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Study on physiological and morphological traits of purple shamrock (*Oxalis triangularis* A. St.-Hil.) as affected by humic acid under salinity stress

Maryam ATHARI [1], Masoud ARGHAVANI [1], Ali SOLEIMANI [1], Mitra AELAEI [1], Pegah SAYYAD-AMIN [2, 3], Somayeh ESMAEILI [4]

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Study on physiological and morphological traits of purple shamrock (*Oxalis triangularis* A. St.-Hil.) as affected by humic acid under salinity stress

Abstract: Salinity is one of the main environmental factors that limit plant growth and productivity. Humic acid (HA) can directly have positive effects on plant growth, and absorption of nitrogen, potassium, calcium, magnesium, and phosphorus by plants. This study aimed to concentrate on the impact of HA under salinity stress on *Oxalis triangularis* to use it as an ornamental plant in green spaces and landscaping. Treatments included three salinity stress levels (0, 40 and 80 mM NaCl) and four concentrations of HA (0, 200, 400, and 600 mg l^{-1}) on *O. triangularis* with four replicates. Applying HA under salinity stress at 40 and 80 mM increased stem length compared to the control, but this increase was lower at medium and high salinity levels. HA at 400 mg l^{-1} had the best effect on rhizome length under salinity stress at 40 and 80 mM. The reduced trend on proline content in HA at 0, 200, 400, and 600 mg l^{-1} under salinity stress at 40 mM was 19.65, 18.45, 16.92, and 13.57 %, respectively. By increasing the concentrations of HA, anthocyanin content was raised when compared to the control. Leaf sodium and potassium decreased by applying HA with or without salinity stress.

Key words: anthocyanin, leaf, proline, rhizome

Preučevanje vplivov huminske kisline na fiziološke in morfološke lastnosti škrlatne zajčje deteljice (*Oxalis triangularis* A.St.-Hil.) v razmerah slanostnega stresa

Izvleček: Slanost je eden izmed glavnih okoljskih dejavnikov, ki omejujejo rast in produktivnost rastlin. Huminska kislina (HA) ima lahko neposreden pozitiven učinek na rast rastlin in na absorbcijo dušika, kalija, kalcija, magnezija in fosforja. Namen te raziskave je bil preučiti vpliv huminske kisline v razmerah slanostnega stresa na škrlatno zajčjo deteljico (*Oxalis triangularis*) pri uporabah kot okrasna rastlina in za ozelenitve v krajini. Obravnavanja so obsegala tri ravni slanostnega stresa (0, 40 and 80 mM NaCl) in štiri koncentracije huminske kisline (0, 200, 400, and 600 mg l^{-1}) s štirimi ponovitvami. Uporaba huminske kisline je pri slanostnem stresu 40 and 80 mM povečala dolžino stebel v primerjavi s kontrolo a to povečanje je bilo manjše pri večjem slanostnem stresu. Uporaba huminske kisline v koncentraciji 400 mg l^{-1} je imela najboljši učinek na dolžino korenike pri slanostnem stresu 40 and 80 mM. Pri obravnavi s koncentracijami 0, 200, 400 in 600 mg l^{-1} huminske kisline pri slanostnem stresu 40 mM se je vsebnost prolina v rastlini zmanjševala od 19,65; 18,45; 16,92 do 13,57 %. S povečevanjem koncentracije huminske kisline se je povečevala vsebnost antocianina v primerjavi s kontrolo. Vsebnosti natrija in kalija v listih sta se pri uporabi huminske kisline zmanjševali pri ali odsotnosti slanostnega stresa.

Ključne besede: antocianin, korenika, list, prolin

1 Department of Horticultural Science, University of Zanjan, Zanjan, Iran
2 Department of Horticultural Science and Landscaping Engineering, Ferdowsi University of Mashhad, Mashhad, Iran
3 Corresponding author, e-mail: pegah.sayyadamin@mail.um.ac.ir
4 Department of Horticultural Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran

1 INTRODUCTION

Plants are faced with different types of environmental stresses, such as salinity, which can decrease plant growth and yield (Zamani et al., 2020). Salinity is an abiotic stress, which usually occurs in arid and semi-arid regions influencing plant growth and productivity (Porcel et al., 2012). Salt stress leads to disruption of physiological and biochemical activities such as ion balance, seed germination, osmotic regulation, photosynthesis, respiration, and disturbance in the absorption of potassium, phosphorus, calcium, and nitrogen ions leading to insufficient levels of those elements in the plant (Porcel et al., 2012; Ulczycka-Walorska et al., 2020).

