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How Does Immersion of saffron Corm in Some Hormones and Humic Acid Affect the Morphological Characteristics of Plant under Salinity Stress

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ABSTRACT

BACKGROUND: The use of growth-promoting hormones and organic acids is one of the ways to deal with environmental stresses.

OBJECTIVES: The present study was conducted to assess the effect of these treatments on saffron vegetative and reproductive traits, to use organic inputs properly, take steps towards sustainable production and increase quality of important medicinal plant.

METHODS: Current research carried out via factorial experiment was conducted in Islamic Azad University, Isfahan (Khorasgan) Branch (2018-2019) in a completely randomized design with three replications. The treatments were control, Salicylic acid (1 and 2 mM), humic acid (15 and 30 mM) and Jasmonic acid (5 and 10 µM) with two salinity levels (1 and 4 $ds.m^{-1}$). The evaluated traits included chlorophyll a, b and total chlorophyll, the number of flowers, flowering stem and leaves, petiole length, fresh and dry weights of leaves, stigma weight, and fresh weight of flowers.

RESULT: The highest levels of chlorophyll a and total chlorophyll were belonging to humic acid 30 and control treatments under salinity 1. The highest amount of chlorophyll b was observed in humic acid 30 and control treatments under salinity 1 and salicylic acid 2 under salinity 4. The highest number and fresh weight of flowers were obtained from the control treatment under salinity 1. The tallest petioles and flowering stems were observed in Jasmonic acid10 under salinity1. The highest leaf length was belonging to salicylic acid2 under salinity1. The highest leaf fresh weight was belonging to salicylic acid2 under salinity1 whereas of Humic acid15 under salinity1 produced the highest dry weight of plant leaves. The highest stigma weight was observed in Jasmonic acid5 and the control under salinity1. As a result, application of plant growth regulators and humic acid can reduce effect of salinity stress in saffron.

CONCLUSION: As a final conclusion of this study, it can be said that the use of growth-promoting hormones (salicylic acid) and organic acids (humic acid) under environmental stress conditions can improve morphological and vegetative characteristics such as chlorophyll and biomass produced in some plants such as saffron.

KEYWORDS: *Chlorophyll, Fresh weight, Jasmonic acid, Salicylic acid, Vegetative.*

