International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.8, No.6, pp 890-897, 2015

The influence of different levels of foliar-application SA on the flowering and some chemical compositions of *Calendula officinalis* L. under salinity irrigation.

Hashish, Kh.I[1*], Azza, A.M.Mazhar[1*]; Nahed, G. Abdel Aziza[1], Mona H.Mahgoub[1]

Ornamental Plants and Woody Trees Dept. National Research Centre, 33 Bohouth st.Dokki, Giza. Egypt.

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, flowering and chemical constituents 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 Stalinized. The untreated plants (control) were irrigated with tap water. Seedlings were sprayed three times (after 30, 50 and 70 days from sowing) there concentrations of salicylic acid namely (0, 50and 100 ppm) as foliar spray to cover completely the plant foliage. Flowers number/ plant, flower diameter (cm), fresh and dry weight of flowers (g)/plant after 1st 2nd and 3rd collection and N and protein % in shoots were decreased by using 3000ppm salinity. All solidity treatments caused a decrement on total carbohydrates contents in leaves, stems and roots. The highest values in both samples are, flowering parameters at 3rd collection and total carbohydrates percentage were obtained from salicylic acid at the level of 200 ppm. Foliar application of 200ppm salicylic acid combined with tap water, increased carbohydrate percentage nitrogen, and protein % in shoots.
Key words: *Calendula officinalis,* salicylic acid*;* salinity; flowering parameters*;* chemical constituents.

Introduction

Calendula is believed to be native to Egypt and has almost worldwide distribution. There are numerous varieties of this species, differing primarily in flower shape and color. Calendula grows to about 0.7 m in height and the wild form has small, bright yellow-orange flowers that bloom from May to October. It is the ligulate florets, incorrectly referred to as flower petals that have been used medicinally. This plant should not be confused with other members of the plant has been grown in European gardens since the 12th century, and its folkloric uses are almost as old. Tinctures and extracts of the florets were used topically to promote wound healing and to reduce inflammation the marigold family[1, 2].

The dried petals have been used like saffron as a seasoning and have been used to adulterate saffron[3]. The pungent odor of the marigold has been used as an effective pesticide. Marigolds are often interspersed among vegetable plants to repel insects[4].

Calendula extracts have been used topically to promote wound healing, and experiments in rats have confirmed a measurable effect. An ointment containing 5% flower extract in combination with allantoin markedly stimulated epithelialization in surgically-induced wounds. On the basis of histological examination of the wound tissue, it was concluded that the ointment increased glycoprotein, nucleoprotein, and collagen metabolism at the site[5].

Salinity is one of the major stresses in arid and semi-arid regions causing adverse effects at physiological, biochemical and molecular levels, limiting flowering and crop productivity[6,7]. Salt Stress can disturb growth and photosynthetic processes by causing changes in the accumulation of $Na^{+,}$ Cl^{-}, and nutrients and disturbance in water and osmotic potential[8]. The increasing concentration of Na^{+} and Cl^{-} in water irrigation suppresses the uptake of essential nutrients N, P and K and alters ionic relationships[9].). In fact, the most serious threat to agriculture is salinity; salinity water is a major harmful factor worldwide[10].

Salicylic acid (SA) 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[11]. Salicylic acid (2- hydroxybenzoic acid) may help regulate several plants functions, including systemic acquire resistance to pathogens and the formation of flowers[12]. Recent review has demonstrated physiological and biochemical processes in plants and it is considered phytohormones[13] among the morphogenetic processes affected by salicylic acid were flowering.

This investigation was carried out to evaluate the influence of different levels of foliar-applied SA on the flowering and some chemical compositions of *Calendula officinalis* L. under salinity irrigation.

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, flowering and chemical constituents 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, EC1.3 dSm^{-1}, CCo_3 2.51%, k^{+} 0.4, Na^{+} 2.3, CO^{++} 1.0, Mg^{++} 0.7, HCO_3 2.3, Cl^{-}1.8 and SO^{-}_{4}0.2meqL^{-}. The physical and chemical properties of the soil were determined [14]. Seeds were sow in pots (30 cm in diameter) filled with 12 kg of sandy soil on the 15th October of each seasons. Three salinity levels were prepared (1000, 2000 and 3000ppm) by adding sodium chloride + calcium chloride (1:1v/v) far irrigating seedlings with previously prepared Stalinized. 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 15th of December seedlings were sprayed three times (30, 50 and 70 days from sowing) there concentrations of salicylic acid namely (0, 50and 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 included5 replicates; each replicate consisted of five plants. Each pot was fertilized twice with 2.5 g nitrogen as ammonium nitrate (33.5 % N) and 209 calcium super phosphate (15% P_2O_5) and 2.0 g potassium sulphate (48.5 K_2O_2) per pot these fertilizers were applied at 30 and 60 days from sowing. Other agricultural processes were performed according to normal practical.