Humic acid (HA) is extracted from different sources such as soil, humus, peat, oxidized lignite, and coal. HA can directly increase the growth of shoots and roots and the absorption of nitrogen, potassium, calcium, magnesium, and phosphorus by plants. HA as a naturally occurring polymeric organic compound is not dangerous for the plant and environment and contains elements that improve soil fertility, reduce soil nutrient deficiency and increase water and nutrient availability by forming chelates of various chemical elements (Motaghi and Sakinejad, 2014). HA increases plant growth through chelating different elements to overcome the lack of nutrients and has valuable effects on growth increase, production, and quality improvement of agricultural products due to having hormonal compounds (Ahmad et al., 2013; Motaghi and Sakinejad, 2014. HA can directly release different elements from minerals, absorb and provide them to the roots at the right time. Also, HA is a feed and growth stimulant for beneficial soil microorganisms, which help to release elements in the soil in various methods and can break aluminum-phosphorus or iron-phosphorus bonds in acidic soils and calcium-phosphorus bonds in alkaline soils and release phosphorus into the soil solution. It has been reported that due to the competitive adsorption between organic acids and phosphate, the adsorption of phosphorus on active surfaces is reduced, which is the reason for increasing the efficiency of phosphorus fertilizer in soils amended with organic materials. Sepehr and Zebardast (2013) reported that humic substances and phosphate fertilizers through competition for absorption sites and as a result reducing phosphorus stabilization in the soil, can reduce the consumption of phosphorus fertilizers as a result of reducing its environmental effects in the soil (Sepehr and Zebardast, 2013).

Oxalis triangularis A. St.-Hil. (syn. *O. regnellii*) is a rhizomateus perennial with a low, moderate growth habit commonly known as "False Shamrock", is an edible perennial plant belonging to the Oxalidaceae family. The cultivated variety *O. triangularis* subsp. *papilionaceae* 'Atropurpurea' with purple-black, triangular-shaped leaves is highly popular as an ornamental pot plant (Šafrankova, 2014). It is native to Brazil and tropical Mexico and propagated by division of the rhizomes (Abd El-Razek et al., 2014). This ornamental plant in purple color can serve as a decoration for salads or foods. It is resistant to high temperature and draught due to tuberous rhizome below the ground (Kim et al., 2018). Thus, this plant can also use in landscape and green spaces. Regarding these advantages, there is no literature on the effect of salinity stress on this plant. Therefore, this study aimed to concentrate on the impact of salinity stress with or without HA on *O. triangularis* to use it as an ornamental plant in green spaces and landscaping.

2 MATERIAL AND METHODS

The research was done at the Research Greenhouse of the Horticultural Science Department of the Agricultural Faculty of Zanjan University. At first, the rhizomes of *O. triangularis* were cultivated in a mixture of sand and perlite and maintained in greenhouse for 4 months and then the salinity stress was applied for 6 weeks. During the cultivation process, the greenhouse temperature was adjusted to 18 ± 2 °C as the daytime temperature and 15 ± 2 °C as the nighttime temperature, and relative humidity was set to 60–70 % (Figure 1). Treatments included three salinity stress levels (0, 40, and 80 mM of NaCl) and four concentrations of HA (0, 200, 400, and 600 mg l^{-1}). HA (HA) was applied by foliar application at six times (once a week). Control treatment was the treatment without salinity stress and HA.

The morphological traits including stem, root, and rhizome lengths and the number of flowers leaves were measured at the end of the experiment. The biochemical traits like ion leakage, proline, anthocyanin, sodium (Na^+) and potassium (K^+) and color indices were investigated in this study Anthocyanin content was assayed according to (Wagner, 1979). Briefly, the mixed methanol and chloridric acid solution with the ratio of 99:1 was applied and the absorption was read by spectrophotometer (model SAFAS MONACO (RS 232)) at 550 nm. Proline content was measured using the methods described by Bates et al. (1973). The leaf extract was homogenized in 10 ml of 30 ml L^{-1} of sulfosalicylic acid, used for the measurement of proline by spectrophotometer at 520 nm. Ion leakage was measured according to Bideshki and Arvin, 2010. To determine potential EC (Electrolyte conductivity) 1, the samples were autoclaved for 20 min at 121 °C, and maintaining the samples at 21 °C overnight, EC2 was measured. The percentage of ion leakage was determined for each treatment according to the following formula:

Figure 1: The pictures of HA under salinity stress

Ion leakage (%) = *EC1* / *EC2* × 100

Sodium (Na^+) and potassium (K^+) were measured by flame photometer (model M410, Corning, Palo Alto, CA, USA) (Tekaya et al. 2014).

2.1 LEAF COLOR PARAMETERS

Leaf color parameters were assessed using Photoshop software, L*, a*, and b*color spaces, and Image J software with Color Space Converter to convert RGB color space to L*, a*, and b* spaces. The colors (L*, a*, and b*) were measured for each image according to the methods of Jajarimi and Taghizadeh, 2015, where L* is 100, it represents a perfect reflecting diffuser, in contrast to zero, which represents black. Positive a* values represent the red color, while the negative is green. Positive b* is yellow and the negative value is blue (Hosseini et al., 2021).

2.2 STATISTICAL ANALYSIS

This experiment was conducted on 3×4 factorial design based on a completely randomized design with four replications. Data were analyzed by SAS software version 9.1 and the means were compared by the Duncan test at 0.05 (p value < 0.05). Additionally, R software version 4.3.2 was used to draw clustering heatmap in multivariate analysis and correlation graph.