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1. BACKGROUND

Saffron (*Crocus sativus* L.) from the Iridaaceae family has a thick, spherical and thin membrane covered corm which is used for cultivation and propagation due to the male infertility of the plant (Moravej Aleali *et al*., 2019, Najwa Ramli *et al*., 2020). Saffron blooms in autumn and falls asleep in spring and summer (Ahmad Baba *et al*., 2015). The flowers have six purple petals and three flagella plus a red-orange stigma (Gismondi *et al*., 2012; Mashmoul *et al*., 2017). About 90% of the world's saffron is produced in Iran (Moallem Banhangi *et al*., 2019). The origin of saffron was the foothills of Alvand and Zagros mountains. This plant has less yield in Iran compared to other countries in the world (Fallhai and Mahmoodi, 2018; Gholami *et al*., 2017). Saffron is a subtropical plant and has a good yield in mild winters and hot and dry summers with temperatures between 35 and 40°C (Manzo *et al*., 2015). This plant prefers fertile sweet soils with medium texture (loamy, clayey and sandy) plus lime and pH 7-8 rather than saline, poor and wet soils (Bayat *et al*., 2016). Salinity affects many physiological processes such as growth, photosynthesis, and protein synthesis, restricts chlorophyll and enzymes activity, and disrupts chloroplast membrane functions and fat metabolism (Porcell *et al*., 2016; Zhu *et al*., 2016). Symptoms of salinity appear as leaf margin necrosis, shrinkage, branch tip drying, premature aging, tubular leaves, reduced branch length, shorter plant height, and less leaf number and area (Satir and Berberoglu, 2016, Jalali *et al*., 2010). In general, the amounts of chlorophyll a and b is decreased by salinity. However, some reports have shown an increase in chlorophyll a under saline conditions (Kiani Pouya and Rasouli, 2014; Kanwal *et al*., 2011). The results of Nadian *et al*. (2013) study on the effect of different salinity levels on growth components showed that salinity increment decreased saffron's dry matter sharply. This decrease was ascribed to the toxicity of sodium ions accumulation and decreased potassium concentration. The results of Torbaghan and Ahmadi (2011) showed that increasing salinity reduced plant height and flowering days. In addition, the fresh weight of flower was increased by increasing salinity up to 100 mM, but the best stigma yield was observed at the salinity level of 50 mM. A substance called jasmonic acid methyl ester was first extracted from the plant *Jasminum grandiflorum* (Kaya and Doganlar, 2016; Sirhindi *et al*., 2015). Jasmonates are derivatives of cyclopentane linoleic acid that are synthesized through the biosynthetic pathway of octadecanoids (Azeem, 2018). It is involved in processes such as seed germination, stomatal closure, ethylene synthesis, root growth, geophysics, hair formation, embryonic development, sex determination, fertility, seedling development, tuber formation, leaf movement, fruit ripening and leaf decay. It also regulates plant defense mechanisms in response to biological and abiotic stresses (De Ollas *et al*., 2015; Chen *et al*., 2016). According to previous studies, plants treated with jasmonic acid had more pigments than the control treatment (Ilyas *et al*., 2017). Salicylic acid, or Ortho hydroxyl benzoic acid (HOC_6H_4COOH), is a harmless internal growth regulator of natural phenolic compounds with an aromatic ring (Sharma *et al*., 2020). Seed germination, nutrient uptake, nutrient transfer, flowering induction, growth development, inhibition of ethylene synthesis, jasmonic acid biosynthesis, opening and closing stomata, transpiration regulation, membrane permeability, respiration, photosynthesis, Chlorophyll increment, fruit production, glycolysis, and resistance against biological and non-biological stresses are important roles of salicylic acid (Luo *et al*., 2019; Wei *et al*., 2018; Zhang *et al*., 2019). Researchers have reported increased flower fresh weight, petal fresh weight, stigma fresh weight and stigma length as a result of immersion of saffron corms in salicylic acid hormone at concentrations of 1 and 2 mM (Jabbari *et al*., 2017). In a study about the effects of corm priming with salicylic acid (control, 1 and 2 mM) on flowering and quality characteristics of saffron stigma, the results showed that priming with 2 mM concentration had the highest fresh weight of flower, dry weight of stigma and the shortest emergence time (Ansarian Mahabadi *et al*., 2019). Humic acid (nature-friendly organic fertilizer) is extracted from various sources such as soil, humus, peat, oxidized lignite, plant and animal residues, and coal (Kaya *et al*., 2020; Zhang *et al*., 2014). Humic acid shows anti-stress effects under abiotic stress conditions by transferring ions to the root zone and increasing the uptake of trace nutrients by preventing leaching and reducing the toxicity of some adsorbed elements (Yigider *et al*., 2016, Garcia *et al*., 2016). Golzari results (2016) showed that application of 3.5 liters per hectare of humic acid increased the yield of dry stigma. Golzari Jahanabadi *et al*. (2017) by examining the effects of some fertilizer sources on the vegetative characteristics of saffron stated that the maximum number of leaves, fresh and dry weight of leaves and the number of corms were obtained from humic acid treatment. This acid can also have positive effects on the number of saffron flowers and fresh and dry weights of the stigma (Mollafilabi and Khorrmodel, 2016). About 77 million hectares out of 1.5 billion hectares of agricultural lands around the world are suffering from high salinity. Fifty percent of the world's land is expected to become unusable by 2050 due to salinity development (Fricke, 2020). Therefore, understanding the mechanisms of plants tolerance to salinity is one of the main issues in the world in order to select suitable species for the region (Hanafy Ahmed *et al*., 2018), preserving the environment and achieving sustainable development. Improper use of chemical fertilizers has reduced the yield and quality of crops. It also has caused contamination of water and soil resources (Pandey and Garg, 2017; Peng *et al*., 2016). It is necessary to find a way that can reduce the use of these fertilizers (Parihar *et al*., 2015).

2. OBJECTIVES

Considering that there has been no report on the simultaneous evaluation of humic acid, salicylic acid and Jasmonic acid effects on leaf pigments and morphological characteristics of saffron, the present study was conducted to assess the effect of these treatments on saffron vegetative and reproductive traits, to use organic inputs properly, take steps towards sustainable production and increase the quality of this important medicinal plant.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

A study was conducted at the Islamic Azad University, Isfahan (Khorasgan) Branch (2016-2017) as a factorial experiment in a completely randomized design with three replications. Treatments were hormones in seven levels and salinity in two levels. The evaluated traits were chlorophyll a, b and total chlorophyll, the number of flowers, lengths of petiole, flowering stem and leaf, fresh and dry weight of leaves, stigma weight, and fresh weight of flowers. Uniform saffron corms (10-15 g) were collected from a four-year-old farm of Dehaqh-Alavijeh areas of Isfahan. Corms were then immersed in benomyl fungicide and abamectin acaricide for 5 minutes. For humic acid treatment, corms were immersed in 15 and 30 mM concentrations of humic acid for 24 hours. At other treatments corms were immersed in Jasmonic acid (5 and 10 μM) and salicylic acid (1 and 2 μM) for six hours and then for 18 hours in distilled water to assimilate with humic acid.

3.2. Farm Management

Corms were planted in four liter pots filled with field soil on October 15, 2017 without any field care or nutrition. Chemical analysis of soil before and after salinity treatment is reported in table 1. To enforce natural salinity stress, irrigation water of Kabutarabad Agricultural Research Center with a salinity of 4 $ds.m^{-1}$ was selected. Also, irrigation water of university farm with salinity of 1 $ds.m^{-1}$ was used. Immediately after planting the corms, the first irrigation was done Subsequent irrigations were performed after the potting soil had dried.