The flowers were collected biweekly starting the beginning of 1st March2012 and 2013 till1stMay 2012 and 2013 March from transplanting. Meanwhile, two plants samples were taken from each treatment at (150,165 and 180 days from sowing).

The following data were recorded, flowers number/ plant, flower diameter (cm) and fresh and dry weight of flowers. All previous data were subjected to statistical analysis according to procedure out lined [15]. Treatments means were compared by L.S.D. test and the combined analysis of the two seasons were calculated [16]. Total carbohydrates percentage was determined [17]**. (1956)**. Total nitrogen was determined (on a dry matter basis) using the modified Micro kjeldahl method [18] **.**Total protein % was calculated by multiple total Nx 6.25.

Results and Discussion

Flowering characteristics:

Data concerning the effect of various salicylic acid and different water salinity levels on flowering characters are presented in Tables (1-3). Four parameters pertaining to flowering characteristics were evaluated which involved: Flowers number plant, flower diameter (cm), fresh and dry weight of flowers (g) compared with control plants after 1 st, 2 nd and 3 rd collection (150, 165 and 180 days) from transplanting. All flowers parameters significantly decreased with increasing salinity levels, while the lowest values of criteria were obtained unde r that of 3000ppm. 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[19,20].

Table 1:Flowers characters 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	Flowers number/plant	Flowers diameter (cm)	Flowers fresh weight (g)	Flowers dry weight (g)
salicylic acid (SA) ppm				
0	7.80	2.77	12.56	3.15
100	8.93	2.91	14.36	3.49
200	10.04	3.08	15.16	3.68
LSD 5%	0.4	0.02	0.2	0.03
Salinity (S) ppm				
0	12.27	3.45	17.33	4.16
1000	8.71	3.19	13.47	3.64
2000	7.82	2.81	13.19	3.28
3000	6.88	2.12	12.11	2.66
LSD 5%	0.6	0.04	0.4	0.05
Interaction (ppm)				
SA 0 + S0	9.71	3.15	13.31	3.63
SA 0 + S1000	8.12	3.13	13.00	3.50
SA 0 + S2000	7.35	2.67	12.76	3.11
SA 0 + S3000	7.01	2.11	11.15	2.35
SA 100 + S0	12.11	3.45	18.12	4.12
SA 100 + S1000	8.71	3.18	13.51	3.67
SA 100 + S2000	7.76	2.81	13.25	3.35
SA 100 + S3000	7.12	2.19	12.57	2.81
SA 200 + S0	15.00	3.76	20.55	4.73
SA 200 + S1000	9.31	3.25	13.91	3.75
SA 200 + S2000	8.35	2.95	13.57	3.39
SA 200 + S3000	7.51	2.35	12.61	2.83
LSD 5%	1.3	0.06	0.8	0.09

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

Characters / Treatments	Flowers number/plant	Flowers diameter (cm)	Flowers fresh weight (g)	Flowers dry weight (g)
salicylic acid (SA) ppm				
0	10.86	3.24	15.94	3.43
100	13.02	3.35	18.70	4.07
200	14.13	3.45	21.36	4.67
LSD 5%	0.3	0.01	0.4	0.03
Salinity (S) ppm				
0	16.73	4.31	25.94	5.72
1000	13.10	3.71	18.47	4.09
2000	11.07	2.8	16.84	3.63
3000	9.78	2.56	13.41	2.79
LSD 5%	0.5	0.02	0.6	0.06
Interaction (ppm)				
SA 0 + S0	12.71	4.11	18.85	4.35
SA 0 + S1000	11.53	3.67	18.00	3.56
SA 0 + S2000	10.50	2.71	15.61	3.17
SA 0 + S3000	8.71	2.46	11.31	2.65
SA 100 + S0	17.36	4.31	26.35	5.67
SA 100 + S1000	13.61	3.69	18.11	4.07
SA 100 + S2000	11.00	2.83	16.73	3.73
SA 100 + S3000	10.11	2.56	13.61	2.81
SA 200 + S0	20.11	4.51	32.63	7.15
SA 200 + S1000	14.17	3.76	19.31	4.63
SA 200 + S2000	11.71	2.86	18.17	4.00
SA 200 + S3000	10.51	2.65	15.31	2.91
LSD 5%	0.8	0.04	0.9	0.09

Table 3: Flowers characters of *Calendula officinal*_L. plant of the third sample as affected by salicylic acid (SA) and irrigation with saline water (average two seasons).

