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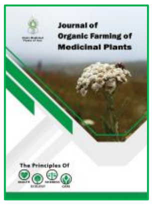

Original Article

Evaluation of humic acid and iron and zinc nanochelates effect on Italian basil (*Ocimum basilicum* L.) in salinity stress condition

Melika Hoseini[a], Farzad Paknejad[b], Mohammad Nabi Ilkaee[b]

[a] *Department of Horticulture Science, Karaj branch, Islamic Azad University, Karaj, Iran.*
[b] *Department of Agronomy and Plant Breeding, Karaj branch, Islamic Azad University, Karaj, Iran*

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Corressponding Author:
Farzad Paknejad
farzadpaknejad@yahoo.com

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ABSTRACT

The salinity tolerance of basil is considered low and application of humic acid and micronutrient elements are the environmentally friendly way to alleviate the negative effects of salinity stress. For this purpose, a factorial experiment was conducted base on a completely randomized design in three replications on the Italian basil (*Ocimum basilicum* L.) in a greenhouse located in Karaj, Iran, in April 2022. The factors of this research included humic acid in three concentrations (0 (control), 500 and 1000 mg l^{-1}), application of micronutrient elements in three levels (0 (control), iron nanochelate, zinc nanochelate) and salinity stress in two levels (without stress (normal condition), and 30 mM NaCl). The results showed that salinity stress decreased the yield and essential oil of Italian basil. Increasing the concentration of humic acid as well as the use of micronutrients increased the quality and qualitative yield of this plant. The highest dry matter yield (151.12 g m^{-2}) was seen in interaction of humic acid 1000 mg l^{-1} +iron nanochelate in normal condition and also (149.74 g m^{-2}) was observed in humic acid 1000 mg l^{-1}+ zinc nanochelate in normal condition. The highest essential oil (1.89%) was seen in interaction of humic acid 1000 mg l^{-1} + iron nanochelate, in normal condition. Therefore, humic acid and micronutrients (iron and zinc nanochelate) reduced the negative effect of salinity stress and improves Italian basil tolerance with stress condition and these are good environmentally friendly way to deal with saline conditions.

1. Introduction

Medicinal plants need essential nutrients for growth and expansion, also the production of secondary metabolites to defend against stresses. Basil (*Ocimum basilicum*) is an annual plant from the mint family of Lamiaceae and one of the most important medicinal plants and leafy vegetables. Its aerial body contains essential oil, polyphenol, flavonoid and phenolic acid. Its medicinal uses include anticancer activity, antimicrobial activity, anti-inflammatory effects, immune system regulating activity, anti-stress, anti-diabetes, anti-arthrosis and antioxidant activity (Shahrajabian et al., 2020). It is also common to use as a tea, spice and fresh vegetable (Moghadam et al., 2013).

Hydroponic cultivation is an easy method to produce medicinal plants that are grown in a solution containing nutrients without soil. Among the advantages of this cultivation, we can mention the easy control of nutrients, lack of soil pollution, fast plant growth, short cropping period, high product quality, and increased marketability. Hydroponic systems also allow easier management of the complementary irrigation with saline water compared to the cultivation in soil (Singh et al., 2020; Gashgari et al., 2018).

Salinity stress is a major concern in several ecosystems and has a significant impact on global agriculture. The expansion of saline areas has significantly increased. Based on severity and duration of the salinity stress, the plants show different reactions. Salinity stress affects all the main processes including growth, photosynthesis, protein synthesis and lipid metabolism and causes a decrease in root and stem growth, ion toxicity, disturbance in the absorption and the balance of nutrients inside the plant and ultimately a decrease in photosynthesis and also reduce plant yield (Negrão et al., 2017). It is long established that salinity stress and other environmental stress conditions have a strong impact on the essential oil and also accumulation of secondary compounds in medicinal plants (Miras-Moreno et al., 2020; Corrado, et al., 2021). Other researchers reported that salinity stress reduced yield and yield components of basil (Lazarević et al., 2021) and purslane (*Portulacaoleracea* L.) (Dehghan et al., 2018).

