova et al. (2003) found that salicylic acid applications alleviated the harmful influences of salinity on plant growth.

In this context, it can be accepted that the damaging belongings of salinity on plant growth parameters and other pertinent physiological activities can be mitigated by foliar plants by the suitable levels of salicylic acid (Greenway and Munns, 1980; Afzal et al., 2006). The improved effects of SA have been well documented in making salt tolerance in several plants. Furthermore, salicylic acid is an endogenous phytohomone of phenol nature, which contributes to the regulation of physiological processes in plants (Baby et al., 2010). The inhibition influences of salinity stress on vegetative growth parameters might be owing to salinity which inhibits growth parameters through decrease water absorption, diminished metabolic activities cause to Cl^- and Na^+ toxicity and nutrient lack produced by ionic interference (Delacerda et al., 2003; Hashish et al., 2015). The found results display similarity to findings by Nofal et al., (2015) Abou El-Ftouh et al., (2018) and Abd El-Gayed (2020) on Calendula plants.

Foliar application of SA at 250 ppm under well water gave the highest significant increases in flowering parameters. Alike trend was found in other characters. This positive influence of SA was attributed to enhanced CO_2 assimilation, chlorophylls, carotenoid and anthocyanin contents, photosynthetic rate, transpiration rate, and increased elements uptake by stressed plants treated with SA (Zohreh et al., 2009 in Violet plant; Hashish et al., 2015 in Calendula plant).

Regarding the role of salicylic acid on raised negative influence of salinity water of Calendula plants, data in Table (3) showed that spraying of SA at the 250 level with well water increased all flowering parameters. In this setting, it can be presumed that the application of exogenous SA improved the salt stress tolerance of plants (Deef, 2007), but the data were opposite and depended on the developed stage of plants (Borsani et al., 2001). Stevens et al. (2006) mention that SA has been recommended to be physiologically important in stress resistance since exogenous, SA brought about plants resistance to many abiotic stress with

TABLE 3. Effect of salicylic acid (0, 250, 500, and 750 ppm) foliar applications under three levels of salinity water irrigation tap water, mixture water, and well water on the Calendula flowering parameters.

Salinity	Salicylic acid (ppm)	Time flowering per plant(days)		Number of inflo-rescences per plant		Diameter of inflores-cences per plant		Dry inflorescences weight (g/ plant)	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Tap water	0	141.36A	142.73A	3.13E	3.16D	4.03F	4.03DE	0.94D	0.88E
	250	138.73A	137.06B	3.06E	3.46D	4.23EF	4.30CD	1.25D	1.69CD
0.6 ds·m-1	500	129.66BC	131.33CDE	3.70DE	3.33D	4.16EF	3.96DE	1.41CD	1.55D
	750	135.03AB	136.36BC	3.66DE	3.66D	4.13F	3.86E	1.74BCD	1.32DE
Mix water	0	130.66B	132.70BCD	5.73B	5.73BC	4.80BCD	4.26CDE	1.00D	1.46D
	250	128.40BC	126.36EF	5.06BC	5.40BC	4.56CDE	5.16B	1.49CD	2.12C
2.2 ds.m-1	500	128.00BC	128.03DEF	5.46B	6.86A	4.93BC	4.90B	1.52CD	2.90B
	750	130.36BC	129.00DEF	4.40CD	4.73C	4.58CDE	4.43C	1.81BCD	2.95B
Well water	0	131.03B	127.33DEF	5.76B	6.13AB	4.40DEF	3.96DE	1.71BCD	1.49D
	250	117.36D	109.70H	7.73A	7.13A	5.70A	5.83A	2.49AB	3.58A
4.1 ds.m-1	500	122.66CD	116.66G	7.40A	6.36AB	5.62A	5.73A	2.97A	3.17AB
	750	130.06BC	124.73F	5.07BC	5.73BC	5.10B	5.66A	2.26ABC	2.87B