Table 1. The amount of soil chemical components

EC ($ds.m^{-1}$)	pH	Na (ppm)	Cl (ppm)	N (%)	P (ppm)	K (ppm)	Mg (ppm)	Ca (ppm)	Soil Texture
7.93	7.3	311	11.72	0.16	90.7	639	274	768	Loamy Silty

3.3. Measured Traits

To measure chlorophyll, 0.2 g of fresh leaf tissue was gradually abraded with acetone 80%, and finally the volume of the solution was increased to 10 ml using acetone 80%. The optical absorption of the solution was then read at 663, 645 and 470 nm using a spectrophotometer (Model D 6320) previously calibrated with 80% acetone. The following equations were used to calculate the amount of chlorophyll a, b and total chlorophyll (mg/g of fresh leaf tissue) (Li *et al*., 2009).

Equ.1. $Chl.a\ (mg.g^{-1}) = [(12.7\times Abs_{663}) - (2.6\times Abs_{645})] \times V/W \times 1000.$

Equ.2. Chl.b (mg.g^{-1}) = [(22.9×Abs $_{645}$) - (4.68×Abs $_{663}$)] × V/W × 1000

Equ.3. Chl.total (mg.g^{-1}) = Chl.a + Chl.b

3.4. Statistical Analysis

Obtained data were analyzed using SAS statistical program and means were compared by Duncan test at 5% probability level. Excel 2010 was used to draw the graphs.

4. RESULT AND DISCUSSION

Variance analysis results showed that the effects of salinity, hormone and their interactions were very significant ($p<0.01$) on chlorophyll a, b and total chlorophyll (Table 2), the number of flowers, petiole length, flowering stem length, leaf length, fresh and dry weights of leaves, stigma weight and fresh weight of flowers (Table 3).

Table 2. Variance analysis results of chlorophyll a, b and Total chlorophyll

S.O.V	df	Chlorophyll a	Chlorophyll b	Total chlorophyll
Salinity	1	0.16**	0.04**	0.38**
Hormone	6	0.07**	0.02**	0.18**
Hormone × salinity	6	0.08**	0.02**	0.18**
Error	28	0.001	0.0003	0.003

ns, * and **: no significant, significant at 5% and 1% of probability level, respectively.

Table 3. Variance analysis results of some morphological characteristics of saffron

S.O.V	df	Number of flowers	Petiole length	Flowering stem length	Leaf length
Salinity	1	214.9**	437.9**	3971**	32.93**
Hormone	6	33.91**	35.07**	368.0**	15.89**
Hormone × salinity	6	16.10**	35.92**	241.4**	10.63**
Error	28	0.29	0.40	3.83	0.22

ns, * and **: no significant, significant at 5% and 1% of probability level, respectively.

Continue table 3.

S.O.V	df	Fresh weight of leaves	Dry weight of leaves	Stigma weight	Fresh weight of flowers
Salinity	1	0.09**	0.07**	0.002**	0.02**
Hormone	6	0.03**	0.02**	0.0001**	0.004**
Hormone × salinity	6	0.04**	0.016**	0.0001**	0.008**
Error	28	0.0005	0.0002	0.000001	0.00004

ns, * and **: no significant, significant at 5% and 1% of probability level, respectively.

The highest chlorophyll a content was observed in humic acid30 and control treatments under salinity1. Also, the highest amount of chlorophyll b (0.32 mg.g^{-1}) was belonging to salicylic acid2 and salinity4. The lowest amount (0 mg.g^{-1}) was obtained from the control treatment and salinity4 (Fig. 1, 2 and 3).

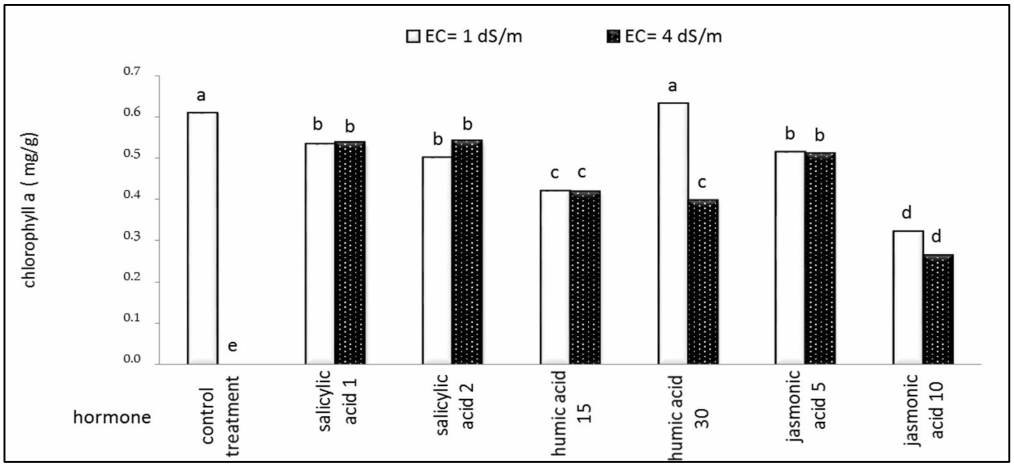

Fig.1. The interaction effect of salinity and hormone on chlorophyll a
Means with at least one common letter are not statistically different at 5% probability level according to Duncan test.