Spraying of micronutrient elements can be used as an emergency or supplementary method in critical times during the growth and development of plants. It has been reported that the response of plants to foliar application of micronutrient elements depends on various factors, including the species, variety and the state of plant nutrients (Shahhoseini et al., 2020). Micronutrient elements are among the elements that have important effect in the growth and development of plants. When these elements are present at a sufficient level, the physiological, biochemical and metabolic characteristics of the plant improve, while their deficiency causes abnormal growth of plants. Low organic matter is common in many regions due to the nature of the soil, high pH, salinity stress, continuous drought, high bicarbonate ion in irrigation water and imbalance of nutrients (Jatav et al., 2020). Micronutrient elements increased yield and yield components of chamomile (*Tanacetum parthenium* L.) (Shahhoseini et al., 2020). Nanotechnology can involve using nano-scale fertilizer particles which physiologically provide new methods for alleviating nutritional problems in plants. The use of Nano chelates leads to an increased efficiency of the elements, to at least reach the negative effects caused by the consumption of excessive use of fertilizers and reduce the frequency of application of fertilizers (Chinnamuthu & Boopathi, 2009).

Humic acid is one of the economical and environmentally friendly organic fertilizers, which a rich provenance of macro and micronutrient elements, vitamins, enzymes and growth stimulating hormones that provide nitrogen and phosphorus elements for the plant and increasing the yield and quality of crops and medicinal plants (Boveiri Dehsheikh et al., 2020). The usage of humic acid reduces the negative effects caused by salinity stress. In this regard, it has been reported that humic acid increased the yield of basil plants even under salinity stress compared to non-use (El Gohari et al., 2023). Salinity stress is one of the most important factors in the reduction of agricultural products in Iran, and it is very important to provide solutions to combat it. Thus, considering the importance of humic acid and micro-nutrients in improving the yield of medicinal plants and reducing the negative effects of salinity stress, this research aimed to investigate the effect of foliar application of humic acid and nano-nutrient of iron and zinc to alleviate plants responses under salinity conditions.

2. Materials and Methods

2.1 Experimental design and plant material

This experiment was carried out in a factorial form in a completely randomized design in three replications in a greenhouse located in Nazar Abad Karaj, Iran, on the Italian basil (*Ocimum basilicum* L.), in April 2022. Italian basil cultivar was Genova. The factors of this research included spraying of humic acid in three different concentrations (0 (control), 500 and 1000 mg l^{-1}), spraying of micronutrient elements in three levels (0 (control), iron nanochelate and zinc nanochelate), and salinity stress in two levels (No stress (control), 30 mM NaCl).

Laboratory sodium chloride was used to apply salinity stress. The concentration of nano zinc fertilizer was 12% and the concentration of nano iron fertilizer was 9% from company of Khazra, and with a registration numbers 29936 and 34428, respectively. Each experimental unit consisted of 5 pots in which the culture medium consisted of 60% by cocopeat and 40% perlite. First, the seedlings were planted in the seedling tray, and after three weeks, three-leafed seedlings were planted in pots (height 12 cm and diameter 15 cm) with the mentioned substrate. One plant was planted in each pot. Plants are watered with Hoagland's 1/2 solution regularly every day (once a day) (Utmazian et al., 2007).

In order to determine the electrical conductivity, the pots were drained and, if necessary, washed with water. Basil plants were sampled and harvested after two months. Salt stress was applied with Hoagland nutrient solution. The duration of salt stress was one month. Control plants received only 1/2 Hoagland nutrient solution (without salt). In order to prevent shock caused by salinity stress to the plants, in the first irrigation after the salinity stress started, 20 mM salt was used together with Hoagland's nutrient solution. Once a week, the root environment of the plants was completely washed with distilled water so that the changes in EC and pH resulting from the accumulation of salts in the planting bed due to the washing process were as low as possible.

The measured traits were: plant dry weight, plant height, stem thickness, essential oil and essential oil yield, chlorophyll index, relative water content, membrane stability index, proline content, catalase and ascorbate peroxidase activity.

