

## Application of salicylic acid on *Calendula officinalis* L is to alleviate the adverse effects of salinity stress

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**Abstract:** The experimental trials were carried out during two successive seasons (2011/2012 and 2012/2013) at the green house of the National Research Centre, Cairo, Egypt. It was intended to find out the individual and combined effect of different levels of salinity and salicylic acid doses as foliar applications on growth and photosynthetic pigments of *Calendula officinalis* L. Three salinity levels were prepared (1000, 2000 and 3000ppm) by adding sodium chloride + calcium chloride (1:1v/v) for irrigating seedlings with previously prepared salinized. The untreated plants (control) were irrigated with tap water. Seedlings were sprayed three times (30, 50 and 70 days from sowing) there concentrations of salicylic acid namely (0, 50 and 100 ppm) as foliar spray to cover completely the plant foliage. The lowest values of stem length, number of branches/ plant, number of leaves / plant, root length and fresh & dry weight of all plant organs, were obtained from the plants irrigated with the highest level of salinity(3000 ppm) in the first sample. In the second sample the decreased in stem length, number of branches / plant, number of leaves / plant and root length at levels of 1000, 2000 and 3000 ppm respectively. Leaves, stems and roots fresh and dry weight were significantly decreased gradually by increasing salinity levels in the first and second sample. The highest values in both samples are, chlorophyll (a), chlorophyll (b), chlorophyll (a+b) and carotenoids were obtained from salicylic acid at the level of 200 ppm. Foliar application of 200ppm salicylic acid combined with tap water, increased all growth parameters, pigments content.

**Key words:** *Calendula officinalis*, vegetative growth; salicylic acid; salinity.

### Introduction

*Calendula officinalis* (pot marigold) is a plant in the genus *calendula*, in the family Asteraceae. It is probably native southern Europe through its long history of cultivation makes its precise origin unknown, and may possibly be of garden origin. It is also widely naturalized further north in Europe and elsewhere in warm temperate regions of the world<sup>1</sup>.

It is a short-lived aromatic perennial plant, growing to 80cm tall, with sparsely branched lax or erect stems. The leaves are oblong-lanceolate, 5-17cm long, hairy on both sides, and with margins entire or occasionally waved or weakly toothed. *Calendula officinalis* is widely cultivated as a herb and be grown easily in sunny locations in most kinds of soils. Although perennial, it is commonly treated as an annual plant, particularly in colder regions where its winter survival is poor, or in hot summer locations where it also does not survive<sup>2</sup>.

*Calendula* is considered by many gardening experts as one of the most versatile flowers to grow in a garden, especially since they are easy to grow, and tolerate most soils<sup>3</sup>.

Salinity is a common abiotic stress factor seriously affecting plants production in different regions, particularly in arid and semi-arid regions. It is estimated that over 800 million hectare of land in the world are affected by both salinity and sodicity<sup>4</sup>. There are various detrimental effects of salt stress in plants, responsible for severe decrease in the growth and yield of plants. Osmotic stress (drought problem), ion imbalance, particularly with Ca, K and the direct toxic effects of ions on the metabolic process are the most important and widely studied physiological impairments caused by salt stress<sup>5,6,7</sup>. Salt stress, like many abiotic stress factors, reduces the ability of plants to take up water, leading to growth reduction as well as metabolic changes similar to those caused by the water stress<sup>6</sup>. On *Moringa oleifera*, fresh and dry weights, chlorophyll a and b content decreased with increase in soil sodicity<sup>8</sup>.

Salicylic acid has been defined as a new potential plant hormone and found to play an important role in disease resistance and a biotic stress tolerance<sup>9,10</sup>. Exogenous application of SA improved plant tolerance to salt stress in dicotyledons<sup>11</sup>. Salicylic acid caused a significant increase on the plant density and dry weight of root and shoot<sup>12</sup>. Spraying plants *Single hybrid* with SA increased dry weight of stem, leaves and whole plant, SA increased the most amino acids, except methionine, in the plants<sup>13</sup>. SA plays an important role in the resistance of old leaves against pathogens, which acts as an induction signal for specific defense responses of plants. It acts by producing low weight proteins which play an interesting role in the resistance<sup>14,15</sup>. On *Rosa hybrid* found that SA reduced the synthesis of ethylene and it is able to block the synthesis of auxins<sup>16</sup>. As mentioned above SA increased the dry matter and consequently vase life<sup>17</sup>.