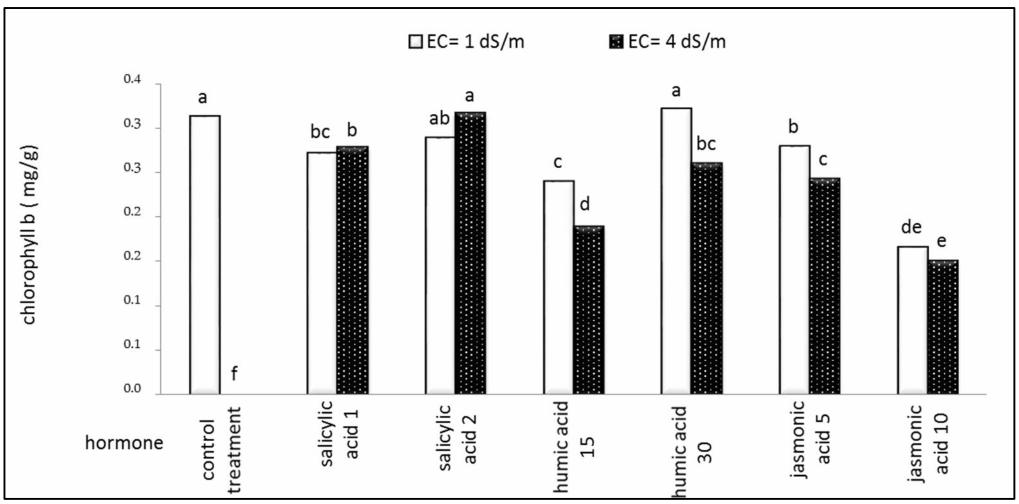

Fig. 2. The interaction effect of salinity and hormone on chlorophyll b
Means with at least one common letter are not statistically different at 5% probability level according to Duncan test

Salinity in low ranges can increase the concentration of chlorophyll per unit leaf area. However, with excessive salinity and destruction of chloroplasts, chlorophyll content will be decreased (Ashrafi *et al*., 2018). Stimulating effects of salicylic acid have been shown to be associated with changes in hormonal conditions or improved photosynthesis, transpiration, or stomatal conduction (Kumar *et al*., 2013). Salicylic acid prevents the breakdown of chlorophyll oxidase by inhibiting the activity of chlorophyll oxidase enzymes; thereby it Increases chlorophyll and Rubisco enzyme activity, and then total photosynthesis. By increasing photosynthesis, quantitative indicators such as flower, stigma and plant weights will be affected (Ghaderi *et al*., 2015).

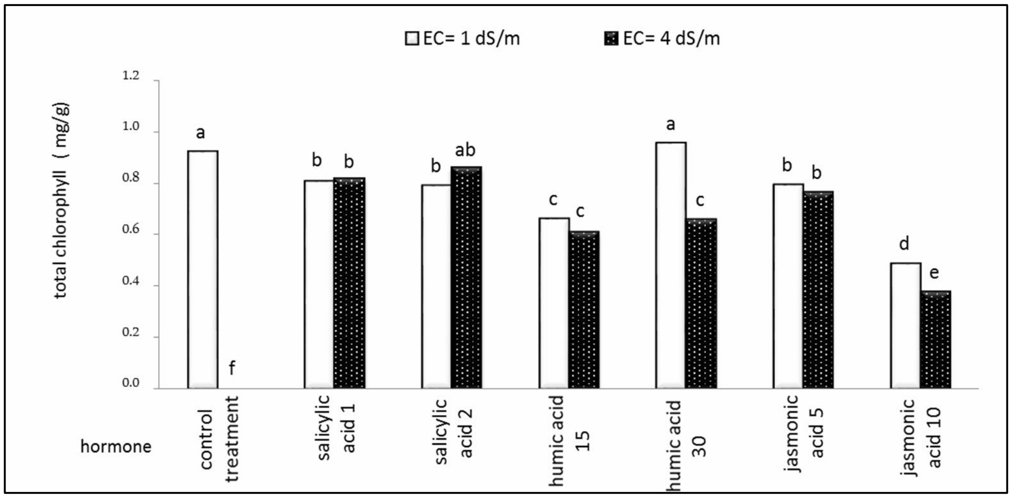

Fig. 3. The interaction effect of salinity and hormone on total chlorophyll
Means with at least one common letter are not statistically different at 5% probability level according to Duncan test.

