Original article

Variations of phytochemical properties of *Calendula officinalis* L. under salinity stress and treatment with humic acid and selenium

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Summary

The present study was designed to investigate the phytochemical composition and yield of essential oil of *Calendula officinalis* L. (Marigoldisa) under NaCl-induced salinity stress using humic acid and selenium as a factorial experiment in the form of a completely randomized design with two factors of NaCl-induced salinity stress (50 and 100 ppm) as the main factor and foliar application with selenium (at both 5 and 10 ppm levels) as well as soil application of humic acid (at both 50 and 100 ppm levels) as side factors and their interactions. The experiment consisted of 19 treatments each with 3 replicates, each replicate containing 3 plants and a total of 171 pots. In this study, *Calendula officinalis* L. seeds were cultivated in a tray containing cocopeat and then transferred to a pot of size 15 after 20 days (six-leaf stage) with the pot soil containing loamy soil, sand, and compost with a 1-1-1 ratio. The treatments were applied after 20 days of transfer to the main pot (early stage of stem emergence). The humic acid was added at the time of bed preparation with sodium chloride applied in combination with irrigation water twice in three consecutive weeks. Foliar application with selenium was also performed after each irrigation with sodium chloride. Fertilization with the required elements was normally performed for plants with the pots treated without control. Sampling and evaluation of traits were done about 20 days after flowering stage. The experiment was repeated for two consecutive years for greater confidence and better results. The results revealed that in both years, the control treatment had the highest effect on traits including fresh and dry weight of aerial parts and root, plant height and flower number, petal carotenoid content and total leaf chlorophyll content, protein content, phenol and flavonoid content, and it lead to longer longevity. The highest proline content was associated with the NaCl 100ppm treatment and the highest activity of superoxide dismutase and peroxidase enzymes as well as highest percentage of essential oil were observed in the NaCl 50 ppm + H 200ppm + Se 10ppm treatment. In addition, in the first and second years, the control treatment with 10.9 and 11.2 days, respectively, had the highest while NaCl 100ppm with 5.4 and 6.1 days had the lowest longevity of *Calendula officinalis* L. flowers on the plant.

Significance of this study

What is already known about this subject?

- The phytochemical description of plant organs with humic acid and selenium used under salinity stress.

What are the new findings?

- This research deals with some ways for increasing the salinity tolerance of different plants, including medicinal and ornamental *Calendula officinalis* L.

What is the expected impact on horticulture?

- Organic materials are a key step for good management of salinity as the main problems in the agricultural sector in arid and semiarid regions, actors limiting the growth and development of agricultural production in the world.

Keywords

antioxidant activity, humic compounds, marigold, selenium element, sodium chloride stress, volatile oils

Introduction

Calendula officinalis L. is known as Pot Marigoldisa plant from the family Asteraceae and the species *Calendula*. Humic compounds cause increased salinity, enhanced dehydration, augmented cold resistance, reduced toxicity of fertilizers and extra elements in the soil. Greater effect permanency up to several years in the soil, increased plant resistance to various diseases and a significant reduction in the need for pesticides, increased seed germination rate, improving soil texture, improved rooting, and more water retaining in the soil. It also contributes to the rapid growth of beneficial bacteria in the soil, to assist the dissolution and release of trace and major elements, thereby reducing the need for chemical fertilizers, supports modification of alkaline soils due to acidic pH, is environmentally friendly causing no harm to the plant or environment, helps maintain soil balance and improve crop quality, is the best natural chelator and act as a mechanism against stress (Garcia et al., 2012). Selenium is predominantly absorbed as selenate in the middle of plant roots, while selenite and organic selenium compounds are also readily absorbed. The chemical form of selenium affects its uptake and distribution in different parts of the plant (Sorris et al., 2005). Higher solubility of selenate as compared