Thus, the aim of the present study is to investigate the effect of salicylic acid application on growth and photosynthetic pigments of *Calendula officinalis* plants to alleviate the adverse effects of salinity stress.

## Materials and Methods

The experimental trials were carried out during two successive seasons (2011/2012 and 2012/2013) at the green house of the National Research Centre, Cairo, Egypt. It was intended to find out the individual and combined effect of different levels of salinity and salicylic acid doses as foliar applications on growth and photosynthetic pigments of *Calendula officinalis* L.

*Calendula officinalis* L. seeds were secured from Medicinal and Aromatic plants Research section, Ministry of Agriculture. The soil of the experimental site was sandy. The investigated soil characterized by 77.5% coarse sand, 10.4% fine sand, 5.6% silt and 6.5% clay, pH 7.7, EC 1.3 dSm<sup>-1</sup>, CO<sub>3</sub> 2.51%, K<sup>+</sup> 0.4, Na<sup>+</sup> 2.3, CO<sup>++</sup> 1.0, Mg<sup>++</sup> 0.7, HCO<sub>3</sub> 2.3, Cl<sup>-</sup> 1.8 and SO<sub>4</sub> 0.2 meqL<sup>-1</sup>. The physical and chemical properties of the soil were determined<sup>18</sup>. Seeds were sown in pots (30 cm in diameter) filled with 12 kg of sandy soil on the 15<sup>th</sup> October of each season. Three salinity levels were prepared (1000, 2000 and 3000 ppm) by adding sodium chloride + calcium chloride (1:1 v/v) for irrigating seedlings with previously prepared salinized. The untreated plants (control) were irrigated with tap water. One liter of water to each pot twice a week through the course of the study (7 months). On the 15<sup>th</sup> of December seedlings were sprayed three times (30, 50 and 70 days from sowing) with concentrations of salicylic acid namely (0, 50 and 100 ppm) as foliar spray to cover completely the plant foliage. The statistical layout of the experiment was completely Randomized Design of 2 factors (3 salicylic acid x 4 salinity concentrations) each treatment included 5 replicates, each replicate consisted of five plants. Each pot was fertilized twice with 2.5 g nitrogen as ammonium nitrate (33.5 % N) and 20g calcium super phosphate (15% P<sub>2</sub>O<sub>5</sub>) and 2.0 g potassium sulphate (48.5 K<sub>2</sub>O<sub>2</sub>) per pot; these fertilizers were applied at 30 and 60 days from sowing. Other agricultural processes were performed according to normal practice.

The following data were recorded; stem length (cm), number of branches/plant, number of leaves / plant, root length (cm), fresh and dry weight (g) of plant organs. All previous data were subjected to statistical analysis according to procedure outlined<sup>19</sup>. Treatments means were compared by L.S.D. test and the combined analysis of the two seasons were calculated according to the method of<sup>20</sup>. Chlorophyll (a, b, a+b) and carotenoids contents were determined in fresh leaves according to<sup>21</sup>.

## Results and Discussion

### Growth characters:

The growth parameters as affected by saline water irrigation treatments are showed in Table (1). The lowest values of stem length, number of benches plant, number of leaves / plant, root length and fresh & dry weight of all plant organs were obtained from the plants irrigated with the highest level of salinity(3000 ppm), while the highest values occurred by irrigation with tap water Moreover, the differences between the concerned salinity concentrations were significant. Numerically, stem length, number of branches / plant, number of liquids/ plant and root length were decreased by (8.12, 19.52 and 52.58,23.28, 39.31 and 54.71, 27.78, 39.27 and 50.55%) and ( 23.29, 29,90 and 46.04%) as a results of irrigation with saline waters having the concentrations of 1000,2000and 3000ppm , respectively in comparison with the tap water irrigated plant in the first sample. In the second simple the data in Table 12 showed that, the values were decreased by (8.08, 18.69, 29.97) in stem length, (32.79, 40.76 and 46.74%) in number of branches / plant, (15.14, 23.89 and 38.19%) in number of leaves / plant and (22.68, 32.94and 47.65%) in root length at levels of 1000, 2000and 3000 ppm respectively as compared with untreated plants. The differences among the treatments were significant in both stages.