Increased chlorophyll content by organic stimuli is because of better availability and absorption of nutrients by plant roots which affect the process of photosynthesis and synthesis of pigments (Bhattarai *et al*., 2020). Also, to justify the results, growth stimuli in humic acid, trans-zatin cytokines, auxin and betaine, which increase chlorophyll content or prevent chlorophyll degradation, can be known as yield enhancer via photosynthesis and production of assimilates by chlorophyll (Ahmad *et al*., 2018, Suh *et al*., 2014). Humic acid increases soil microscopic organisms by meeting their nutritional needs. As a result, it reduces the pH of the soil and increases the absorption of microelements such as manganese, iron and magnesium, which also play an important role in chlorophyll synthesis (Ekin, 2019). The highest number of flowers and fresh weight of flowers (10.33 and 0.18 g, respectively) were obtained from control treatment under salinity1 (Fig. 4 and 5). The tallest petioles and flowering stems were observed in Jasmonic acid10 under salinity1 (11.41 mm and 33.94 mm, respectively) which wont statistically different from salicylic acid 1 (Fig. 6 and 7). The highest leaf length (9.08 cm) was belonging to salicylic acid2 under salinity1 (Fig. 8), which was not statistically different from salicylic acid1. The lowest dry and wet leaf weight and leaf length were obtained from control treatment under salinity4. The highest leaf fresh weight (0.46 g) was belonging to salicylic acid2 under salinity1 whereas of humic acid15 under salinity1 produced highest dry weight of plant leaves (0.399 g) (Fig. 9 and 10). The highest stigma weight (0.02 g) was observed in jasmonic acid5 and control under salinity1 (Fig.11), which was not statistically different from some other treatments. The lowest stigma weight, No. flowers, fresh flower weight, petiole length and flowering stem length were observed in control, humic acid15 and Jasmonic acid5 and 10 treatments under salinity4.

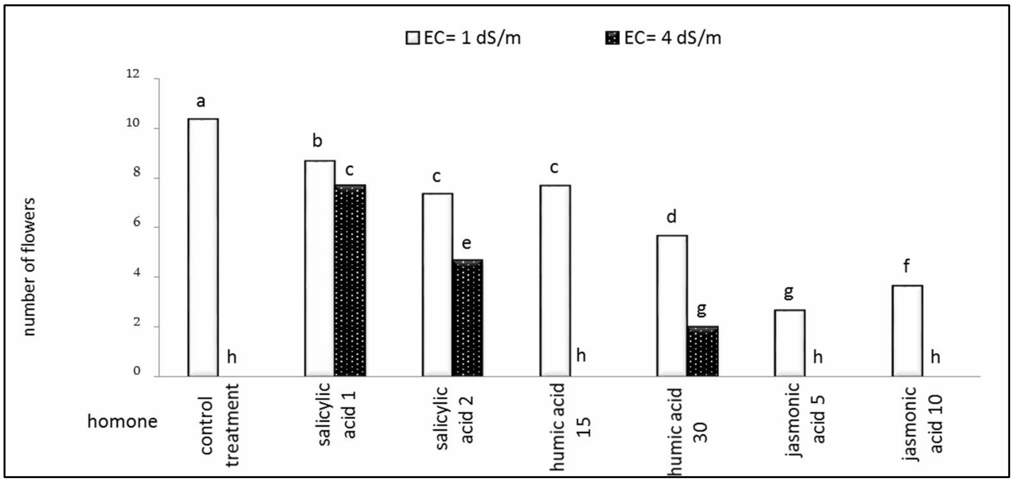

Fig. 4. The interaction effect of salinity and hormone on the number of flowers
Means with at least one common letter are not statistically different at 5% probability level according to Duncan test.

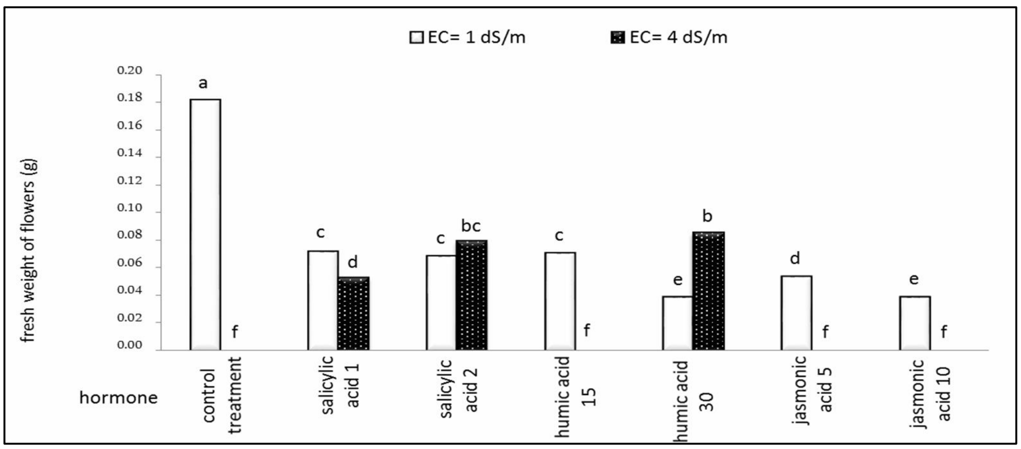

Fig. 5. The interaction effect of salinity and hormone on fresh weight of flowers
Means with at least one common letter are not statistically different at 5% probability level according to Duncan test.

Weight loss due to the salinity stress is because of reduced water absorption and inhibition of synthesis of photosynthetic products and carbohydrates. Growth reduction is main effect of salinity on plants (Sytar *et al*., 2017). One of the reasons for wet and dry weight loss of saffron under stress conditions can be decrease in biochemical capacity for carbon sequestration and limitation of gas emission which affects transfer of photosynthetic materials and causes leaves to saturate by these materials. So the process of plant photosynthesis will be limited (Hernandez, 2019). Photosynthesis and cell growth and consequently leaf growth are among processes that are initially affected by salinity (Ji *et al*., 2018). Results of Avarseji *et al.* (2013) also showed dry weight and leaf length of saffron was decreased significantly by salinity increment.