2.2 Yield and some physiological traits

The plant height was measured by a graduated ruler, the plants were dried for 20 days at room temperature (25°C) after harvesting, and the dry weight of the plants was weighed by a digital scale and the diameter of the stem was measured by a digital caliper. To measure chlorophyll index Spad chlorophyll meter (SPAD-502, Konica Minolta, Japan) was used.

2.3 *Essential oil content and essential oil yield*

The essential oil content was measured by extracting it from the dry plant (leaves and basil flowers) using the distillation method with water by the Clevenger device (for 3 hours), and finally, the essential oil content was calculated in milliliters per 100 g of dry matter. Essential oil yield was obtained from the product of the dry matter yield in the essential oil content.

2.4 Relative water content (RWC)

The amount of RWC was calculated as the equation 1.

$$RWC = \frac{FW-DW}{SW-DW} \times 100 \qquad (Eq.1)$$

In the above equation, FW = leaf fresh weight, DW = leaf dry weight, SW = leaf saturated weight (Ferrat & Loval, 1999). Membrane stability index was evaluated by measuring the leakage rate of leaf electrolytes. For this purpose, each sample was placed in distilled water with a volume of 20 ml and kept at 25°C for 24 hours. The electrical conductivity of the distilled water with the sample was measured as the primary leakage. Secondary leakage was also measured by measuring the electrical conductivity of the samples after heating them for 2 minutes at 100˚C. The membrane stability index was calculated through equation 2 (Bertin et al., 1996).

$$\text{Membrane Stability Index (MSI)} = [1- C1/C2] \times 100 \qquad (Eq2)$$

In this regard, MSI = membrane stability index, C1 = primary leakage, C2 = secondary leakage.

2.5 Proline content

Proline quantity was measured by Bates et al (1973) method. 0.3 g of fresh leaves was combined with 5 ml of sulphosalicylic acid 3%, then homogenized. Then centrifuged at 4°C for 10 minutes at 15000 rpm. Finally that was read with spectrophotometer (PG Instruments Itd VIS/UV+T model) at 520 nm wavelength and compared with a control sample.

2.6 Catalase and ascorbate peroxidase activity

To measure catalase enzyme activity, 1500 µl of 100 mM sodium phosphate buffer containing 2% PVP and 1.3 mM EDTA was added to 350 mg of plant tissue and finally, absorbance changes were recorded with a spectrophotometer (PG Instruments Itd VIS/UV+T model) at 240 nm for 3 minutes (Aebi, 1984). Ascorbate peroxidase enzyme was measured from fresh plant leaves at a temperature of 25˚C by spectrophotometric (PG Instruments Itd VIS/UV+T model) method (Sairam et al., 1998).

2.7 Date analysis

The data obtained from this research were analyzed using SAS software (Ver. 9.4) and the mean comparison was done using Duncan's multi-range test with a 5 % error probability.

3. Results

3.1. Dry matter yield

ANOVA results for dry matter yield presented that the effect of salinity stress, humic acid, and micronutrient elements and their interactions were significant ($p < 0.01$) (Table 1). The results of mean comparison indicated that salinity stress reduced the dry matter yield of Italian basil. The use of humic acid and increasing its concentration increased the yield of dry matter. Foliar spraying of zinc and iron was also associated with an increase in the dry matter yield of the plant compared to the control (without foliar spraying). The highest dry matter yield (151.12 g m^{-2}) was seen in the interaction of foliar application of humic acid 1000 mg l^{-1}+ foliar application of iron in without salinity stress (control) and also (149.74 g m^{-2}) was observed in humic acid 1000 mg l^{-1}+ iron in in control condition. The lowest value of this index (105.21 g m^{-2}) was observed in stress condition and the control treatments (Fig. 1).