Characters / Treatments	Flowers number/plant	Flowers diameter (cm)	Flowers fresh weight (g)	Flowers dry weight (g)
salicylic acid (SA) ppm				
0	26.88	4.57	29.35	6.74
100	30.18	5.22	32.10	7.81
200	34.53	5.55	35.30	8.91
LSD 5%	09	0.02	0.9	0.5
Salinity (S) ppm				
0	39.78	5.64	38.83	10.86
1000	31.92	5.22	33.06	8.21
2000	27.23	5.02	29.95	6.52
3000	23.59	4.57	27.16	5.66
LSD 5%	1.3	0.04	1.2	0.7
Interaction (ppm)				
SA 0 + S0	33.11	5.00	36.00	8.85
SA 0 + S1000	30.11	4.61	29.54	7.16
SA 0 + S2000	24.31	4.50	27.11	5.81
SA 0 + S3000	20.00	4.15	24.75	5.12
SA 100 + S0	38.91	5.75	38.75	10.93
SA 100 + S1000	31.31	5.37	32.91	8.17
SA 100 + S2000	26.35	5.10	29.61	6.51
SA 100 + S3000	24.13	4.67	27.11	5.61
SA 200 + S0	46.11	6.16	41.73	12.81
SA 200 + S1000	34.35	5.68	36.73	9.31
SA 200 + S2000	31.03	5.45	33.13	7.25
SA 200 + S3000	26.64	4.89	29.61	6.25
LSD 5%	2.1	0.06	2.2	1.0

Foliar application of salicylic acid treatments on marigold plants significantly increased number of flowers / plant, flower diameter (cm) and fresh and dry weight of flowers (g) compared with the control plants treatments. Foliar application of salicylic acid at 200ppm gave the highest significant increases in flowering parameters. The number was obtained at 3rd collection, while the lowest was obtained after at 1st collection. Similar trend was found in other characters. This positive effect of SA were attributed to enhanced CO_2assimilation, chlorophyll concentration, photosynthetic rate and increased mineral uptake by stressed plants treated with SA[21,22] .

Concerning the role salicylic acid on elevated negative effect of salinity water of *Calendula officinalis* plants, data in Table (1-3) indicated that spraying of SA at the two levels increased all flowering parameters.

In this context, it can be assumed that application of exogenous SA enhanced the salt stress resistance of plants[23], but the results were contradictory and depended on the developmental phase of plants[24] . The SA has been suggested to be physiologically important in stress tolerance since exogenous, SA brought about plants tolerance to various a biotic stress including salt[25] .

Chemical constituents:

Total carbohydrates contents:

According to the data in Table (4), it is evident that all solidity treatments caused a decrement on total carbohydrates contents in leaves, stems and roots compared with the control means with clear effect when high levels were used. The reduction on in total carbohydrate as solidity levels increasing might have relation to respiration processes since the free sugar were the main sugar pattern involve in the mechanism of respiration[26.]

As for the effect of salicylic acid on carbohydrates percentages, application of SA at 200ppm gave the highest valves of total carbohydrates percentage as compared with the untreated plants. The increment was 41.24% in leaves, 53.9% in stems and 30.38% in roots compared with control. This effect might be due to the effect of SA on enzymatic activity and translocation of the metabolites to the plants organs. These results are agreement with those obtained by[27,28] .

The significant interaction effect among the two studied factors (salinity x salicylic acid) on carbohydrate percentage values were found when using salinity at zero ppm combined with 200 ppm SA. The increases total carbohydrate contents fallowing SA application could be the indicator of build up of a protective mechanism to reduce oxidative damage induced by salt stress.

Table 4: Nitrogen, protein and carbohydrates of *Calendula officinal* L. plant as affected by salicylic acid (SA) and irrigation with saline water (average two seasons).