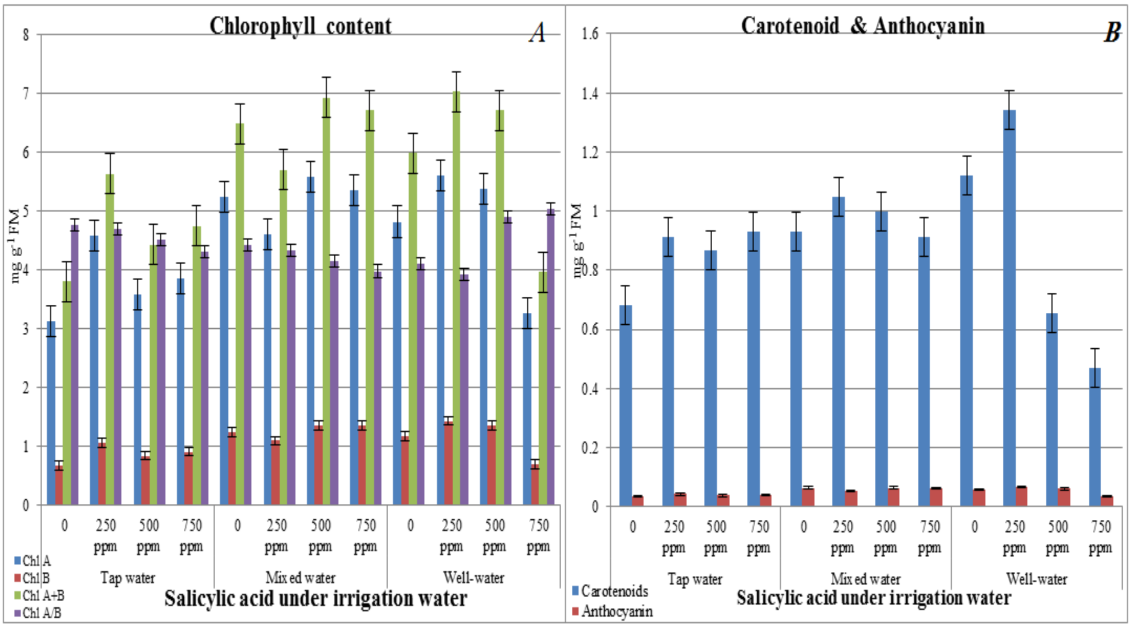

Fig. 1. Effect of salicylic acid (0, 250, 500, and 750 ppm) foliar applications under three levels of salinity water irrigation tap water, mixture water, and well water on the Calendula (A) chlorophyll content; (B) carotenoid and anthocyanin.