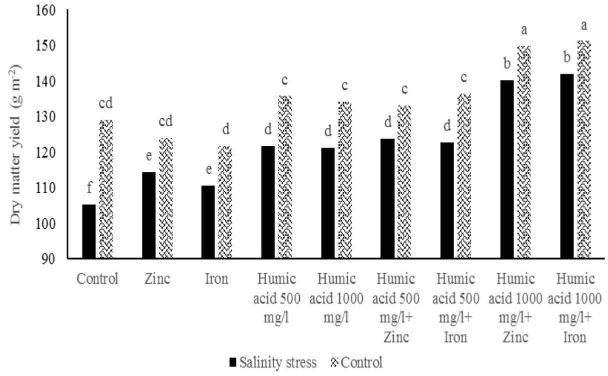

Fig. 1. Interaction effect of humic acid, micronutrient and salinity stress on dry matter yield

3.2. Plant height

The effect of salinity stress, humic acid, micronutrient elements, as well as their interaction effects, had a significant effect on plant height ($p < 0.01$) (Table 1). The results of mean comparison indicated that salinity stress reduced plant height. The application of humic acid and increasing its concentration improved the plant height. Foliar spraying of zinc and iron was also associated with an increase in plant height compared to the control (without foliar spraying). The highest plant height (43.52 cm) was observed in interaction of without salinity stress (control) + foliar spraying of humic acid 1000 mg l^{-1} + foliar spraying of iron, and also (43.51 cm) was related to no salinity stress (control) + foliar spraying of humic acid 1000 mg l^{-1} + iron foliar spraying. The lowest plant height (28.98 cm) was related to stress condition and the control treatments (Fig. 2).

3.3. Stem diameter

The effect of salinity stress, humic acid and the effect of micronutrient elements had a significant effect on stem diameter ($p < 0.01$) (Table 1). The results of the mean comparison showed that the salinity stress decreased the stem diameter. Foliar spraying of zinc and iron, as well as the usage of humic acid and improving its content increased stem diameter. The maximum stem diameter (0.63 cm) was seen in without stress, and (0.82 cm) related to humic acid 1000 mg l^{-1}. In micronutrient treatments the highest stem diameter (0.79 cm) was seen in iron spraying, and also (0.81 cm) was related to zinc (Table 3).

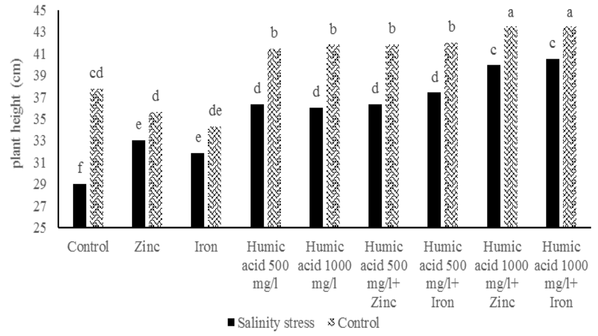

Fig. 2. Interaction effect of humic acid, micronutrient and salinity stress on plant height

3.4. Essential oil content and essential oil yield

ANOVA results for essential oil content and essential oil yield showed that the effect of salinity stress, humic acid, and micronutrient elements as well as their interaction, were significant ($p < 0.01$) (Table 1). The results of mean comparison indicated that salinity stress reduced the essential oil content and essential oil yield of Italian basil. The use of humic acid and increasing its concentration increased the essential oil content and essential oil yield. Foliar spraying of zinc and iron increased the essential oil content and essential oil yield of plants compared to the control (without foliar spraying). The highest essential oil content (1.89%) and essential oil yield (151.12 g m^{-2}) were seen in the interaction of foliar application of humic acid 1000 mg l^{-1}+ foliar application of iron in without salinity stress (control) and the lowest essential oil content (0.55%) and essential oil yield (0.61 g m^{-2}) were observed in stress conditions and the control treatment (Fig. 3 and 4).