to selenite plays an important role in selenium transport and metabolism in plants; thus, treatment of a plant with selenate causes a major transfer to the leaves. However, in the plant treated with selenite, selenium is mainly accumulated in the roots and a small amount is transmitted to the aerial organs (Longchamp et al., 2013). The role of selenium in plants as an essential element has not been fully understood. High concentrations of this element are toxic to plants with the symptoms of toxicity including diminished growth, chlorosis, withering, and drying of leaves, as well as reduced protein synthesis and death before plant puberty. On the other hand, low concentrations of selenium have beneficial effects on plant cell metabolism and, according to evidence, selenium soil or foliar application can increase crop growth, yield, and quality (Xu et al., 2003; Longchamp et al., 2013). In an experiment, the effects of sodium chloride were investigated on vegetative and reproductive traits of three Narcissus cultivars including 'Dutch Master', 'Ice Follies', and 'Tete-a-Tete'. The results of this study presented that the reduction of quality and all traits (leaf length, number of flowers, and number of leaves, petals and leaves, flower fresh weight, fresh weight of aerial organs, and flower longevity) was associated with increasing sodium chloride level. This decline in quality, growth, and flowering was less in 'Dutch Master' cultivar than in other cultivars (Veatch-Blohm et al., 2014). In a research, foliar application of potassium selenate and gibberellin garlic was used to figure out the product quality and yield. According to the results, the malondialdehyde content in potassium selenate treatment was lower than that of gibberellin and control treatments. In addition, the activity of catalase, superoxide dismutase, glutathione reductase, ascorbate peroxidase, lipoxygenase enzymes in potassium selenate treatment was higher than in the gibberellin and control treatments. Carotenoid, chlorophyll, and carbohydrate contents in potassium selenate treatment were also higher than in gibberellin and control treatments (Stetef et al., 2016). Salinity treatment is one of the most important and damaging environmental stresses, which reduces the quality and yield of the product. The effects of humic acid and sodium chloride were used to the germination percentage, growth, quality, and yield of alfalfa. After treatment, traits such as seed germination power, fresh and dry weight, plumule and radical length, photosynthesis rate, and plant yield were estimated. The results demonstrated that the application of humic acid had a significant effect on negative aspects of salinity stress at two concentrations of 2 and 12 dS m⁻¹ (Sofi et al., 2018). The impact of foliar application and soil application of sodium selenate on basil was compared. Then, the phenol content, essential oil percentage, selenium content, total antioxidant capacity, and crop yield were described. According to the results of this experiment, it can be concluded that sodium selenate in foliar and soil application improved the basil selenium content (Liubov Skrypnik et al., 2019). The spread of soil and water salinity throughout the world and Iran is expanding due to the scarcity of groundwater; increasing the concentrations of soluble elements in the water. *Calendula officinalis* L. has also excellent medicinal properties and nutritional value. Accordingly, this research deals with some ways for increasing the salinity tolerance of different plants, including medicinal and ornamental *Calendula officinalis* L.

Materials and methods

Plant material and experimental location

The present study was carried out in commercial green-

houses in Eslamshahr with average temperature of about 22 to 23°C, relative humidity of about 50 to 60%, and light intensity of about 60 to 70 μmol s⁻¹ m⁻², in 2018 and 2019. *Calendula officinalis* L. seeds were purchased from Pakan Seed Company in Isfahan; sodium chloride was purchased from Merck Germany; and acid humic from Ariashimi Company.