3 RESULTS

The maximum rhizome length (6 cm) was observed at 200, 400 and 600 mg l^{-1} of HA at 40 mM salinity. The minimum rhizome length (2.5 cm) was obtained in plants treated to 80 mM NaCl alone. Salinity stress decreased the rhizome length by 5 % and 41 % at 40 and 80 mM compared to the control (without HA and salinity stress), respectively (Figure 2A). Plants treated by HA at 400 mg l^{-1} alone and salinity stress at 80 mM alone had the highest (5 cm) and lowest (3 cm) rhizome length, respectively (Figure 2B). Salinity stresses at 40 and 80 mM reduced rhizome length by 22 % and 33 % when compared with the control. Although HA at 200 and 400 mg l^{-1} alone increased this trait by 5 % and 11 % as compared to control, respectively. The decreased trend in rhizome length at all concentrations of HA under salinity was found, but this decrease was lower than salinity stress alone. The highest (17.5 cm) and lowest (11.5 cm) stem lengths were observed in plants treated with HA at 400 mg l^{-1} and salinity stress at 80 mM alone, respectively (Figure 2C). Increased salinity decreased stem length. This decrease at 40 and 80 mM was 11.3 and 13.2 % as compared to the control, respectively. Application of HA alone led to increasing of stem length by 24, 32 and 13 % at 200, 400, and 600 mg l^{-1} in comparison with control. Application of HA under salinity stress also increased stem length compared to control, respectively, but this increase was lower at high levels of salinity. HA at 200 mg l^{-1} had the highest number of leaves (16.5) and followed by 400 mg l^{-1} (Figure 2D). HA (16.25). By applying HA at 200 and 400 mg l^{-1}, the number of leaves increased by 11 % and 19 % under salinity stress at 40 mM and by 1.9 % and 1.9 % at 80 mM salinity stress. Although, HA alone at 600 mg l^{-1} led to an increase (7.6 %) in number of leaves, but it had no effect at 40 mM of NaCl and even it had a negative impact (23 %) at 80 mM NaCl on this trait (Figure 2).

The lowest and the highest ion leakage was related to HA at 600 mg l^{-1} (10.05 %) and 80 mM salinity stress (56.55 %), respectively (Figure 2E). By raising salinity stress ion leakage increased by 225 % and 341 % at 40 mM and 80 mM levels as compared to control, respectively. Ion leakage increased by HA application under salinity stress, but this increase was lower at all concentrations of HA than salinity stress alone. Ion leakage decreased by 17, 19, and 21 % compared to the control at concentrations 200, 400, and 600 of HA, respectively.