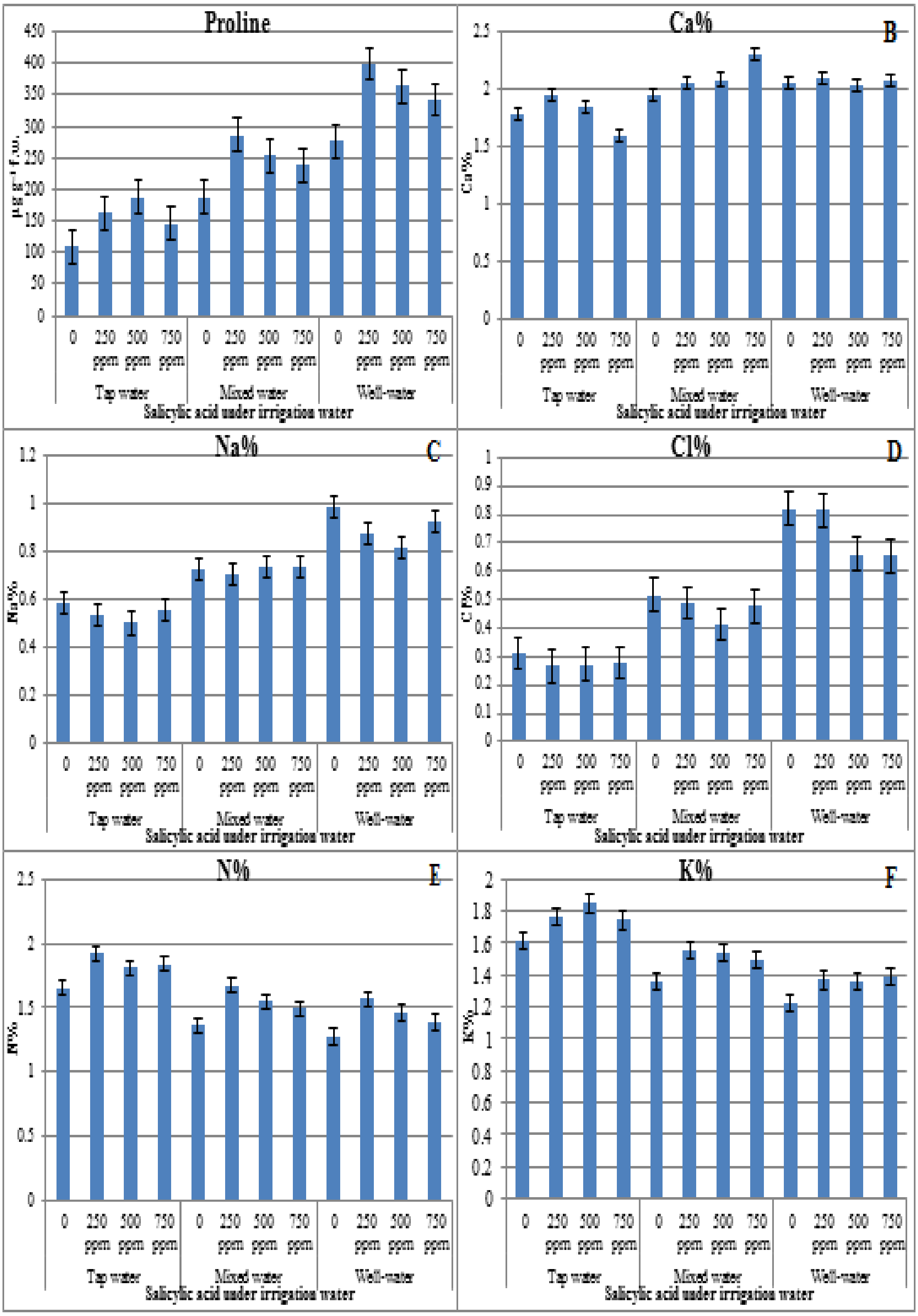

Fig. 2. Effect of salicylic acid (0, 250, 500, and 750 ppm) foliar applications under three levels of salinity water irrigation tap water, mixture water, and well water on the Calendula (A) proline; (B) Ca%; (C) Na%; (D) Cl%; (E) N%; and (F) K%.