Experimental design and treatments

Factorial experiment was designed and implemented in a completely randomized design with two factors of NaCl-induced salinity stress (with 50 and 100 ppm levels) as the main factor along with foliar application with selenium (with 5 and 10 ppm levels) and soil application of humic acid (at both levels 50 and 100 ppm) as the side factors and their interactions. The experiment consisted of 19 treatments each with 3 replicates, each replicate containing 3 plants and a total of 171 pots. In this study, *Calendula officinalis* L. seeds were cultivated in a tray containing cocopeat and then transferred to a pot of size 15 after 20 days (six-leaf stage) with the pot soil containing loamy soil, sand, and compost at a 1-1-1 ratio. The treatments were applied after 20 days of transfer to the main pot (early stage of stem emergence). Then, the humic acid was added at the time of bed preparation and sodium chloride was applied in combination with irrigation water twice in three consecutive weeks. Foliar application with selenium was also performed after each irrigation with sodium chloride. Fertilization with the required elements was normally performed for plants with the pots without treatment considered as the control. Sampling and evaluation of traits including aerial organs and root fresh and dry weight, plant height, flower number, petal carotenoid, total leaf chlorophyll, proline, protein, superoxide dismutase activity, peroxidase, phenol, flavonoid, essential oil percentage, and flower longevity on plant were performed about 20 days thereafter (flowering stage). This experiment was repeated for two consecutive years for greater confidence and better results.

Evaluated traits

1. Fresh weight of the aerial organs (g). The fresh weight of the aerial organs was measured by a digital balance with a precision of 0.01.

2. Dry weight of aerial organs (g). The dry weight of aerial organs on a given day was measured after 72 hours of exposure to 60°C by a digital balance with a precision of 0.01.

3. Plant height (cm). Plant height was measured using a ruler from the soil surface to the top part of the plant.

4. Number of flowers. The number of flowers and buds in each sample were counted visually.

5. Total leaf chlorophyll content. Total leaf chlorophyll content was obtained by Arnon's method (1949) by milling a specific weight of the leaves in 80% acetone solvent, exposing the resulting compound for 24 hours at 4°C and then reading the absorption rate at the wavelengths 645 and 663 nm. Calculation was done by the following formula and finally the total chlorophyll content was expressed in mg g⁻¹ leaf fresh weight.

$$\text{Total leaf chlorophyll content} = 20.2 (A_{645} \text{ nm}) + 8.02 (A_{663} \text{ nm}) + (V1,000 \times 10).$$

A: Light absorption rate; V: Final acetone volume.

6. Proline. Proline content was measured by the method proposed by Bates et al. (1973). The solution was then prepared by 6 M phosphoric acid (41.12 mL 85% sulfuric acid at the density of 1.68 kg L⁻¹ was added to distilled water with solution and then brought to a volume of 100 mL), 3% sulfo-

salicylic acid (3 g of sulfosalicylic acid powder was dissolved in distilled water and brought to the volume of 100 mL) and ninhydrin acid (1.25 g ninhydrin acid powder dissolved in 30 mL of acetic acid (glacial) after which, 20 mL of 6 M phosphoric acid was prepared and added). Specifically, 0.5 g of the plant material was first crushed with mortar and poured into the test tube, then 10 mL of 3% sulfosalicylic acid was prepared and added to the tubes and the samples were placed in ice. The samples were centrifuged at 15,000 rpm for 10 to 15 minutes at 4°C to remove the excess material from the solution. Next, 2 mL of the smoothed extract was poured into a new tube and 2 mL of ninhydrin acid and 2 mL of acetic acid (glacial) were added and mixed well. At the same time, 2 mL of 0, 4, 8, 12, 16, and 20 ppm proline standard solution was poured into new tubes with 2 mL of ninhydrin and 2 mL of acetic acid (glacial) added and then mixed well. The samples were heated in a warm water bath for 1 hour and then placed in an ice bath. Further, 4 mL of toluene was added to the solution and was stirred by a vortex for 20 seconds. Also, 100 ppm proline standard: 0, 2, 4, 6, 8 and 10 mL was taken from the standard 100 ppm proline, respectively and brought to the volume of 50 ppm with distilled water. The soluble proline standards in toluene phase were poured into the spectrophotometer as needed with the proline content read at 520 nm and standard curves plotted. Then, the absorption rate in the plant samples was read and the proline content was calculated by embedding it in a line equation.