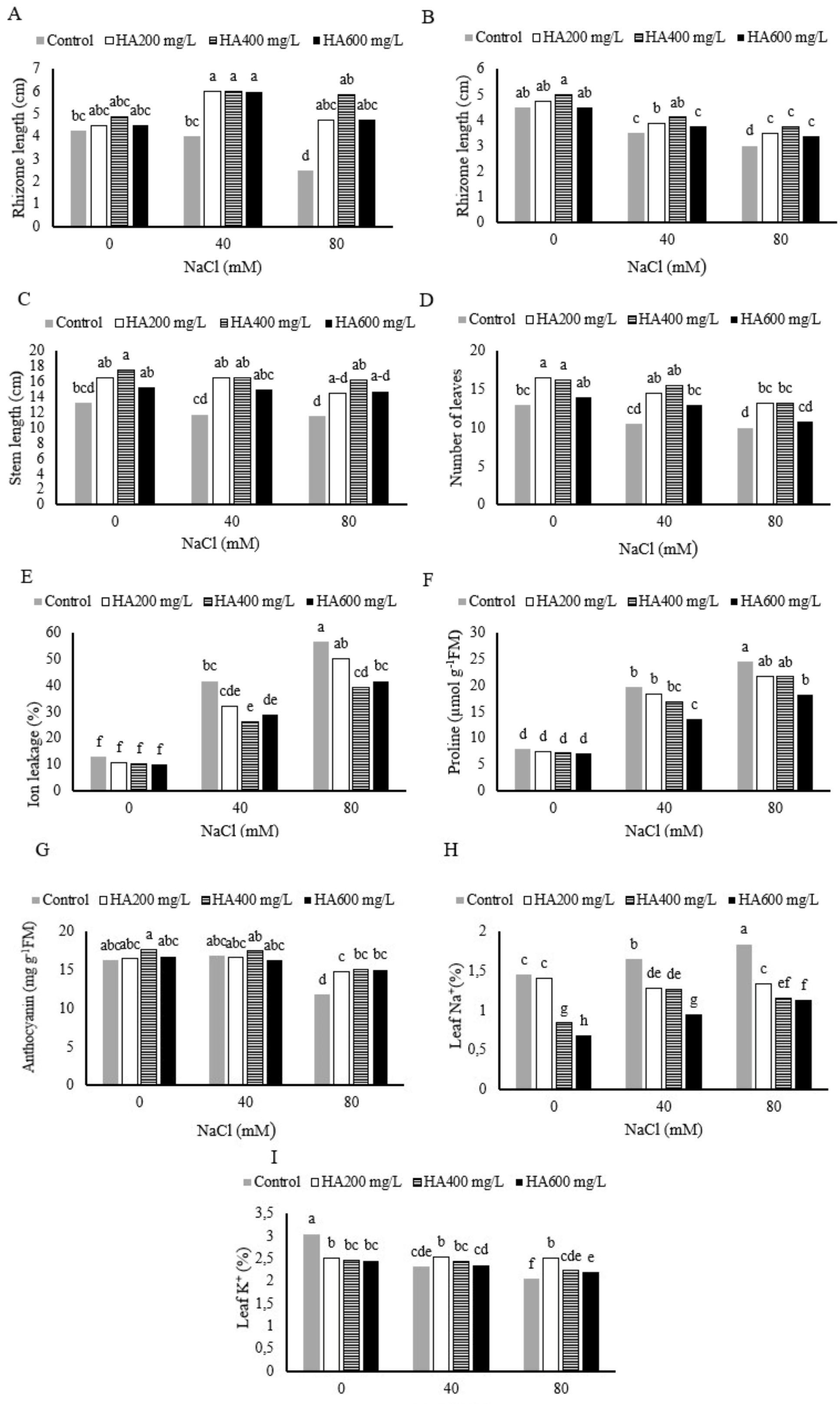

Figure 2: The effect of HA on *Oxalis triangularis* growth parameters, leaf sodium (Na^+) and potassium (K^+), proline and anthocyanin and ion leakage under salinity stress. HA : HA, the columns with the same letter are not significantly different at 0.05 level

The lowest and the highest proline content were observed in HA at 600 mg l^{-1} (7 µmol g^{-1}FM) and severe salinity stress (24.5 µmol g^{-1}FM), respectively (Figure 2F). By applying HA and salinity stress, proline content decreased and increased in comparison with the control, respectively. The reduced trend on proline content was 19.65, 18.45, 16.92, and 13.57 % under salinity stress at 40 mM plus 0, 200, 400, and 600 of HA in comparison with control. The same trend was found under salinity stress at 80 mM at all of the mentioned HA treatments by 24.5, 21.75, 21.65, and 18.25 % compared to the control, respectively.

Considering anthocyanin content, the highest and the lowest ones were demonstrated in HA at 400 mg l^{-1} (17.68 mg g^{-1}FM) and NaCl at 80 mM alone (11.77 mg g^{-1}FM). Salinity stress increased and decreased anthocyanin by 3.55 % and 27 % at 40 mM and 80 mM levels in comparison with control, respectively (Figure 2G). By increasing the concentrations of HA, anthocyanin content raised by 0.9, 8.33, and 2.4 % at 200, 400, and 600 mg l^{-1} when it compared to the control, respectively. Considering HA, anthocyanin content increased by 0.9 % and 1.71 % at 200 mg l^{-1} and 8.3 % and 6.98 % at 400 mg l^{-1} under 0 and 40 mM of NaCl when it compared to control, respectively. On the other hand, this antioxidant compound reduced by 9 %, 7.35 %, and 7.66 % at 200, 400, and 600 mg l^{-1} under 80 mM salinity in comparison with the control, respectively.

Leaf Na^+ showed the highest content (1.84 %) in plants treated with 80 mM salinity. HA at 600 mg l^- alone had the lowest leaf Na^+ (Figure 2H). Salinity stress increased leaf Na^+ by 13 and 26 % as compared to control, respectively. Leaf Na^+ decreased by applying HA with or without salinity stress. By applying HA at 200, 400, and 600 mg l^{-1}, leaf Na^+ decreased by 11, 12, and 34 % under 40 mM NaCl in comparison with control, respectively. This decrease was higher in 80 mM NaCl plus HA at all concentrations.

Plants under control and salinity stress treatments at 80 mM had the highest (3.05 %) and the lowest (2.05 %) leaf K^+, respectively (Figure 2I). Salinity stress decreased leaf K^+ by 23 % and 32 % as compared to control, respectively. Although, leaf K^+ decreased by applying HA with or without salinity stress in comparison with control, but this content was higher than salinity stress alone. By applying HA at 200, 400, and 600 mg l^-, leaf K^+ decreased by 17 %, 20 %, and 22 % under 40 mM NaCl in comparison with control, respectively. This decrease was lower in 80 mM NaCl plus HA at 400 mg l^{-1} and 600 mg l^{-1}.

3.1 MULTIVARIATE ANALYSIS

Cluster I, which included salinity levels at 40 and 80 mM with or without HA at 200, 400, and 600 mg l^{-1}. Within this cluster salinity at 40 and 80 mM alone showed very low Na^+ and K^+ (Figure 3).

Cluster II was controlled through HA alone, which had high anthocyanin.

To determine the dispersion of treatments, principle components analysis (PCA) was used (Figure 4A). According to the first two components, the variances explained by the first two components were 40.5 and 21.30 %, respectively. In addition to the positive correlations with stem and rhizome length, PC1 also showed the strongest positive correlations with proline, leaf Na^+, and ion leakage. Anthocyanin and leaf K^+ were strongly and negatively correlated with PC2 (Figure aA). Regarding the principle cluster analysis on treatment showed that control had a negative correlation with PC1 and PC2. As shown in Figure 4B, stem length had a positive correlation with rhizome length (r = 0.38), rhizome length (r = 0.23), and anthocyanin (r = 0.29), while the negative correlation with K^+ (r = 0.37). There was a strong negative correlation between ion leakage and anthocyanin (r = 0.7).