**Table 1: Growth parameters of *Calendula officinal* L. plant of the first sample as affected by salicylic acid (SA) and irrigation with saline water (average two seasons).**

Characters Treatments	Stem length (cm)	No of branches/ plant	No ofleaves plant	Root length (cm)
salicylic acid (SA) ppm				
0	19.27	3.06	25.70	8.91
100	22.04	6.47	33.95	9.75
200	25.66	7.48	41.52	10.69
LSD 5%	1.3	0.02	3.2	1.2
Salinity (S) ppm				
0	25.98	7.86	47.77	13.01
1000	23.87	6.03	34.50	9.98
2000	20.91	4.77	29.01	9.12
3000	12.32	3.56	23.62	7.02
LSD 5%	1.9	0.03	4.1	1.7
Interaction (ppm)				
SA 0 + S0	22.0	3.88	30.64	10.71
SA 0 + S1000	20.35	3.51	26.74	9.67
SA 0 + S2000	18.43	2.85	24.67	8.51
SA 0 + S3000	16.31	2.00	20.75	6.74
SA 100 + S0	25.31	8.75	45.95	13.00
SA 100 + S1000	23.65	6.71	36.75	9.93
SA 100 + S2000	20.66	5.45	29.75	9.11
SA 100 + S3000	18.53	4.97	23.36	6.96
SA 200 + S0	30.63	10.95	66.73	15.31
SA 200 + S1000	27.61	7.86	40.00	10.35
SA 200 + S2000	23.63	6.00	32.61	9.73
SA 200 + S3000	20.78	5.11	26.74	7.35
L.S.D. at 5%	3.3	0.05	5.9	2.9

**Table 2: Growth parameters of *Calendula officinal*\_L. plant of the second sample as affected by salicylic acid (SA) and irrigation with saline water (average two seasons).**

<b>Characters Treatments</b>	<b>Stem length (cm)</b>	<b>No of branches/ plant</b>	<b>No of leaves plant</b>	<b>Root length (cm)</b>
salicylic acid (SA) ppm				
0	31.31	6.15	57.84	13.93
100	32.87	8.21	67.07	15.99
200	34.98	8.81	75.25	17.58
LSD 5%	1.1	0.4	3.3	1.3
Salinity (S) ppm				
0	38.51	11.04	83.09	21.34
1000	35.40	7.42	70.51	16.50
2000	31.31	6.54	63.24	14.31
3000	26.97	5.88	51.36	11.17
LSD 5%	1.6	0.7	4.2	1.8
Interaction (ppm)				
SA 0 + S0	35.61	8.31	69.61	17.31
SA 0 + S1000	33.11	6.31	64.11	15.34
SA 0 + S2000	30.61	5.61	55.31	13.00
SA 0 + S3000	25.91	4.35	42.31	10.07
SA 100 + S0	38.31	11.61	86.31	21.61
SA 100 + S1000	35.33	7.83	69.74	16.50
SA 100 + S2000	31.11	6.88	64.11	14.74
SA 100 + S3000	26.71	6.53	52.11	11.12
SA 200 + S0	41.62	13.21	93.36	25.11
SA 200 + S1000	37.76	8.12	77.67	17.67
SA 200 + S2000	32.21	7.13	70.31	15.19
SA 200 + S3000				
SA 200 + S3000	28.31	6.76	59.67	12.33
LSD 5%	2.8	1.3	6.2	3.3

**Table 3: Fresh weight and dry weight of *Calendula officinal*\_L. plant at the first sample as affected by salicylic acid (SA) and irrigation with saline water (average two seasons).**