7. Superoxide dismutase enzyme. The enzyme extract was prepared according to the method proposed by Ezhilmathi et al. (2007) from 1 gram of petals and then the enzyme activity was measured by Bayer and Fridovich method (1987) based on the inhibition of photochemical reduction of Nitro-blue tetrazolium (NBT). In this method, 1 mL of the solution, 50 mM of K-phosphate buffer (pH 7.8), 9.9 mM of L-methionine, 57 µM of NBT, and 0.025% (v/v) Triton X-100 were added to a small glass tube with 20 µL of the sample added. The reaction was performed by adding 10 µL of riboflavin solution (4.4 mg 100 mL) and placing the tubes in a box containing two fluorescent lamps for 7 min where the parallel control sample was implemented using a buffer instead of the sample. Next, the absorption rate was read at 560 nm and the control sample was used as the blank. The activity of superoxide dismutase was expressed based on the enzyme unit per gram of petal fresh weight. One unit of superoxide dismutase enzyme is called the amount of enzyme required for 50% inhibition of NBT resuscitation at 560 nm in a spectrophotometer.

8. Total phenol content. To measure the phenol content, 0.5 to 1 gram of the sample was first crushed in Chinese mortar with 10 times its volume by 80% ethanol and centrifuged at 100 rpm for 20 min. The supernatant was dried and evaporated while the remainder was dissolved in a certain amount of deionized water (5 mL). Different 0.2–2 mm samples were poured into different test tubes with the volume of each test tube brought to 3 mL with deionized water. Then, 0.5 mL of Folin-Ciocalteu reagent plus 2 mL of sodium carbonate solution was added to each test tube after 3 min. After mixing thoroughly, the test tubes were placed in boiling water for 1 min, and then cooled. The absorption was determined at 650 nm via a control reagent and expressed in mg of phenol per gram of sample. The standard curve was plotted at different catechol concentrations (Malik et al., 1980).

9. Essential oil percentage. The petals were extracted by Clevenger apparatus. Specifically, 100 g of dried petals was accurately weighed and then crushed by a mill. Next, the

sample was poured into the balloon of the Clevenger apparatus where 500 mL of distilled water was added with the essential oil value expressed in percentage (Mehrafarin et al., 2017).

10. Longevity of flowers on the plant. The longevity of flowers on the plant was measured from the time of flowering and the appearance of color until wilting, staining, deflowering, and further expressed in terms of days (Ezhilmathi, 2007).

Data analysis method

After data collection, data were introduced into Excel software and analyzed by SPSS statistical software. Mean data comparison was performed using Duncan's multiple range test at 1% and 5% levels. Excel software was also used to plot the graphs.

Results

In Table 1, analysis of variance shows the effect of the treatment on the fresh weight of aerial organs, dry weight of aerial organs, plant height, number of flowers, protein content, superoxide dismutase activity, total phenol content, essential oil percentage, and flower longevity on the plant which was significant at 1% level and on the chlorophyll content of leaf and proline content at 5% level. The effect of year on the fresh weight of aerial organs, dry weight of aerial organs, total leaf chlorophyll content, proline and protein content, essential oil percent and flower longevity was significant at 1% level and on plant height and number of flowers, superoxide dismutase activity, and phenol content at 5% level. In addition, the interaction effects of treatment × year on all traits were statistically significant at 1% level.

Fresh weight of aerial organs

According to Figure 1, the fresh weight of aerial parts for all treatments in the second year was higher than in the first year. In the first and second years, the control treatment with 28.64 and 30.32 g had the highest while NaCl 100ppm with 21.41 and 22.05 g had the lowest fresh weight of the aerial organs respectively. In addition, the minimum difference of the fresh weight of the aerial organs between two years was observed in NaCl 100ppm + humic acid 100ppm treatment with 0.26 g and highest in the control treatment with 1.68 g. The difference of fresh weight of aerial organs in the control treatment of the second experiment (highest fresh weight of the aerial organs) with NaCl 100ppm treatment in the first experiment (lowest fresh weight of the aerial organs) was 8.91 g. In the first experiment, the application of humic acid was about 13.47%, selenium 14.88%, and the interaction of both 7.63%, while in the second experiment, the use of humic acid was about 16.18%, and selenium 16.54% and their interaction 10.23%. The fresh weight of the aerial organs under stress condition caused by NaCl was lower than in that the control treatment.