There were differences in leaf color among treatments (Figure 5). Control and HA at 200, 400, and 600 mM belong to a group with the same values for a and b color indices. The highest b value was observed in salinity at 40 mM alone. It was also found that salinity at 40 mM with HA in all used concentrations was placed in the same group.

4 DISCUSSION

Salinity is one of the main environmental factors that limits plant growth (Enteshari and Sharifian, 2012)**.** Saline conditions decrease height, shoot length, leaf area, fresh mass, and dry mass in many plants (Kang et al., 2021). When the concentration of NaCl rises, growth parameters like plant height biomass and uptake of nutrients decreased (Khaled and Fawy, 2011). The length of rhizome and roots are the most important indicators to measure the effect of salinity, because the roots are in direct contact with the soil and provide water transfer from the soil to the shoot. By reducing absorption and reducing the development of roots, salinity reduces the accumulation of dry matter in the roots and reduces

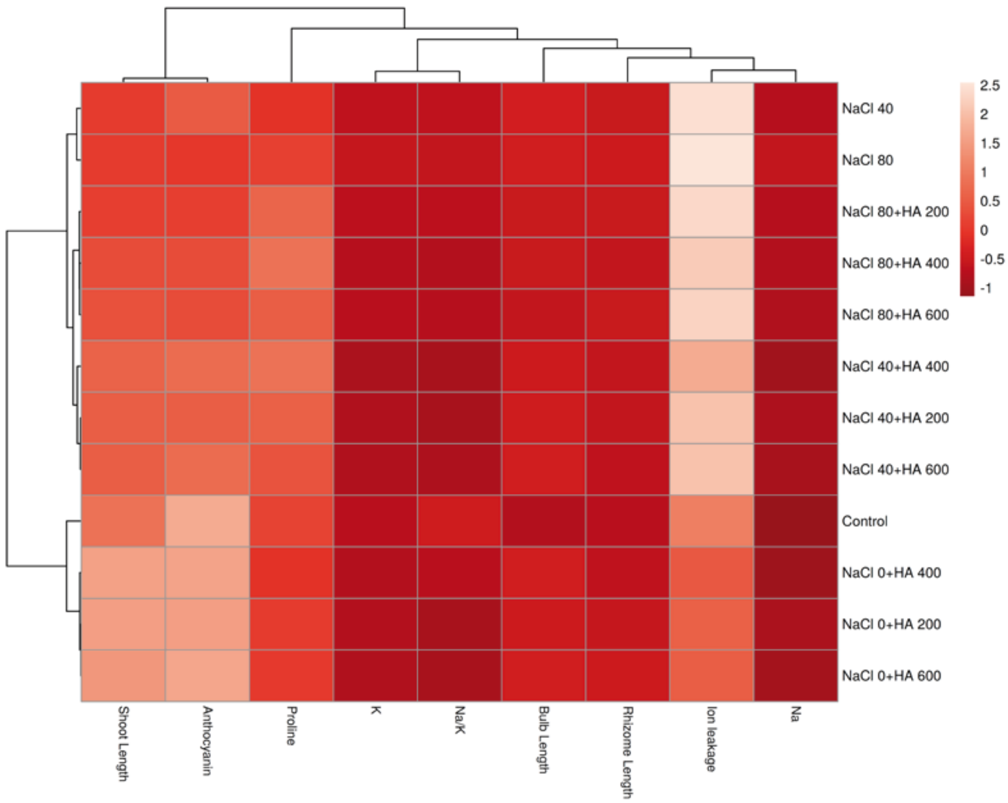

Figure 3: Cluster analysis of *Oxalis triangularis* based on physical and chemical properties of leaf under salinity stress by application of HA (gradient from low (dark red), white (red) to high (pink)). Abbreviations: sodium (Na^+) and potassium (K^+), Na, K = Na^+/K^+