Characters / Treatments	Nitrogen %	Protein %	Total carbohydrates %		
			Leaves	Stems	Roots
salicylic acid (SA) ppm					
0	1.36	8.50	25.80	22.48	23.28
100	1.64	10.25	34.49	31.76	25.75
200	1.74	10.88	36.44	34.60	30.40
Salinity (S) ppm					
0	1.72	10.75	35.96	34.22	29.21
1000	1.61	10.06	33.24	29.99	27.56
2000	1.53	9.56	31.05	28.16	26.04
3000	1.47	9.19	28.72	26.07	23.35
Interaction (ppm)					
SA 0 + S0	1.57	9.81	28.17	25.21	25.61
SA 0 + S1000	1.44	9.00	27.00	23.00	25.00
SA 0 + S2000	1.27	7.94	25.01	21.71	23.00
SA 0 + S3000	1.16	7.25	23.03	20.01	19.51
SA 100 + S0	1.69	10.56	38.11	37.61	29.03
SA 100 + S1000	1.65	10.31	35.71	31.74	26.31
SA 100 + S2000	1.63	10.19	33.11	29.67	25.13
SA 100 + S3000	1.59	9.94	31.02	28.00	25.13
SA 200 + S0	1.89	11.81	41.61	39.83	33.00
SA 200 + S1000	1.73	10.95	37.00	35.24	31.37
SA 200 + S2000	1.69	10.56	35.03	33.11	30.00
SA 200 + S3000	1.65	10.31	32.11	30.21	28.02

Nitrogen contents and protein:

Results in Table (5) indicated that nitrogen, and protein % in shoots gradually increased by increasing salicylic acid levels. This effect might be due to these substances on enzyme activity and translocation of the metabolites to shoots.

All salinity treatments reduced N and protein % in shoots and the greatest reductions resulted from the highest salinity level (3000ppm) [29, 30]. Stalinization impaired N accumulation and in corporation into protein and raised total free amino acid accumulation in sanitized plant. Also, it can be suggested that amino acids can act as comments of salt tolerance mechanism and build up a favorable osmotic potential inside the cell in order to combat at the effect of which replaced nitrate in the vacuoles[31].

The highest nitrogen and protein % in shoots were recorded when foliar application of 200ppm salicylic acid combined with tap water, which recorded (27.94 and 28.0%), respectively, compared with control plants. The increases in the activity of antioxidant enzymes following salicylic acid application could be the indicator of build –up of a protective mechanism to reduce oxidative damage induced by salt stress.