Dry weight of aerial organs

As indicated in Figure 2, the dry weight of aerial organs of all treatments in the second year was higher than in the first year. In the first and second years, the control treatment with 7.03 g and 7.54 g, had the highest while NaCl 100ppm treatment with 4.41 g and 4.59 g had the lowest dry weight of aerial organs. Also, the minimum difference of dry weight of aerial organs between the two years was related to NaCl 100ppm + H 200ppm + Se 5ppm treatment with 0.04 g and highest was associated with NaCl 50ppm + Se 5ppm

TABLE 1. Results of analysis of variance of the first experiment.

Variations source	Mean squared					
	Degree of freedom	Fresh weight of aerial organs	Dry weight of aerial parts	Plant height	Number of flowers	Total leaf chlorophyll content
Treatment	18	51.182**	7.414**	98.039**	11.708**	0.224*
Year	1	52.324**	20.715**	58.517*	0.075*	0.051**
Treatment × year	18	0.590**	0.291**	1.754**	0.345**	0.026**
Experimental error	—	0.126	0.027	0.119	0.022	0.014
CV (%)	—	12.40	10.36	12.27	9.12	8.94

TABLE 1. Continued. Results of analysis of variance of the first experiment.

Variations source	Mean squared					
	Degree of freedom	Proline	Superoxide dismutase	Phenol	Essential oil percentage	Flower longevity on plant
Treatment	18	71.882*	247.13**	158.31**	0.513**	38.601**
Year	1	21.817**	2.019*	154.167**	0.085**	3.667**
Treatment × year	18	2.641**	23.510**	31.712**	0.012**	0.274*
Experimental error	—	0.028	0.205	0.154	0.005	0.033
CV (%)	—	10.45	11.21	10.01	9.70	11.14

* and **: significant at the 5% and 1% probability levels, respectively; 'ns' is not significant.

FIGURE 1. Interaction of time and treatment on the fresh weight of aerial organs. Vertical bars indicate standard error. Means followed by the same letter are not different according to Duncan's test.

FIGURE 2. Interaction of time and treatment on dry weight of aerial organs. Vertical bars indicate standard error. Means followed by the same letter are not different according to Duncan's test.

treatment with 0.77 g. The difference of fresh weight of aerial organs in the control treatment of the second experiment (highest dry weight of aerial organs) with NaCl 100ppm in the first experiment (lowest dry weight of aerial organs) was 3.13 g. In the first experiment, the application of humic acid was about 29.82%, selenium 31.62%, and their interaction 18.65%, while in the second experiment, the use of humic acid was about 26.12% and selenium 26.87% and their interaction 19.45%. The dry weight of aerial organs under stress conditions caused by NaCl was lower than in the control.

Plant height

As shown in Figure 3, plant height in all treatments in the first year was higher than in the second year. In the first and second years, the control treatment with 30.43 and 28.12 cm had the highest while NaCl 100ppm treatment with 21.75 and 20.36 cm had the lowest plant height, respective-

ly. In addition, the minimum plant height difference between two years was associated with NaCl 100ppm + H 100ppm + Se 5ppm at 0.06 g while the highest was related to the control treatment at 2.31 g. The difference of plant height in the control treatment of the first experiment (highest plant height) with NaCl 100ppm treatment in the second experiment (lowest plant height) was 10.07 cm. Overall, in the first experiment, humic acid use was about 21.36%, selenium 22.88%, and their interaction 11.22%, while in the second experiment, humic acid application was about 18.40%, selenium 18.20% and their interaction 6.47%, where the plant height under NaCl stress conditions was lower than in the control.

Number of flowers

According to Figure 4, the number of flowers in most treatments in the first year was higher than in the second year. In the first and second years, the control treatment with

FIGURE 3. Interaction of time and treatment on plant height. Vertical bars indicate standard error. Means followed by the same letter are not different according to Duncan's test.