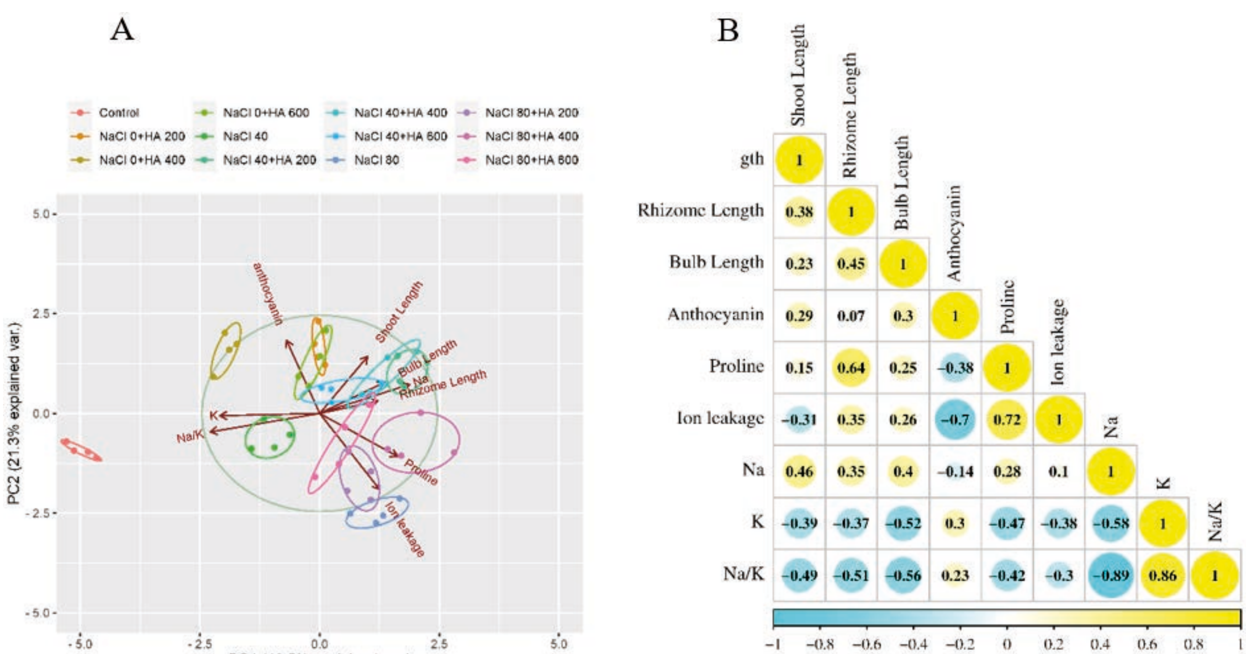

Figure 4: The dispersion of *Oxalis triangularis* based on physical and chemical properties of leaf under salinity stress by application of HA according to the first and the second principal components analysis (PC1/PC2)

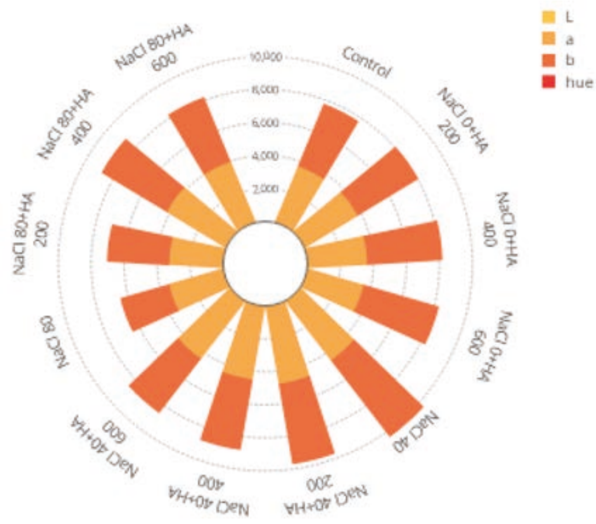

Figure 5: Polar bar chart of color parameters of *Oxalis triangularis* based on physical and chemical properties of leaf under salinity stress by application of HA

root performance. Under the conditions of stress, the air stomata are closed and the rate of photosynthesis is reduced, and as a result, the transfer of photosynthetic materials to the roots is reduced, and finally, salinity can reduce the growth of the roots, and thus the capacity to absorb and transfer water and nutrients from the soil to the aerial parts (Khorasaninejad et al., 2010). Improving the absorption of nutrients such as phosphorus through the roots, humic substances lead to an increase in the longitudinal growth of roots due to the auxin-like effect that affects root growth. The results of the present study are consistent with the results reported in *Poa pratensis* L. and *Capsicum annum* L. that HA effectively increases root length in these plants (Ervin et al., 2008; Çimrin et al., 2010)

Reducing the growth of shoot length is the most obvious effect of salinity on plants. Prevention of plant growth under salinity stress can be due to reduced cell division, ion imbalance, reduced water absorption, impaired absorption of elements, the effect of toxic ions, especially sodium, impaired absorption, recovery, and metabolism of nitrogen and protein, the closing of stomata and reduced by the efficiency of photosynthesis (Oueslati et al., 2010). And as a result, by reducing the height of the shoot, it is possible to transfer a smaller amount of carbohydrates, especially in stressful conditions.

Plant growth of *Lolium perenne* L increased in response to treatments with low and medium concentrations of HA (Nikbakht et al., 2014). HAs stimulate the activity and synthesis of H^+-ATPase enzymes in the plasma membrane of plant cells, which is similar to the auxin effect (Baldotto et al., 2013). In addition, the plasma membrane (PM) proton H^+-ATPase activity in root cells is induced by HAs (Jindo et al., 2012) and promote rooting of ornamental plants (Baldotto et al., 2013). In general, the effect of HAs on plant physiology is recognized with regard to enhancement of root growth and nutrient uptake (Jindo et al., 2012; Khattab et al., 2014 and Kazemi et al., 2019). HA stimulated root growth increased proliferation of root hairs. Enhancing root initiation by HAs, which promotes a guarantee for the growth of the above ground parts of the plant (Fan et al., 2014). Our results on the growth parameters by applying HA under salinity stress were in agreement with Saeedi Pooya et al. (2018) on turfgrass and Jozay et al. (2021) on *Syngonium*.