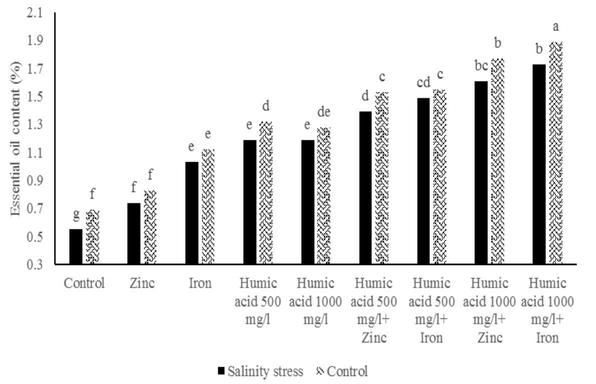

Fig. 3. Interaction effect of humic acid, micronutrient and salinity stress on essential oil content

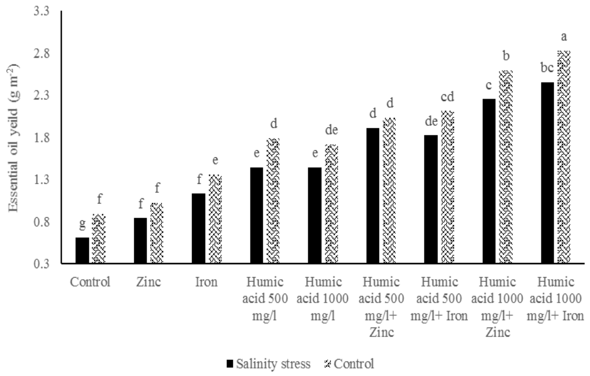

Fig. 4. Interaction effect of humic acid, micronutrient and salinity stress on essential oil yield

3.5. Chlorophyll index

The effect of salinity stress, humic acid, micronutrient elements, as well as their interaction, had a significant effect on chlorophyll index ($p < 0.01$) (Table 2). The results of the mean comparison indicated that salinity stress reduced the chlorophyll index. The application of humic acid and increasing its concentration increased the chlorophyll index. Foliar spraying of zinc and iron were also improved chlorophyll index compared to the control (without foliar spraying). The highest chlorophyll index (22.1) was seen in interaction of without salinity stress (control) + foliar application of humic acid 1000 mg l^{-1} + foliar application of iron, and also (22.9) was related to no salinity stress (control) + foliar application of humic acid 1000 mg l^{-1} + iron foliar spraying. The lowest chlorophyll index (11.56) was related to stress conditions and the control treatments (Fig 5).

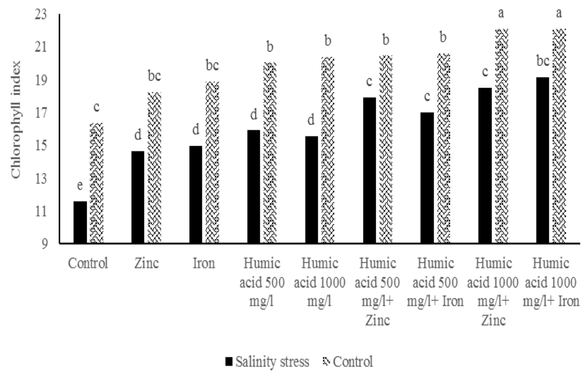

Fig. 5. Interaction effect of humic acid, micronutrient and salinity stress on chlorophyll index

3.6. Relative water content (RWC) and membrane stability index

The effect of salinity stress, humic acid ($p < 0.01$), and micronutrients had a significant effect ($p < 0.05$) (Table 2). The results of the mean comparison showed that the salinity stress decreased RWC and increased membrane stability index. Foliar spraying of zinc and iron, as well as the application of humic acid and increasing its concentration improved RWC and decreased the membrane stability index. In salinity treatments, the

Table 1. ANOVA for dry matter yield, plant height, steam diameter, essential oil content and essential oil yield

S.O.V	d.f.	Dry matter yield	Plant height	Stem diameter	Essential oil content	Essential oil yield
Salinity stress (S)	1	589.72**	729.52**	279.53**	95.04**	7219.48**
Humic acid (H)	2	926.54**	335.87**	311.73**	117.44**	1026.39**
Micronutrient (M)	2	721.65**	821.95**	576.52**	102.88**	3279.58**
S×H	2	619.68**	445.37**	21.38 $^{n.s}$	118.37**	1566.21**
S×M	2	895.27**	576.43**	19.73 $^{n.s}$	125.73**	2642.25**
M×H	4	953.83**	855.31 **	13.52 $^{n.s}$	177.55**	1956.31**
S×M×H	4	1254.62**	926.12**	32.69$^{n.s}$	187.24**	1865.83**
Error	36	18.46	39.53	29.71	10.32	24.32
C.V (%)		10.21	7.83	9.13	9.78	10.59