FIGURE 4. Interaction of time and treatment on the number of flowers. Vertical bars indicate standard error. Means followed by the same letter are not different according to Duncan's test.

8.4 and 7.9 had the highest while NaCl 100ppm treatment with 4.6 and 4.8 had the lowest number of flowers, respectively. Also, the minimum difference in the number of flowers between two years was 0.00 for the NaCl 50 + H 100ppm treatment while the highest was 0.005 in the two control and NaCl 50ppm + H 200ppm + Se 10 treatment. The difference between the number of flowers in the control treatment of the first experiment (the highest number of flowers) and the NaCl 100ppm treatment in the first experiment (the lowest number of flowers) was 3.8. Overall, in the first experiment, the application of humic acid was about 27.08%, selenium 26.19%, and their interaction 16.22%, while in the second experiment, humic acid use was about 23.10%, selenium 19.94% and their interaction 11.87%, where the number of flowers under stress conditions induced by NaCl was less than in the control.

Leaf chlorophyll content

As observed in Figure 5, the total leaf chlorophyll content in NaCl 50ppm + H 200ppm treatment in the first year was more than in the second year. In the first and second years, the control treatment with 0.8329 and 0.8613 mg g⁻¹ fresh

weight had the highest while NaCl 100ppm treatment with 0.4843 and 0.4901 mg g⁻¹ fresh weight had the lowest total leaf chlorophyll content. Also, the lowest difference in the total leaf chlorophyll content between two years was associated with NaCl 100ppm treatment at 0.0058 and the highest was related to NaCl 100ppm + H 200ppm + Se 5ppm treatment at 0.0760. The difference of the total leaf chlorophyll content in the control treatment of the second experiment (highest leaf chlorophyll content) with NaCl 100ppm treatment in the first experiment (lowest leaf chlorophyll content) was 0.377. In the first experiment, the application of humic acid was about 27.58%, selenium 33.78%, and their interaction 11.52%, while in the second experiment, the use of humic acid was about 27.68%, and selenium 32.65%. The total leaf chlorophyll content under NaCl-induced stress was 11.05% and lower than in the control.

Leaf proline content

As observed in Figure 6, the proline content in the NaCl 100ppm + H 200ppm and NaCl 100ppm + Se 5ppm treatment in the first year was higher than in the second year. In the first and second years, 100ppm NaCl treatment with

FIGURE 5. Interaction of time and treatment on total leaf chlorophyll content. Vertical bars indicate standard error. Means followed by the same letter are not different according to Duncan's test.

FIGURE 6. Interaction of time and treatment on leaf proline content. Vertical bars indicate standard error. Means followed by the same letter are not different according to Duncan's test.

11.26 and 12.09 mg g⁻¹ fresh weight had the highest while NaCl 50ppm + H 200ppm + Se 10ppm treatment with 4.52 and 5.42 mg g⁻¹ fresh weight had the lowest proline content. In addition, the lowest difference of proline content between two years was associated with NaCl 50ppm + H 200ppm treatment with 0.09 while the highest was related to the NaCl 50ppm + Se 10ppm treatment with 0.95. The difference of proline content in NaCl 100ppm treatment in the second experiment (highest proline content) with NaCl 50ppm + H 200ppm + Se 10ppm treatment in the first experiment (lowest proline content) was 7.57. In the first experiment, the application of humic acid was about 19.03%, selenium 19.32%, and their interaction 45.69%, while in the second experiment, the use of humic acid was about 23.47%, and selenium 21.90% and their interactional effect 44.39%. The proline content in NaCl-induced stress conditions was about less than others.