Also, the decrease in the growth of shoot length under salt stress conditions is due to excessive absorption of sodium chloride in the tissues and around the root, which leads to an increase in osmotic potential and a decrease in water absorption by the plant and a decrease in tissue water, which leads to the lack of cell elongation. Other reasons for growth reduction due to salinity stress include the reduction of water absorption and the inhibition of photosynthetic products, carbohydrate synthesis and the toxicity caused by the high amount of sodium in the cell (Bayat et al., 2012; Taiz et al., 2015). There have been several reports on the reduction of plant shoot length under salt stress. One of the mechanisms that humic substances lead to an increase in longitudinal growth is related to its gibberellin-like compounds. In a report, the effect of HA on stem growth was due to its effect on root H-ATPase activity and the distribution of root nitrate in the stem, which in turn led to changes in the specific distribution of cytokinins, polyamines and ATP, therefore, it had an effect on stem growth (Rubio et al., 2009). On the other hand, HA forms stable and dissolved complexes that increase the absorption of elements, soil fertility, and production in plants (Sajadian and Hokmabadi, 2015). The main effect of HAs on plant physiology includes enhanced root growth (Jindo et al., 2012). Enhanced growth was due to better-developed root systems by applying humic substances, may affect membrane permeability. It also interacts with the phospholipid structures of the cell membranes and react as carriers of nutrients through them. As a result, the plants take more mineral elements (Khaled and Fawy, 2011).

The decrease in the number of leaves as a result of salinity treatment in our experiments is due to the inhibitory effect of salinity stress on the absorption and transfer of photosynthetic materials, followed by a reduction in the level of photosynthesis and photosynthetic pigments such as chlorophyll a and b, net absorption of CO_2 and stomatal conduction and stomatal closure. Also, the decrease in the number of leaves due to salinity stress can be caused by other factors such as osmotic stress, ionic toxicity, nutrient imbalance, and oxidative stress (Anser et al., 2012). In a research, Kumar et al. (2009) stated that salinity by increasing the osmotic pressure of the soil solution leads to a decrease in water absorption and as a result to a decrease in cell division, elongation and differentiation and as a result to a decrease in the number of leaves. The decrease in chemical potential in saline environments initially causes an imbalance of water potential between the apoplast and symplast, which itself causes a decrease in the cell pressure potential, which ultimately leads to a decrease in vegetative growth and the number of leaves. Therefore, it seems that in the present study, salinity caused a decrease in the number of leaves in *O. triangularis* by causing ionic toxicity, osmotic stress, disrupting the balance of nutrients, reducing photosynthesis, and reducing water absorption (Said-Al et al., 2011). Therefore, the results of the present research are consistent with the results of the research conducted on *Spinacea oleracea* L. (Sogoni et al., 2021) and *Rosa canina* L. (Bilal et al., 2020). The positive effects of HA application may be due to the presence of hormone-like compounds from the group of auxins, cytokinins and gibberellins in the plant (Abel-Mawgoand et al., 2007). HA is a polymer that can increase the metabolism of micro-organisms in the soil by increasing the absorption of nutrients through chelating and regenerating properties and maintaining membrane permeability (indirect effect) physical soil and increasing the number of leaves (Ahmad et al.. 2013). The results of this research are consistent with the results of the effect of HA on *Solanum melongena* L., *Capsicum annum* L. and gladiolus, that spraying HA on eggplant and pepper plants (Ahmad et al., 2013) increases the number of leaves in these plants.

Membrane stability is among the physiological characteristics that are affected by environmental stresses. At high concentrations of salinity, the permeability of the cell membrane increases and as result the stability of the membrane decreases and finally leads to leakage of electrolytes. The amount of ion leakage of the membrane is one of the factors that can show the intensity of damage caused by stress in the plant. Membrane leakage can be used as an indicator to measure the level of resistance to salt stress in different plants and even different organs of the same plant. In response to oxidative damage caused by salt stress, plants use different enzymatic and non-enzymatic antioxidant systems. Antioxidant enzymes such as catalase and peroxidase remove free radicals. HA increases the activity of antioxidant enzymes under salinity stress conditions, increasing the level of antioxidant enzymes, causes plants to better tolerate stress conditions and reduce ion leakage (Garcia et al., 2016).

Proline is a storage amino acid in the cytoplasm and plays an effective role in protecting the structure of macromolecules inside the cell during stress conditions. In other words, proline is an indicator in determining the sensitivity to stress in plants. When the plant is exposed to stress, the breakdown of proteins and, as a result, the increase of amino acids and amides is accelerated, one of these amino acids is proline. It is osmotic and osmotic protective and proline is one of these compounds. Proline increase in plants during stress is a kind of defense mechanism (Lotfi et al., 2015). Proline increases plant tolerance to stress through several mechanisms such as osmotic regulation and preventing enzyme degradation. Proline prevents oxidation inside plant cells under stress conditions. The use of organic compounds such as HA is one of the methods that may improve the efficiency of water and food consumption and reduce the effect of salinity stress in plants. Similar to present results, HA can be increase the activity of antioxidants and decrease proline in rapeseed (Lotfi et al., 2015).