Characters Treatments	Fresh weight (g)			Dry weight (g)		
	Root	Stem	Leaves	Root	Stem	Leaves
salicylic acid (SA) ppm						
0	1.82	10.49	28.12	0.90	5.60	5.68
100	2.26	11.40	36.95	1.15	6.06	7.33
200	2.61	12.92	40.59	1.34	6.50	9.01
LSD 5%	0.02	1.1	1.3	0.2	0.6	1.2
Salinity (S) ppm						
0	3.04	15.55	51.93	1.67	8.02	11.33
1000	2.54	11.61	31.66	1.18	6.43	7.03
2000	1.84	10.55	28.82	0.96	5.48	6.02
3000	1.50	8.70	24.94	0.73	4.29	5.24
LSD 5%	0.03	1.5	2.2	0.3	1.3	1.7
Interaction (ppm)						
SA 0 + S0	2.17	13.88	34.12	1.12	7.67	6.23
SA 0 + S1000	2.11	10.30	30.61	1.00	5.67	6.06
SA 0 + S2000	1.63	9.76	25.13	0.82	4.96	4.41
SA 0 + S3000	1.35	8.00	22.61	0.67	4.11	5.00
SA 100 + S0	3.18	15.67	55.17	1.77	7.98	12.15
SA 100 + S1000	2.63	11.31	35.31	1.16	6.77	6.99
SA 100 + S2000	1.77	10.13	29.71	0.97	5.50	5.88
SA 100 + S3000	1.47	8.50	27.61	0.70	4.00	5.11
SA 200 + S0	3.7	17.11	66.51	2.11	8.41	15.61
SA 200 + S1000	2.88	13.21	39.61	1.37	6.86	8.03
SA 200 + S2000	2.12	11.76	31.63	1.08	5.97	6.77
SA 200 + S3000	1.62	9.61	24.61	0.81	4.75	5.61
L.S.D. 5%	0.06	2.3	3.1	0.6	1.9	2.5

The depressive effect on stem length by salinity might be mainly attributed to reduction in cell division and enlargement induced by salinity, also causes of stomata which reduced the supply of carbon dioxide for photo synthesis<sup>22</sup>. In this respect, there was attributed the undesirable effect of high salinity levels on plant growth to disturbance in mineral balance or utilization<sup>23</sup>. Additionally, natural hormones might be affected due to the saline conditions leading to unbalanced growth of the plant, consequently, the decrease in number of leaves / plant<sup>24</sup>. In this context, increasing concentrations of NaCl can reduce the endogenous level of IAA in root. Therefore can be suggested that IAA concentration under saline conditions may be insufficient to induce root elongation<sup>25</sup>. The obtained data also cleared that, leaves, stems and roots fresh and dry weight were significantly decreased gradually by increasing salinity levels. Leaves stems and roots fresh weights were decreased by (39.03, 44.50 and 51.97%), (25.33, 32.15 and 44.05%) and (16.45, 39.47 and 50.66%), where leaves, stems and roots dry weight were decreased by (37.95, 46.87 and 53.75%), (19.83, 31.67 and 46.51%) and (29.34, 42.51 and 56.29%) as a result of irrigation with waters having the concentrations of 1000, 2000 and 3000 ppm respectively comparing with the top water irrigated plant in the first sample, (Table 3). In the second sample in Table (4) data mentioned that, the values were (14.54, 18.44 and 27.46%) in leaves fresh weight and (9.60, 14.62 and 22.74%) in leaves dry weight, (8.12, 22.92 and 33.47%) in stem fresh weight, (16.26, 29.40 and 43.95%) in stem dry weight, (22.52, 35.99 and 52.48) in root fresh weight, (23.59, 37.88 and 48.92%) in root dry weight in the plants treated with (1000, 2000 and 3000 ppm) as compared with control plants. The decrease in fresh weight of leaves might be due to that salinity increased osmotic pressure which caused a drop in plant water content as found<sup>26</sup>. Also they indicated that high salinity levels could cause a depression in photosynthetic activities resulting in low CO<sub>2</sub> fixation.