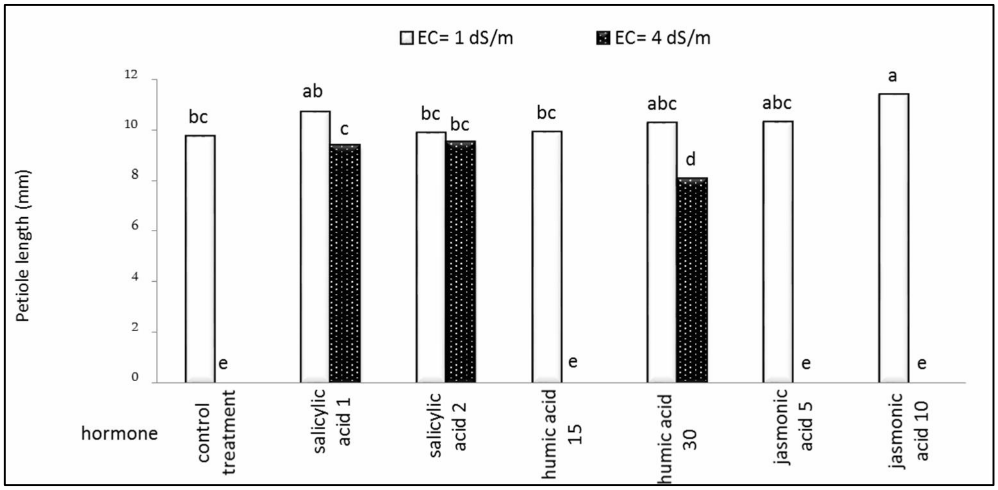

Fig. 6. The interaction effect of salinity and hormone on Petiole length
Means with at least one common letter are not statistically different at 5% probability level according to Duncan test.

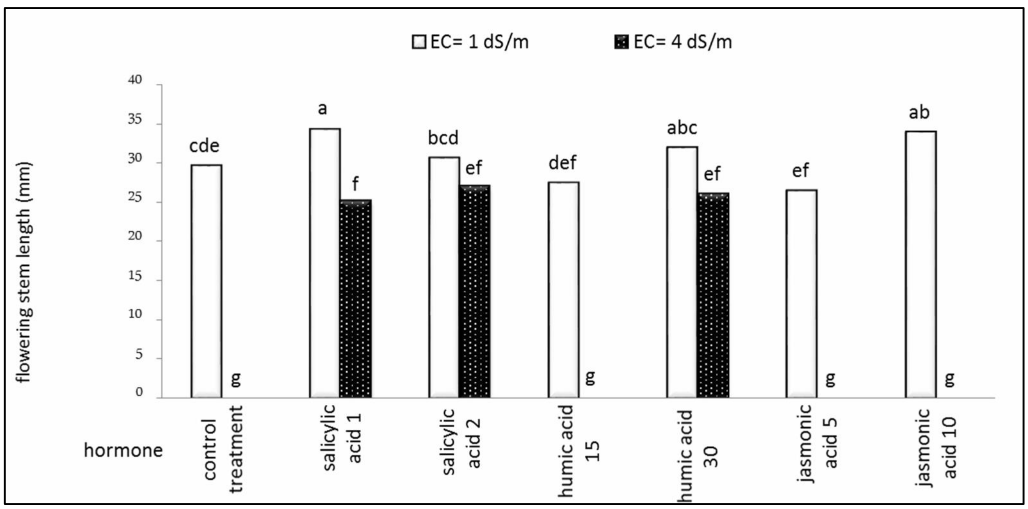

Fig. 7. The interaction effect of salinity and hormone on flowering stem length
Means with at least one common letter are not statistically different at 5% probability level according to Duncan test

Given that salinity stress prevents water absorption and causes consequent drought stress, the water potential for cell inflammation decreases, which will be led to reduced leaf development and leaf fresh weight (Dilnur *et al*., 2019). The results of this study regarding the effect of salinity stress on the reduction of saffron growth parameters are consistent with the results of Tavakoli *et al*. (2011), Chaudhary *et al*. (2013), Talat *et al*. (2015), Nadem *et al*. (2014) and Kang *et al*. (2014). Salicylic acid increases cell division within the apical meristem of the seedling and thereby improves seedling growth.