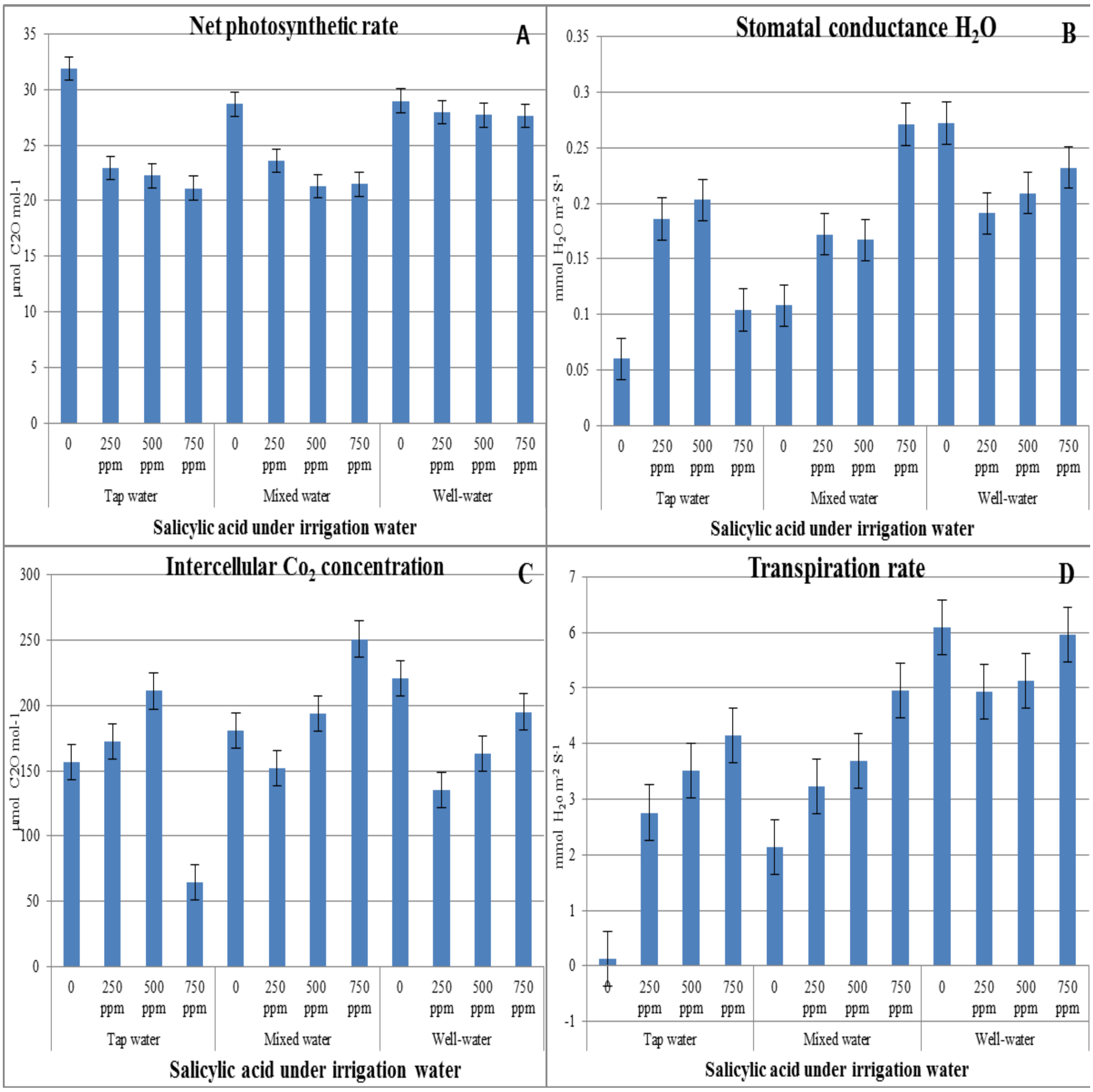

Fig. 3. Effect of salicylic acid (0, 250, 500, and 750 ppm) foliar applications under three levels of salinity water irrigation tap water, mixture water and well water on the Calendula (A) net photosynthesis rate (P_N), (B) stomatal conductance (g_s), (C) intercellular CO_2 concentration (C_i), and (D) transpiration rate (E).

salt.

Spray applications of SA to shoots of calendula significantly augmented the growth of roots and shoots in the open field circumstances the findings of Abd El-Gayed (2020). Increases in dry mass of salt stressed Calendula plants in response to exogenously applied SA might be connected to the initiation of antioxidant response and defensive role of membranes that increase the tolerance of the plant to damage. Salicylic acid foliar applicate wheat plants displayed higher dry mass compared to the control plants with water-stress (Singh and Usha, 2003) and Abou El-Ftouh et al., (2018) in Calendula plant. Ma et al. (2017) reported that SA significantly moderated the salt-stress response of Dianthus and SA can action as a potential regulator to enhance plant morphological with salt-stress.