**Significant at $p < 0.01$,*Significant at $p < 0.05$, ns: no significance (at $p < 0.05$).

Table 2. ANOVA for quality traits

S.O.V	d.f.	Chlorophyll index	RWC	Membrane stability index	Proline content	Catalase activity	Ascorbate peroxidase activity
Salinity stress (S)	1	526.79**	812.75**	326.95**	196.73**	8.42**	30.66**
Humic acid (H)	2	724.11**	664.29**	225.89**	214.18**	5.75**	42.05**
Micronutrient (M)	2	864.23**	1401.52*	673.46**	152.56*	11.28*	21.42*
S×H	2	553.49**	13.86$^{n.s}$	24.79$^{n.s}$	13.95 $^{n.s}$	1.33$^{n.s}$	13.52$^{n.s}$
S×M	2	912.63**	17.33$^{n.s}$	18.43$^{n.s}$	16.89 $^{n.s}$	0.94$^{n.s}$	11.79$^{n.s}$
M×H	4	782.41**	15.85$^{n.s}$	9.65$^{n.s}$	9.21 $^{n.s}$	0.79$^{n.s}$	10.03$^{n.s}$
S×M×H	4	895.73**	11.16$^{n.s}$	10.28$^{n.s}$	1.38$^{n.s}$	1.03$^{n.s}$	7.52$^{n.s}$
Error	36	19.16	19.52	21.19	26.73	1.82	13.24
C.V (%)		10.21	13.21	10.42	8.15	5.64	6.73

**Significant at $p < 0.01$,*Significant at $p < 0.05$, ns: no significance (at $p < 0.05$).

Table 3. Effects of salinity stress, humic acid and micronutrients on plant height, carotenoid, relative water content (RWC) and enzymes.

Treatments	Stem diameter (cm)	RWC (%)	Membrane stability index (%)	Proline Content (mg gFW^{-1})	Catalase)µmole FW min^{-1}(	Ascorbate peroxidase (µmol H_2O_2 $min^{-1}mg^{-1}$ protein)
Salinity stress						
Control	0.63 a	72.21 a	1.75 b	0.114 b	0.012 b	0.35 b
30 mM	0.32 b	45.74 b	4.62 a	0.326 a	0.016 a	0.81 a
Humic Acid						
1000 mg l^{-1}	0.82 a	73.85 a	1.02 c	0.102 c	0.017 a	0.41 c
500 mg l^{-1}	0.67 b	65.27 b	1.63 b	0.135 b	0.014 b	0.49 b
Control	0.41 c	54.38 c	2.58 a	0.206 a	0.011 c	0.73 a
Micronutrients						
Iron	0.79 a	71.35 a	1.09 b	0.149 b	0.015 a	0.43 b
Zinc	0.81 a	70.89 a	1.13 b	0.152 b	0.015 a	0.41 b
Control	0.49 b	54.41 b	3.82 a	0.226 a	0.011 b	0.59 a

Dissimilar letters indicate significant differences at the 5% level according to Duncan's test.

highest RWC (72.21%) was observed in control. In humic acid treatments, the highest RWC (73.85%) was related to humic acid 1000 mg l^{-1}. In micronutrient treatments, the highest RWC (71.35%), was seen in iron foliar spraying, and (70.89%) was observed in zinc foliar spraying. In salinity treatments, the lowest membrane stability index (1.75%) was seen in control. In humic acid treatments, the lowest membrane stability index (1.02%) was related to humic acid 1000 mg l^{-1}. In micronutrient treatments, the lowest membrane stability index (10.9%) was seen in iron foliar spraying, and (1.13%) was observed in zinc foliar application (Table 3).