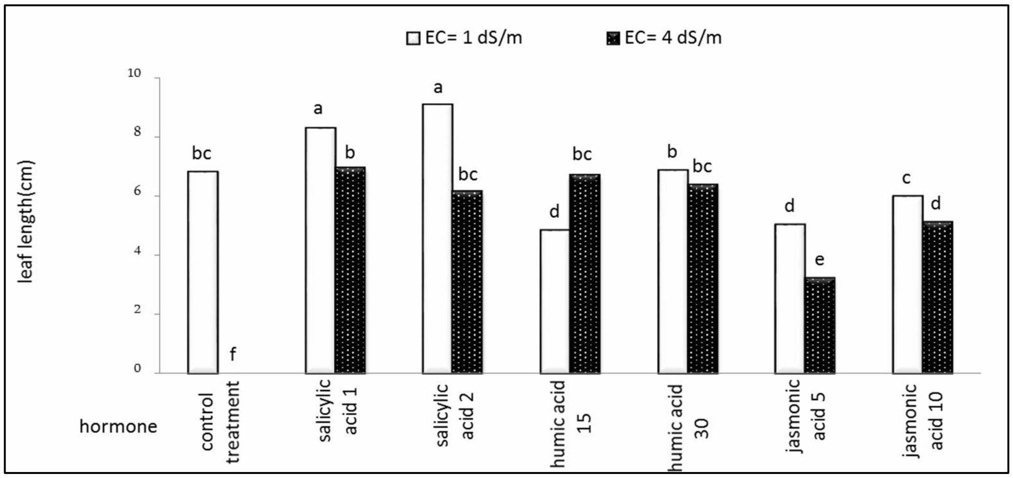

Fig. 8. The interaction effect of salinity and hormone on the Leaf length
Means with at least one common letter are not statistically different at 5% probability level according to Duncan test.

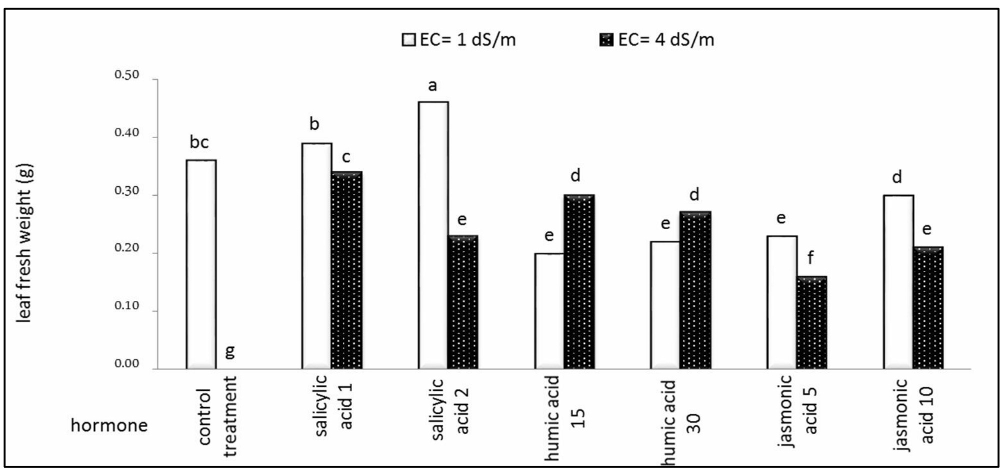

Fig. 9. The interaction effect of salinity and hormone on leaf fresh weight
Means with at least one common letter are not statistically different at 5% probability level according to Duncan test

Fresh weight gain due to salicylic acid treatment under salinity stress is because of antioxidant activity of this substance in cell membranes. On the other hand, salicylic acid treatment increases the amount of lignin in the cell wall structure. This can be the reason of fresh and dry weight increment under salinity (Kovacik *et al*., 2009; Herati *et al*., 2016). The results of this study on salicylic acid are consistent with the results of Martin-Mex and Larqué-Saavedra (2001) on gloxinia and violet and Mardani and Azizi (2011) on cucumber. The positive effect of salicylic acid on plant growth and development has been reported in several studies (Ghaderi *et al*., 2015; Rahimi *et al*., 2013; Mozaffari *et al*., 2018).

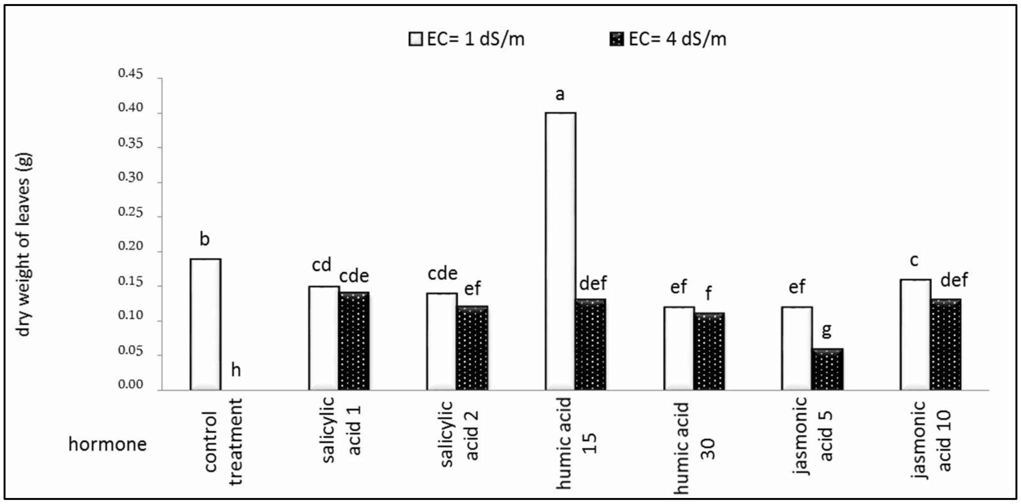

Fig. 10. The interaction effect of salinity and hormone on dry weight of leaves
Means with at least one common letter are not statistically different at 5% probability level according to Duncan test.