Salinity stress increased ion leakage, proline content which was in agreement with Tiwari et al. 2010; Chen et al. (2013) and García-Caparrós et al. (2016) due to enhanced membrane damage, cell membrane permeability and oxidative damage. They also stated that ion leakage from the plasma membrane and proline accumulation has been reported as one of the most important selection criteria for the identification of salt-tolerant plants. Total anthocyanin in pomegranate trees was significantly improved by increasing the amount of humic (Khattab and Shaban, 2014), which was in agreement with our results. The activation of H^+-ATPase improves the uptake of plant nutrients by enhancing electrochemical proton gradient that drives ion transport across cell membranes via secondary transport systems (Jindo et al.; 2012). Potassium accumulation decreased at higher levels of HA (500 and 1000 mg l^{-1}), but it increased at 100 mg l^{-1}. This is in accordance with our results. The possible reason for increasing the absorption of nutrients by plants can be attributed to the increase in root growth. (Nikbakht et al., 2008).

Anthocyanins are one of the phenolic compounds that play an important role in flower color. One of the biochemical changes that occur in environmental stress, including salinity stress, is the production of ROS. Plants must use antioxidant systems such as anthocyanin to resist the oxidative stress. Several reports show that salinity stress reduces physiological indicators such as anthocyanin. The reduction of anthocyanin in salinity stress is due to receiving more photosynthetically active rays, ultraviolet rays cause the reduction of anthocyanin because most of the anthocyanin substances are located in the surface layers of the mesophyll and the epidermis of the leaf. The use of HA increases the amount of anthocyanin in saffron (Ahmadi et al., 2017). It has been reported that HA can cause the synthesis of phenolic compounds such as anthocyanins by stimulating alpha-amylase. The results of this research are consistent with the results obtained in lilium and *Borago officinalis* L., that the application of different concentrations of HA has effectively increased the amount of anthocyanin in these plants (Amiri et al., 2017; Parandian and Samavat, 2012)

Sodium is not present as a necessary element of the plant and its accumulation in the plant in saline conditions leads to the reduction of calcium and potassium ions. Although sodium can help to increase the turgor pressure, it cannot replace potassium ion in special activities such as the activation of enzymes and protein synthesis. The reduction of potassium absorption as a result of the increase of sodium is a competitive process and has nothing to do with the predominant type of salt in the soil (Nikbakht et al., 2014). Potassium is the most prominent dissolved element to keep down the osmotic potential of root cells and is a prerequisite for cell turgescence, and it is necessary to maintain a sufficient level of potassium and plant survival in saline environments. Under saline and alkaline conditions, the high concentration of sodium not only causes disturbances in the absorption of potassium by the root, but also affects the membrane of the root cells and its selective properties (Nikbakht et al., 2014). Due to its strong chelating property, HA keeps sodium ions on itself and prevents the absorption of this ion by the plant. Humic compounds indirectly by providing high-consumption and low-consumption mineral elements for the root, improving the soil structure, increasing the permeability of the substrate to water and air, increasing the soil microbial population and beneficial microorganisms, increasing the cation exchange capacity and the ability to buffer the pH of the substrate or solution. Food, providing some materials for plant roots such as nucleic acids, acetamides and providing HA as carriers of low consumption elements and other growth factors, increase soil fertility.

Salinity not only causes osmotic stress but also leads to disturbances in the absorption of other elements and transfer to the plant's aerial organs and nutritional disturbances, followed by a decrease in plant growth (Said-Al et al., 2011). The activation of PM H^+-ATPase via HA application improves the uptake of plant nutrients by enhancing electrochemical proton gradient that drives ion transport across cell membranes via secondary transport systems (Jindo et al., 2012). Salinity stress decreased leaf K^+ in our experiment which were in accordance with Jaleel et al.(2008) and Khaled and Fawy (2011). Ali et al. (2012) stated that high sodium levels disturb potassium (K^+) nutrition and the reduction of K percentage could be attributed to the competition that exists between Na^+ and K^+, especially at high external NaCl concentrations. Foliar application in 0.1 % HA treatment increased N, P, K, Ca, Mg, Na, Fe, Zn, and Mn amounts under salinity stress at 60 mM when compared with the control and 0.2 % HA treatment (Khaled et al., 2013).

5 CONCLUSION

Plant growth parameters and leaf K^+ decreased under salinity stress, but the content of leaf proline, anthocyanin, and Na^+ increased. The application of HA helped to decrease ion leakage and proline content and improved leaf anthocyanin and growth parameters. In general, the results of this research showed that salinity stress has a negative effect on the growth and performance of *O. triangularis*. High levels of salinity are more affected the growth and performance of the plant. The application of HA was able to partially reduce the harmful effects of salinity on the *O. triangularis* due to its adjustment effect on the physiological parameters of the plant, such as the amount of proline, the ratio of sodium to potassium, and ion leakage. The results of this research showed that the application of HA, especially at a concentration of 600 mg l^{-1}, can moderate the negative effects of salinity on the morphological and physiological characteristics of *O. triangularis* and increase the resistance to salinity stress.

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