References

1. Calendula. USDA, NRCS. 2007. The PLANTS Database (**Error! Hyperlink reference not valid.** Plant Data Center, Baton Rouge, LA 70874-4490 USA.
2. Hamburger M, Adler S , Baumann D, Förg A , Weinreich B . Preparative purification of the major anti-inflammatory triterpenoid esters from Marigold (Calendula officinalis). Fitoterapia. 2003; 74(4):328-338.
3. Duke JA. Handbook of Medicinal Herbs. Boca Raton, FL: CRC Press; 1985.
4. Lewis WH, Elvin-Lewis MP. Medical Botany: Plants Affecting Man's Health. New York, NY: John Wiley & Sons; 1977
5. Klouchek-Popava E , Popov A , Pavlova N , Krusteva S . Influence of the physiological regeneration and epithelialization using factions isolated from Calendula officinalis. Acta Physiol Pharmacol Bulg . 1982; 8(4):63-67.
6. Munns R. Comparative physiology of salt and water stress. Plant Cell Env 2002; 25:239-50. (PubMed).
7. Tester M., Davenport R. Na^+ tolerance and Na^+ transport in higher plants. Ann Biol 2003; 91:503-7 (PubMed).
8. Parida AK, Das AB. Salt tolerance and salinity effects on plants. A Rev Eco Env Safety 2005; 60:324-49. (PubMed).
9. Munns R. Genes and Salt tolerance together together: bringing them together. New Phytol 2005; 167:645-63.
10. Azza A.M. Mazher; Mona H,Mahgoub and Nahed G.Abd El Aziz (2011). Response of *Schefflera arboricola* to gypsum and sulphur application irrigated with different levels of saline water. Australian Journal of Basic and applied Sciences 5(10):121-129.
11. Baby Joseph, D. Jini and S. Sujatha, Insight into the Role of Exogenous Salucylic Acid on Plants Grown under Salt Environment, Asian journal of Crop Science 2 (4): 226-235, 2010.
12. Heitholt, J. J., J. H. Schmidt and J. E. Mulrooney, 2001. Effect of foliar-applied salicylic acid on cotton flowering, boll retention and yield. Materials and methods, 46:105-109.
13. Raskin, I.., 1992. Role of salicylic acid in plants, Annu. Rev. Plant Physiol. Plant Mol. Biol., 43:439-463.
14. Chapman, H.D. and P.E. Prott, 1961. Methods of Analysis for soils, plant and water. Div. of Agric. Sci.,Univ of calif, pp: 309.
15. Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods,7thed Iowa stat. Univ., Press Amer, Iowa, USA.
16. Steel, R.G.D and Torrie, 1980. Principles and procedures of statistics. McGrow- hillbookCo., Inc., New York, Toronto, London.
17. Dubios, M.; A.Giolles, Hamelton P.A.Robers and P.A. Smith, 1956. A clorimetric method for determination of sugar and related substances. Anal. Chem., 28: 350-356.
18. 18. A.O.A.C., 1980. Officinal Method of Analysis of Association of Official Analytical Chemists.12th Ed. Washington, D.C.
19. Greenway, H. and R. Munns,(1980). Mechanism sof salt tolerance in nonhalophytes. Annu. Rev. Plantphysiol., 31: 149-190.
20. Nahed, G. Abd ElAziz; Azza. A. M. Mazher and Mona, H. Mahgoub: Influence of using organic fertilizer on vegetative growth, flowering and chemical constituents of *Matthiola incana* plants grown under saline water irrigation. World Journal of Agricultural Sciences 7(1):47-54(2011).
21. Karlidage, H.; E. Yildirim and ,.Turan, 2009. Exogenous application of salicylic acid effects quality and yield of strawberry grown under antifrost heated greenhouse conditions. J. Plant Nut. Soil sci.,172: 270-276.
22. Zohreh, J; M.Khosh-kui and H.Salehi, 2009. The effectof Foliar- applied salicylic acid on flowering of African violet.Australian Journal of Basic and Applied sciences, 3 (4): 4693-4
23. Deef, E.E., 2007. Influence of salicylic acid on stress tolerance during seed germination of *Triticum aestivum* and *Hordeum vulgare.* Adv. Biol. Res., 1: 40-48.
24. Borsani, O.;V.Vlapmesta and M.A.Botell a, 2001. Evidence for a role of salicylic acid acidin the oxidative damage generated by Na Cl and osmotic stress in Arabidopsis seedlings. Plant physiol. 126:1024-1030.
25. Stevens, J.;T.seneratna and K.Sivasithamparam,. Salicylic acid induces salinity tolerance in tomato (*Lycopresicon esculentum* cv.Roma). Associated changes in gas exchange, water relations and membrane estabilization. Plant growthregul., (2006) .49: 77-83.

26. Bernstein, L.; L.E. François and R A.clark, 1972. Salt tolerance of ornamental shrubs and ground covers. J. Amer. Soc. Hort. Sci., 97: 550-556.
27. Shakirova, F.M.; A.R.Sakhabutdinovex;M.V.Berzukova; R. Fatkhutdinova and D.R.Fatkhutdinova, 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. Plant sci., 164: 317-325.
28. Amin, A.A; El- Sh.M.Rashed and H.M.H.El.Abagy, 2007. Physiological effect of indole– 3- Butyric acid and salicylic acid on growth, yield and chemical constituents of onion plants. Journal of Applied sciences Research, 3 (1):1554-1563.
29. Farahat, M.M.;Azza, A.M.Mazhar; Mona, H. Mahgoub and Sahar M. Zaghloul, (2013): Salt tolerance in Grevillea robusta seedlings viapoliar application of Ascorbic acid Middle- EastJournal of Scientific Research 14 (1): 09-15.
30. Ibrahem E. Habba,NahedG. Abd El- Aziz, Sami A.Metwally and Azza. AM.Mazhar (2103): Response of growth and chemical constituents inkhayasenglensisto salinity and Gypsum under zaleaeaus soil conditions. World Applied sciences journal 22 (4): 447–452.
31. HanafyAhmed, A.H. (1996): Physiological studies tiploun and nitrate accumulation in lettuce plants. J. Agric. Sci. Mansoura Univ., 21: 3971-3994.

*****