Abd El-Gayed (2020) illustrated that several studies indicate that the leaf pigment is one of the important factors to measurement photosynthetic effectiveness and plant growth. In this investigation, salt stress clearly decreased the Chl A, Chl B, Chl A+B (total), Carotenoid and Anthocyanin contents; however, the SA spray application diminished this decrease of the leaf pigment contents of Calendula with salt-stress (Fig. 1). These results are in agreement with researches on *D. superbus* and *T. grandis* (Ma et al., 2017 and Li et al., 2014). The reactive oxygen species can damage lipids, DNA, membranes, and photosynthetic pigments proteins (Fahmy et al.,1998) for stress protection, plants have developed enzymatic and non-enzymatic scavenging mechanisms for the reactive oxygen species (Demiral and Turkan, 2005). These scavenging mechanisms, such as the manufacture of catalase enzyme to decrease hydrogen peroxide (Herandez et al., 2000; Nofal et al., 2015) allow the plant to keep growing with stress conditions. Exogenously applied SA might support the activity of enzymes connected to photosynthetic pigments biosynthesis or might relieve the weakening of the photosynthetic system, thus decreasing photosynthetic pigments degradation.

Regarding this Hamada et al. (2016) mention that proline activate is one of the important components of resistance responses of plants to salinity it might be predictable that pre-treatment by SA contributes to increase of this amino acid under salinity stress through preserving an improved concentration of ABA in maize plants. Deficit water and salinity stress induce the addition of proline to activate in plants. Hussein et al. (2007) mentioned that the influence of SA on diminishing the damages of salt stress on maize plants information recommends that proline is an important constituent in the spectra of SA induced ABA arbitrated protective reactions of plants in response to water deficit and salinity, Sakhabutdinova et al., (2003) which contribute to a decrease of harmful belongings of stress factors and hastening of repair processes during the period after action of stress, which might be an appearance of the protective act of SA on plants. These results are resembled (Abraham et al., 2003) they reported that proline accumulates in plants subjected to salt stress.

The application of SA flourished in significantly diminishing Cl^- and Na^+ concentrations in Calendula plant. Reducing concentrations of Cl^- and Na^+ ions to clarify the bettering influence of SA, prospective owing to the augmented antioxidant action of SA, on the growth of NaCl stressed Calendula plants. These results are in consistent with the discoveries of Al-Hakimi and Hamada (2001), who mention that SA reduced Na^+ content of wheat plant under salinity stress. Additionally, SA treatment significantly augmented N, Mg, Ca, Mn, and K concentrations of the plants. Nevertheless, after the uptake of these elements was measured, we observed that SA augmented Ca, K, and N uptake of the plants below salinity. Several physiological and biochemical belongings of SA on plant systems have been well recognized (Raskin, 1992; Cameron, 2000). Nonetheless, researches associated with ion level and uptakes are comparatively deficient. Moreover, like belongings of SA in the Na, K, Ca, N, and Mg content of wheat shoot and root plants grown below salinity stress (Al-Hakimi and Hamada, 2001). Gunes et al. (2007) studied the effect of active belongings of SA on the ion uptake, and inhibitory influences on Cl and Na uptake would also be accountable for management salinity of *Zea mays* L. crop.