**Table 4: Fresh weight and dry weight of *Calendula officinalis* L. plant at the second sample as affected by salicylic acid (SA) and irrigation with saline water (average two seasons).**

Characters Treatments	Fresh weight (g)			Dry weight (g)		
	Root	Stem	Leaves	Root	Stem	Leaves
salicylic acid (SA) ppm						
0	5.34	18.68	156.26	2.57	6.83	41.34
100	6.78	20.70	162.86	3.53	8.52	44.98
200	8.00	21.67	176.01	3.94	9.28	55.68
LSD 5%						
Salinity (S) ppm	0.3	0.9	2.7	0.1	0.3	1.2
0						
1000	9.28	24.26	194.42	4.62	0.58	51.36
2000	7.19	22.29	166.15	3.53	8.86	46.43
3000	5.94	18.70	158.56	2.87	7.47	43.85
LSD 5%	4.41	16.14	141.04	2.36	5.93	39.68
Interaction (ppm)	0.5	1.3	3.6	0.2	0.5	1.7
Interaction (ppm)	7.34	21.35	178.31	3.31	8.00	47.31
SA 0 + S0	5.67	21.00	158.33	2.93	7.61	43.35
SA 0 + S1000	4.35	17.61	151.64	2.17	6.76	40.17
SA 0 + S2000	4.00	14.76	136.76	1.88	4.96	34.53
SA 0 + S3000	9.51	24.77	186.63	4.81	11.13	49.67
SA 100 + S0	7.74	22.53	163.50	3.67	9.30	45.61
SA 100 + S1000	6.50	18.81	159.57	3.17	7.55	43.63
SA 100 + S2000	3.35	16.67	141.73	2.45	6.11	40.99
SA 100 + S3000	10.99	26.67	218.31	5.73	12.61	57.11
SA 200 + S0	8.17	23.34	176.63	4.00	9.67	50.33
SA 200 + S1000	6.97	19.69	164.45	3.26	8.11	47.76
SA 200 + S2000	5.88	16.98	144.63	2.76	6.71	43.51
SA 200 + S3000						
LSD 5%	8.83	1.8	5.2	0.4	1.0	2.2

Under saline conditions, the plants fail to maintain the required balance of organic and inorganic constituents leading to suppress the plant growth<sup>27</sup>. Moreover, NaCl induced oxidative damage in light was predominately mediated by the inhibition of protein synthesis<sup>28</sup>. Furthermore, the depression in DNA, RNA and protein synthesis. In this context, the reduction in plant mass under salinity treatment to the retardation of the production of proteins and under salinity treatment to the retardation of the production of proteins and nucleic acids<sup>29</sup>. 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<sup>+</sup> and Cl<sup>-</sup> toxicity and nutrient deficiency caused by ionic interference<sup>30,31</sup>.

Meanwhile, the decrease in fresh and dry weight of roots due to salinity might be due to the reduction in water and dry weight of roots due to salinity might be due to the reduction in water and minerals absorption and / or the reduction in upper ground<sup>32</sup>. The depressive effect of high salinity levels on the above mentioned plant traits are the same on *Cassia angustipolia*<sup>33</sup>, *Ceiba pentandra L.*,<sup>34</sup> *Moringa oleifera Lam*<sup>35</sup> and *Schefflera arboricola L.*<sup>36</sup>.

With regard the effect of salicylic acid treatments on *Calendula officinalis* plants, data in Table (1) emphasized that foliar application of salicylic acid on marigold significantly promoted all growth parameters compared with control plants. In the first sample, the averages were (14.37 and 33.16%) in stem length, (11.44 and 144.44%) in number of branches / plant, (32.10 and 61.56) in number of leaves / plant and (9.43 and 19.98%) in root length in the plant sprayed with salicylic acid at levels of 0.100 and 200 ppm, respectively compared with control plants. Meanwhile, the same trend was obtained in the second sample. In addition, data in Table (3 & 4) indicated that the highest values of the weights of all plant organs were obtained from the application of salicylic acid at level of 200 ppm followed by the treatment of 100 ppm in both samples. The positive effect of spraying plants with salicylic acid in all growth parameters might be due to that salicylic acid

is an endogenous growth regulator, which participates in the regulation of physiological processes in plants. Exogenous application of salicylic acid may influence stomata closure, ion uptake and transport, inhibition of ethylene biosynthesis, and transpiration<sup>37, 38, 39</sup>. In this context, the SA, an endogenous plant growth regulator has been found to generate a wide range of metabolic and physiological responses in plants there by affecting their growth and development<sup>40</sup>.