Superoxide dismutase activity

As shown in Figure 7, the activity of superoxide dismutase enzyme was higher in the first year than in the second year in most treatments. In the first and second years, treatment of NaCl 50ppm + H 200ppm + Se 10ppm with 71.06 and 70.12 units of enzyme per gram of fresh weight had the highest while NaCl 100ppm treatment with 25.68 and 27.25 units of enzyme per gram of fresh weight had the lowest activity of superoxide dismutase. Also, the lowest difference of superoxide dismutase activity between two years was associated with NaCl 100ppm + H 10ppm + Se 10ppm treatment with the value of 0.17 while the highest was related to the NaCl 100ppm + Se 10ppm treatment with 5.24. The difference of the activity of superoxide dismutase enzyme in NaCl 50ppm + H 200ppm + Se 10ppm of the first experiment (highest superoxide dismutase activity) with NaCl 100ppm in the first experiment (the lowest activity of superoxide dismutase) was 45.38. In the first experiment, the application of

FIGURE 7. Interaction effect of time and treatment on superoxide dismutase activity. Vertical bars indicate standard error. Means followed by the same letter are not different according to Duncan's test.

FIGURE 8. Interaction of time and treatment on total leaf phenol content. Vertical bars indicate standard error. Means followed by the same letter are not different according to Duncan's test.

humic acid was about 38.91%, selenium 35.45%, and their interaction 12.93% while in the second experiment, humic acid use was about 36.33%, selenium 35.93% and their interaction 12.60%, where the superoxide dismutase activity under NaCl stress conditions was lower than in NaCl 50ppm + H 200ppm + Se 10ppm treatment.

Total leaf phenol content

As displayed in Figure 8, the phenol content in most treatments was higher in the first year than in the second year. In the first and second years, the control treatment with 50.23 and 44.87 mg g⁻¹ fresh weight and NaCl 100ppm with 13.65 and 16.23 mg g⁻¹ fresh weight had the highest and lowest phenol content values, respectively. Also, the lowest difference of phenol content between two years was in NaCl 50ppm + Se 5ppm with 0.07 while the highest was associated with NaCl 50ppm + H 200ppm + Se 5ppm treatment with the value of 5.72. The difference of phenol content in the control

(highest phenol content) with NaCl 100ppm treatment in the first experiment (the lowest phenol content) was 36.58. In the first experiment, humic acid was about 39.82%, selenium 45.63%, and their interaction 15.49%, while in the second experiment, humic acid use was about 34.29%, selenium 37.49% and their interaction 12.14%, where the phenol content under stress conditions caused by NaCl was lower than in the control treatment.

Essential oil percentage

As shown in Figure 9, the percentage of essential oil in most treatments was higher in the first year than in the second year. In the first and second years, NaCl 50ppm + H 200ppm + Se 10ppm treatment with 1.37% and 1.45% and NaCl 100ppm treatment with 0.86% and 0.79% had the highest and lowest percentages of essential oil, respectively. Also, the lowest essential oil percentage difference between two years was associated with NaCl 100ppm + H 100ppm

FIGURE 9. Interaction of time and treatment on plant essential oil percentage. Vertical bars indicate standard error. Means followed by the same letter are not different according to Duncan's test.

FIGURE 10. Interaction effect of time and treatment on the longevity of flower on plant. Vertical bars indicate standard error. Means followed by the same letter are not different according to Duncan's test.

with 0.00 while the highest was related to the NaCl 50ppm + H 100ppm + Se 10ppm with 0.10. The difference of essential oil percentage in NaCl 50ppm + H 200ppm + Se 10ppm treatment of the second experiment (highest essential oil percentage) with the NaCl 100ppm treatment of the second experiment (lowest essential oil percentage) was equal to 0.66. Overall, in the first experiment, the application of humic acid was about 24.45%, selenium 25.36%, and their interactional effect 6.93%, while in the second experiment, humic acid use was about 25.17%, selenium 28.62%, and their interaction 53.5%, where the essential oil percentage under NaCl stress conditions was less than in the case of NaCl 50ppm + H 200ppm + Se 10ppm treatment.