Through reports presented that SA induced increases in growth might be owing to the SA enhanced decrease in the P_N and increase E *and* C_i under salt-stress, spatially at the 0.5 mM SA concentration (Li et al., 2014). Noreen and Ashraf, (2008) mention that salinity stress significantly efficient the photosynthesis processes in *Helianthus annuus* L., particularly efficient E and P_n the same our result. The *Pn* decreased by all applications of SA from 0.6 to 4.1 ds.m^{-1} compared with the untreated Calendula plants (Fig. 3A). Photosynthesis system is a critical metabolic path in plants. The conservation of a promising photosynthetic rate implies the conservation of growth under salt-stress. Borsani et al., (2001) reported that SA can efficiently improve photosynthetic rate damage in Arabidopsis plants. Dubey (2005) found that the noted changes in the photosynthetic rate could be considered owing to non-stomatal or/and stomatal limits. Salt-stress produced an indirect influence, such as stomatal conductance closure, which affected a diminished in the CO_2 supply, in that way causing in the decrease in the photosynthetic rate. The exogenously applied SA relieved the adverse influences of salinity-stress on photosynthesis rate with modifying G_s and P_n. Nevertheless, non-stomatal limits, i.e., the membrane structural integrity of the photosynthetic apparatus, the energy supply of the photosynthetic reaction center, and the related enzyme actions, too affected the *Pn* and E under salt stress (Ma et al., 2017). Our investigation indicated that the *Pn* diminished constantly in the SA foliar application, however the changes in *Gs* also existed nonetheless not with similar response of *Pn*. Ma et al., (2017) mention that either non-stomatal limitations or both non-stomatal and stomatal factors might have impacted the photosynthetic process under salt-stress.

Conclusions

In this investigation, results displayed that foliar applied salicylic acid (SA) plays a significant role in plant growth parameters and improving flower quality. They used exogenously foliar applied SA of solutions twice at intervals of seven days, in this context the best result was noted in well water with spray SA 250 ppm closely followed by SA 500 ppm than SA 500 ppm under mix irrigation water. Through that the above data, it might be determined the possibility of exogenously foliar applied salicylic acid of solutions used to improve the adverse influence of salinity on calendula plants.

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دور حمض الساليسيليك في تقليل تأثير الملوحة على نبات الكلانديولا (*Calendula officinalis* L.)

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يرجع زيادة استخدام حمض الساليسيليك المطبق خارجيًا كمنظم للنمو بسبب تحمل الضغوط المختلفة مثل إجهاد الملوحة. وكان الغرض من أجراء التجربة دراسة تأثير رش حامض السلسيلك على نبات الكلانديولا (النمو الخضري والزهري) النامي تحت إجهاد الملوحة بالصوبة الزجاجية. لذلك تم تصميم التجربة كقطع منشقة مقسمة بأربعة تركيزات من حامض السلسيلك (صفر و٢٥٠ و٥٠٠ و٧٥٠ جزء في المليون) تحت ثلاثة مستويات من مياه الري الملحة ، وهي ماء الصنبور ($EC1 = 0.6$ ds.m^{-1}) ، والماء المخلوط ($EC2 = 2.3$ ds.m^{-1}) ومياه الآبار ($EC3 = 4.1$ ds.m^{-1}) بثلاث مكررات. أظهرت النتائج أن الملوحة قللت من زمن التزهير وقيم أصباغ الأوراق. أدت تطبيقات الرش لحامض السلسيلك إلى النمو الحضري (ارتفاع النبات ، عدد الأوراق ، مساحة الأوراق ، الكتل الجافة للنبات والجذر) ، والنمو الزهري (عدد النورات ، قطر النورات وكتلة النورات الجافة) لنبات الكلانديولا تحت إجهاد الملح. تم الحصول على أعلى قيم أصباغ الأوراق من ٢٥٠ جزء في المليون من تطبيق حامض السلسيلك تحت المعاملة بمياه الآبار. تم تحديد نظام تبادل الغازات في مرحلة الإزهار. الري بمياه الآبار مع حامض السلسيلك أدي لزيادة البرولين ، الكالسيوم ، الصوديوم ، و الكلور؛ لكنها قللت من البوتاسيوم والنيتروجين تحت إجهاد الملوحة. كان للنباتات المعاملة بـ ٢٥٠ جزء في المليون حامض السلسيلك أفضل معايير النمو الخضري والزهري مع مياه الآبار. تشير هذه النتائج إلى أنه يمكن استخدام ٢٥٠ جزء في المليون من حامض السلسيلك كهرمون نباتي لتحسين نباتات الكلانديولا التي تروى بمياه الآبار.