The interaction of salinity and salicylic acid on vegetative growth of *Calendula officinalis* plants were illustrated in Tables (1-4). All growth parameters were affected by the interaction compared with control plants. Under irrigation with zero saline water conditions, spraying 200 ppm SA increased all growth parameters compared with control plants and other treatments concerned in this study. In this respect, it can be assumed that the depressive effects of salinity on plant growth and other relevant physiological activities can be alleviated and / or modified to some extent, by spraying plants by the appropriate concentrations of SA<sup>41, 42</sup>.

The ameliorative effects of SA have been well documented in inducing salt tolerance in many plants<sup>43, 44</sup>. 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<sup>45</sup>.

### Photosynthetic pigments:

**Table 5: Potosynthetic pigments content of *Calendula officinal*\_L. plant as affected by salicylic acid (SA) and irrigation with saline water (average two seasons).**

Characters Treatments	Chl (a) (Mg/g)	Chl(b) (Mg/g)	Chl(a+b) (Mg/g)	Carotenoids (Mg/g)
salicylic acid (SA) ppm				
0	0.30	0.21	0.50	0.39
100	0.33	0.24	0.57	0.45
200	0.34	0.26	0.60	0.51
Salinity (S) ppm				
0	0.35	0.26	0.62	0.50
1000	0.33	0.24	0.57	0.46
2000	0.32	0.23	0.54	0.43
3000	0.29	0.20	0.49	0.39
Interaction (ppm)				
SA 0 + S0	0.32	0.23	0.55	0.45
SA 0 + S1000	0.31	0.21	0.52	0.40
SA 0 + S2000	0.30	0.20	0.50	0.37
SA 0 + S3000	0.26	0.18	0.44	0.33
SA 100 + S0	0.36	0.27	0.63	0.51
SA 100 + S1000	0.33	0.25	0.58	0.46
SA 100 + S2000	0.32	0.23	0.55	0.43
SA 100 + S3000	0.29	0.21	0.50	0.40
SA 200 + S0	0.38	0.29	0.67	0.55
SA 200 + S1000	0.34	0.27	0.61	0.52
SA 200 + S2000	0.33	0.25	0.58	0.50
SA 200 + S3000	0.31	0.22	0.53	0.48

Data presented in Table (5) showed that the content of chlorophyll (a), chlorophyll (b), chlorophyll (a+b) and carotenoids were decreased by 17.14, 23.08, 20.47 and 22.0% respectively, owing to 3000ppm salinity, compared to control. Chlorophyll and carotenoids were affected by salinity, and decreased steadily with increasing salt concentration. 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<sup>46,47</sup>. Similar response were previously observed in other plants, salinity caused a decrease in pigment content of *Erythrina variegates* and a reduction in chlorophyll concentration of *Matthiola incana* plants<sup>48, 49</sup>.

The effect of SA on photosynthetic characteristics was positive under the nonsaline condition. The increase in chlorophyll (a), chlorophyll (b), chlorophyll (a+b) and carotenoid with the application of 200 ppm SA were 13.33%, 23.81%, 20.0% and 30.77% in comparison to the water-sprayed control. Such findings showed a similar trend, changes in photosynthetic content due to stomatal inhibition but were associated with metabolic factors other than photosynthetic pigments or leaf carotenoids<sup>50</sup>. The application of the two rates of SA resulted in alleviating the effects of salt stress, due to the fact that, SA application reduced stomata conductance, intercellular CO<sub>2</sub> concentration and transpiration<sup>51</sup>.

Regarding the effect of interaction, SA application (200ppm) was more effective on pigments content under irrigation with Zero ppm saline water, photosynthetic pigments content were greatly induced.

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