3.7. Proline content

The effect of salinity stress, humic acid ($p<0.01$) and micronutrient elements had a significant effect ($p < 0.05$) on proline content (Table 2). The results of mean comparison presented that salinity stress raised proline content. Usage of zinc and iron, as well as the application of humic acid and increasing its content, decreased the proline content. In salinity treatments, the lowest proline content (0.114 mg gFW^{-1}) was related to control. In humic acid treatments, the lowest proline content (0.102 mg gFW^{-1}) was seen in humic acid 1000 mg l^{-1}. In micronutrient treatments, the lowest proline content (0.149 mg gFW^{-1}) was observed in iron foliar application, and also (0.152 mg gFW^{-1}) was seen in zinc application (Table 3).

3.8. Enzymes (catalase and ascorbate peroxidase) activity

The effect of salinity stress, humic acid ($p<0.01$), and micronutrient elements had a significant effect ($p< 0.05$) on catalase and ascorbate peroxidase enzymes activity (Table 2). The results of mean comparison indicated that salinity stress improved ascorbate peroxidase and catalase activity. The application of humic acid and increasing its concentration, raised catalase activity and decreased ascorbate peroxidase activity. In saline stress, the lowest catalase activity (0.012 µmole FW min^{-1}) and also ascorbate peroxidase activity (0.35 µmol H_2O_2 $min^{-1}mg^{-1}$ protein) were seen in salinity condition. In humic acid treatments, the highest catalase activity (0.017 µmole FW min^{-1}) and the lowest ascorbate peroxidase activity (0.41 µmol H_2O_2 $min^{-1}mg^{-1}$ protein) were related to humic acid 1000 mg l^{-1}. Usage of zinc and iron increased catalase and decreased ascorbate peroxidase activity. The highest catalase activity (0.015 µmole FW min^{-1}) was related to iron foliar spraying and zinc foliar spraying. The lowest ascorbate peroxidase (0.43 µmol H_2O_2 min^{-1} mg^{-1} protein) was related to iron foliar application and also (0.41 µmol H_2O_2 $min^{-1}mg^{-1}$ protein) was seen in zinc foliar application (Table 3).

4. Discussion

In this study, usage of humic acid and zinc and iron nanochelates increased dry matter yield, basil height and basil stem diameter, which can be say that the presence of hormones in humic acid and creating suitable growth conditions and also due to the main role of iron in the increase in chlorophyll synthesis has occurred. In the present research, under the conditions of salt stress, the dry weight of the whole plant decreased, which can be due to the fact that under salt stress, water absorption from the soil was limited, and as a result, the amount of water in the leaf cells and then the leaf surface decreased, and these factors lead to a decrease in photosynthesis and finally decrease in the dry weight of the plant and its performance components. Humic acid provides nutrient and growth hormones for plants (Amer et al., 2021; Lazarević et al., 2021). Humic acid increased the yield and yield components of basil and increased the resistance of this plant under salinity stress conditions (El Gohari et al., 2023). Humic acid and micronutrient elements increased the yield and yield compounds of basil even in salinity stress compared to control, so it can suggest to use humic acid and micronutrient elements for increasing the plants resistance to stress.

The results of the present study showed that salinity stress decreased the chlorophyll index. Salt stress increases ROS in chloroplast and destroys chlorophyll molecule and chloroplast membrane, which leads to reduction of photosynthesis and growth. The decrease in the amount of chlorophyll can be due to the decrease in the synthesis of chlorophyll and also due to its destruction. Also, the destruction of chlorophyll molecule is done by separating the phytol chain from the porphyrin ring due to ROS or chlorophyllase enzyme. Salt may accumulate in chloroplasts and directly exert its toxic effect on the photosynthesis process and photosynthetic system. The decrease in the amount of chlorophyll may be caused by the increase in the destruction and decrease in the production of chlorophyll or both of them, and also as a result of the decrease in the integrity of the thylakoid membrane. Salinity stress by increasing the amount of superoxide radicals and hydrogen peroxide leads to the decomposition of chlorophyll pigments and subsequently causes the destruction and destruction of thylakoid structures in chloroplasts. Humic acid increases the performance of basil plants even in the conditions of salt stress compared to non-use (control) (El Gohari et al., 2023; Amer et al., 2021).