Dry weight gain of summer savory (Faraji Mehmani *et al*., 2016) and fennel (Hashemi *et al*., 2012) under the influence of salicylic acid has been reported. Immersion of salicylic acid had a compensatory effect on the morphological characteristics of saffron under salinity stress (Rivas-San Vicente and Plasencia, 2011). It seems that facilitating the growth and increase of plant growth factors can be due to the presence of growth-promoting compounds as well as macro and micro elements in humic acid (Ali *et al*., 2020). This substance improves plant growth via direct and indirect ways (Canellas *et al*., 2019). Indirect effects include the effect of this substance on soil biochemical structures and its direct effects include increased respiration rate, chlorophyll concentration and hormonal responses. In addition, it has other positive effects such as the production of substances such as superoxide dismutase and alpha-tocopherol, which play important roles in stress tolerance and provide suitable soil and physiological conditions for the plant (De Aquino *et al*., 2019). Similar to the results of this study, researchers reported that foliar application of 200 $mg.l^{-1}$ humic acid increased the weight of saffron leaves (Golzari, 2016) and foliar application of humic acid significantly affected the dry weight of saffron leaves (Malafilabi and Khorramdel, 2016). Kouchaki *et al*. (2016) by examining effects of humic acid application on saffron, stated that the stigma yield increased by 154 and 92%, respectively, compared to the control in two years of project implementation. Golzari Jahanabadi (2017) also reported that consumption of 3.5 $l.ha^{-1}$ of humic acid increased stigma yield during a two-year study. The positive effects of humic acid on morphological characteristics of saffron plant are consistent with the results of Golzari Jahanabadi (2017), Kouchaki *et al*. (2016) and Malafilabi and Khormodel (2016).

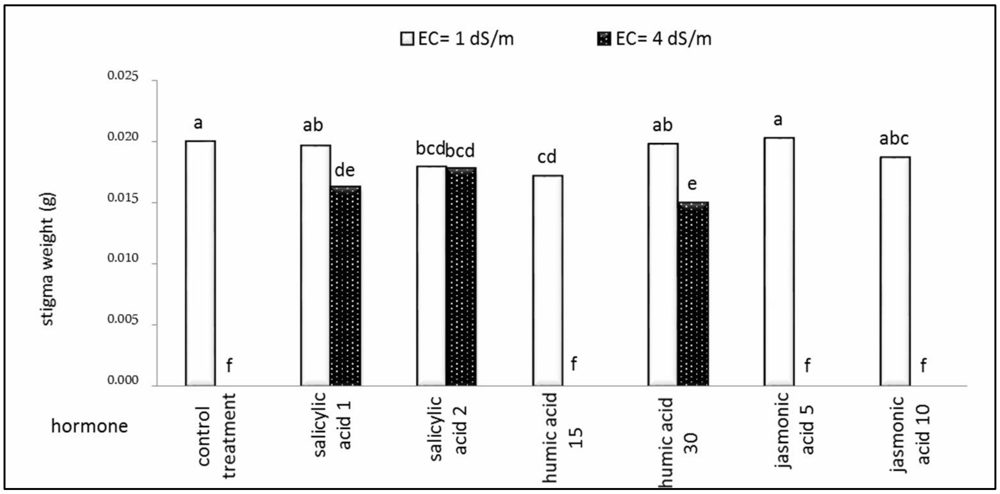

Fig. 11. The interaction effect of salinity and hormone on stigma weight
Means with at least one common letter are not statistically different at 5% probability level according to Duncan test

Heidari and Khalili (2014) also introduced humic acid as one of the stimulants of vegetative growth, improving reproductive growth and increasing quantitative and qualitative yield in a variety of plants which increases growth and consequently weight through the formation of complexes between humic acid and mineral ions, decomposition of humic acid into growth enzymes (Mart, 2007), affecting respiration and photosynthesis (Ahmad *et al*., 2011) and stimulating hormones activites (El-Sherbeny *et al*., 2012).

5. CONCLUSION

Although plants try to maintain themselves in ideal conditions under stress, this trend only in the first days of stress prevents serious changes in plant composition and after a few days, the plant's compounds will be strongly affected by the stresses and show significant differences in terms of quantity and quality. What was observed in this study also confirms this. As a final conclusion of this study, it can be said that the use of growth-promoting hormones (salicylic acid) and organic acids (humic acid) under environmental stress conditions can improve morphological and vegetative characteristics such as chlorophyll and biomass produced in some plants such as saffron. As a result, it may increase secondary metabolites in saffron.

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FOOTNOTES

AUTHORS' CONTRIBUTION: All authors are equally involved.

CONFLICT OF INTEREST: Authors declared no conflict of interest.

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