Longevity of flowers on the plant

According to Figure 10, the longevity of *Calendula officinalis* L. flowers on the plant in most treatments in the first year was less than in the second year. In the first and second years, the control treatment with 10.9 and 11.2 days and NaCl 100ppm with 5.4 and 6.1 days had the highest and lowest longevity, respectively. Also, the lowest essential oil percentage difference between two years was associated with NaCl 50ppm + Se 10ppm with 0.00 while the highest was observed in NaCl 100ppm treatment with 0.70. The difference of longevity of *Calendula officinalis* L. flowers in the control treatment of the second experiment (highest longevity of the *Calendula officinalis* L. flowers on the plant) with NaCl 100ppm was equal to 5.8 in the first experiment (lowest longevity of *Calendula officinalis* L. flowers per plant). In the first experiment, the use of humic acid was about 30.50%, selenium 31.88%, and their interactional effect 22.22%, while in the second experiment, the use of humic acid was about 31.03%, and selenium 33.26%, and their interactional effect 15.29%, where the longevity of *Calendula officinalis* L. flowers on plant under NaCl stress condition was less than in the case of control treatment.

Discussion

Salinity is one of the first stresses that plants face. Today, as one of the most important environmental stresses, it affects the plant growth and it is one of the factors limiting the growth and development of agricultural production in the world. Indeed, it is one of the main problems in the agricultural sector in arid and semiarid regions, including most of Iran (Munns, 2002). Salinity increases the energy required to maintain the cell's natural state, resulting in decreased energy remaining for growth. Thus, plants are generally weaker in salinity and have smaller leaves than normal plants. Vegetative growth of plants decreases with increasing osmotic pressure of the environment under saline conditions. The reduction in the leaf area is due to salinity either as a result of reduced leaf number caused by reduced photosynthesis or diminished leaf size in response to the decline in turgidity pressure. In addition, with increasing salinity stress, the amount of pigments in the plant drops, which is sometimes due to their oxidation by reactive oxygen and their structure degradation. Increasing stress further causes increased oxygen free radicals and consequently antioxidant activity and accumulation of toxic free radicals. Salinity tolerance is the ability that enables plants to grow and complete their life cycles in an environment with high concentrations of soluble salt. The diminished effects of different environmental stresses on acidic and selenium treatments have been shown in different studies. The results of all studies indicate that humic acid creates an environment rich in minerals which

are essential for plant growth through stimulating plant growth by accelerating and intensifying cell division, increasing cell membrane permeability, enhancing root and product dry matter development, raising chlorophyll content in leaves, thereby enhancing photosynthesis rate and nutrient production. It also enjoys a high ion exchange capacity, transforming a number of elements into forms available to plants, helping to dissolve and release both trace and major elements (Jindoo, 2012; Garcia et al., 2012). Some nutrients, such as selenium, are also essential to increase the plant tolerance to stress and plant growth. Low concentrations of selenium have beneficial effects on plant cell metabolism and can increase the growth, yield, and quality of crops (Xu et al., 2003). Butnau and Coradini (2019) found that simultaneous application of humic acid and nanoselenium inhibited the detrimental effects of salt stress caused by sodium chloride in this plant. In order to gain results that are more reliable for the first year, all experiments were repeated in the second year where the investigation and comparison of the results of both experiments were consistent. They were also in line with the results of earlier research on the effects of stress on some ornamental or medicinal plants mentioned in the introduction.

Conclusion

As a general conclusion, we can state:

- In both years, the control treatment had the highest fresh and dry weights, plant height and flower number, total leaf chlorophyll content, phenol content, and flower longevity on the plant.
- The highest proline content was associated with NaCl 100ppm treatment as well as the highest activity of superoxide dismutase enzymes, where the highest percentage of essential oil was related to the NaCl 50ppm + Se 10ppm + H 200ppm.
- In the first and second years, the control treatment with 10.9 and 11.2 days and NaCl 100ppm with 5.4 and 6.1 days had the highest and lowest longevity of *Calendula officinalis* L. flowers on the plant, respectively. According to the biennial results of this study, the use of humic acid along with selenium can reduce the detrimental effects of salinity stress on the plant and increase the *Calendula officinalis* L. resistance to salinity stress.

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