As the membrane instability index decreased, the relative water of the leaf increased. The relative water content of leaves is mentioned as a parameter to measure the water level of plants and it shows the metabolic activities in the tissues. The decrease in the relative amount of leaf water and the increase in the stability index of the plant membrane under the conditions of salt stress may be caused by the decrease in the amount of water absorbed by the plant, which is caused by the increase in osmotic potential caused by the presence of salt in the plant bed, which causes it to collapse. The balance between the two processes of water absorption and transpiration is reached, and as a result, the relative water content of the plant decreases, or it may be because the root systems are not able to compensate for the water lost by transpiration due to the reduction of the absorption level (Negrão et al., 2017).

A decrease in the relative water content of plant leaves indicates a decrease in turgor, which causes a decrease in water required for morph physiological processes such as opening of stomata, cell elongation, and photosynthesis. The increase of proline in salt stress can be attributed to the maintenance of osmotic balance at the cellular level in many plants grown in salt stress treatments. The cell responds to long-term and short-term salinity stress by synthesizing and accumulating osmotic protective compounds such as proline. These compounds are small and non-toxic molecules that decreased the osmotic potential of the cell. The increase in proline caused by the amount of sodium chloride can be explained by the fact that glutamate pathway enzymes are activated under sodium chloride salt stress and proline synthesis increases, because sodium chloride stimulates the genes that synthesize these enzymes (Dehghan et al., 2018). It was reported that application of salinity stress and its increase caused an increase in proline content in Sarkhargol (Azadbakht et al., 2020).

In the present study, application of salt stress increased the activity of enzymes (catalase and ascorbate peroxidase). In this regard, it has been reported that plants increase the activity of antioxidant enzymes to defend against oxidative stress caused by environmental stress (Jahani et al., 2022; Rajput et al., 2021). One of the effects of salt stress is the increase in ROS production and the induction of oxidative stress, which leads to peroxidation of membrane lipids, changes in membrane permeability (ion leakage) and cell damage. Ascorbate peroxidase is a key enzyme in inhibiting ROS and a plant defense mechanism against stress that can clear H_2O_2 produced in chloroplast. Ascorbate is found in chloroplasts and other cell components, which are very important in the defense mechanisms of plants during oxidative stress. The increase in the activity of antioxidant enzymes during salt stress is probably due to the fact that salt stress increases the production of ROS, which is highly reactive and toxic and damages the vital biomolecules of the cell such as lipids, DNA and proteins. It causes damage and eventually disrupts cell metabolism, which increases the oxidative stress induced by sodium, and the antioxidant enzymes produced by plant cells neutralize and reduce ROS, protecting the cell and tolerance against stress conditions in the plant (Zelm et al., 2020; Hao et al., 2021). Increasing the activity of antioxidant enzymes may be a way for the plant to tolerate environmental stress, including salt stress. Ascorbate peroxidase uses ascorbate as an electron donor and has a great affinity for hydrogen peroxide. In most cellular organelles, it clears radicals that are not accessible to catalase (Zelm et al., 2020; Hao et al., 2021; Jahani et al., 2022).

5. Conclusion

The results of the present study showed that salinity stress decreased the yield, enzymes activity, RWC and essential oil of Italian basil. The use of humic acid and increasing its concentration, as well as the use of iron and zinc nanochelates, increased the yield and essential oil of treated plants. In addition, these treatments increased the resistance of Italian basil to salinity stress, which is believed to be the reason for this increase in the plant's access to nutrients, which made the plant better able to withstand the stress conditions. Therefore, application of humic acid (1000 mg l^{-1}) and micronutrients (iron or zinc) can be suggested to farmers to raise Italian basil yield and also plant resistance to salinity stress in greenhouse condition. Considering the expected positive impact on the nutraceutical properties of basil and, potentially, on its market value, it is necessary to have more detailed analyses of the health-related and essential oil properties of basil in salinity stress especially in field conditions.

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