Journal of Ornamental Plants
https://sanad.iau.ir/en/Journal/jornamental
ISSN (Print): 2821-0093 ISSN (Online): 2783-5219

Research Article
Volume 15, Number 1: 11-24, March, 2025
https://doi.org/10.71645/jop.2025.140307141185929

Humic Acid and Iron Fertilizers Enhance the Growth Responses and Antioxidant Enzyme Activity of Cineraria (*Pericallis* × *hybrida* L.)

Lili Fatehnezhad[1] and Abolfazl Jowkar[2*]

[1]Department of Horticultural Science, Shiraz University, Shiraz, Iran
[2*]Nuclear Agriculture Research School, Nuclear Science and Technology Research Institue (NSTRI), Atomic Energy Organization of Iran

Received: 05 October 2024 **Accepted:** 03 November 2024

***Corresponding author's email:** abjowkar@aeoi.org.ir

Abstract

Cineraria (*Pericallis hybrida* L.) has faced growth hindrance and chlorosis. Humic acid, iron sulfate and iron chelate fertilizers were investigated for cineraria cultivation in alkaline soil. The study was arranged in a factorial experiment based on a completely randomized design with three replications and 4 samples in each replication. The media were enriched using humic acid (0, 0.5, 1 g/kg soil) and iron fertilizers (5 and 10 mg/kg iron sulfate and 5 and 10 mg/kg iron chelate). The plants that were treated with soil supplemented with 1 g/kg humic acid in combination with 10 mg/kg iron chelate exhibited improvements in plant height (86%), stem diameter (100%), fresh root weight (170%), flowering period (166%), flower number (182%), inflorescence number (252%), flower diameter (59%) and total chlorophyll content (300%). The application of 1 g/kg humic acid and 10 mg/kg iron chelate increased the mineral element content of potassium, nitrogen and phosphorous by 179%, 193% and 675%, respectively. Combinations of 0.5 g/kg humic acid and 10 mg/kg iron chelate enhanced the anthocyanin (131%), leaf area (140%), TSS (332%) and starch (642%). Fertilization with 0.5 g/kg humic acid in combination with 5 mg/kg iron chelate resulted in the highest activity of the antioxidant enzymes SOD (238%), POD (324%), and CAT (667%), and reduced ion leakage by 60%. 1 g/kg humic acid as a biofertilizer in combination with 0.5 mg/kg iron chelate are suggested for use in the production of plants in soils with stressful high pH conditions.

Keywords: Antioxidant enzymes activity, Cineraria, High soil pH, Leaf chlorosis, *Pericallis* × *hybrida* L.

INTRODUCTION

Cineraria (*Pericallis hybrida*) is a flowering plant that belongs to the Asteraceae family and is full of numerous small daisy-like flowers surrounded by large green foliage (Kasem and El-baset, 2016). It is a popular New Year's Day and Spring Festival decorative potted flower and bedding plant (Jin-gang *et al*., 2014). The main advantage of this plant is its wide range of flower colors (white, pink, blue, red, purple, etc.). The capitulum sizes and types of flowers also vary widely (single, semidouble and double) (Kasem and El-baset, 2016). Although, cineraria is a perennial plant, it is used in commercial production as an annual plant where it needs a neutral to slightly acidic medium for optimal growth and development (Dole and Wilkins, 1995).

One of the main hurdles for plant growth and development in alkaline soils is microelement shortage, especially iron deficiency, which results in leaf chlorosis. Even though micronutrient elements are only required in trace amounts for sufficient plant development and production, their absence can result in micronutrient shortages during physiological and metabolic processes. As a result, micronutrient fertilizer applied in cultivation zones may not fulfil crop needs for root growth and nutrient absorption (Pirzad and Shokrani, 2012).

Plants require iron for growth and development. Biochemical processes such as respiration, photosynthesis, and symbiotic nitrogen fixation all require iron as an activator. Iron is a crucial element in the production of chlorophyll, and a lack of iron causes chlorosis. Many ideal landscape and agricultural plants are affected by iron shortages and thus exhibit chlorosis symptoms on their leaves. Iron deficiency in plants causes chlorophyll to be produced in insufficient quantities, resulting in pale green and yellow leaves (Pirzad and Shokrani, 2012; Nemati Lafmejani *et al*., 2018; Ali *et al*., 2021). Ferrous sulfate, which contains 98% ferrous sulfate heptahydrate, is a regularly utilized substance for this purpose. Instead, an equivalent amount of chelated iron can also be utilized (Mousa *et al*., 2015). Plants can absorb and use the iron chelator Fe-EDTA through the tissues of their leaves or through the roots if it is supplied to the soil. However, soil conditions, notably pH, affect nutrient absorption (Nemati Lafmejani *et al*., 2018; Gabra, 2021). Furthermore, the efficacy of spraying leaves with inorganic and chelated Fe fertilizers (Fe-SO_4, Fe-EDTA, Fe-DTPA, Fe-EDDHA, and Fe-citrate) for overcoming Fe deficiency is highly variable and depends on the solubility, stability, leaf cuticle penetration ability, mobility, and translocation of the fertilizers following diffusion into leaf tissues (Ghafari and Razmjoo, 2013).

On the other hand, biofertilizers are replacing chemical fertilizers due to environmental concerns. Humic acid can be used as a direct replacement for synthetic fertilizers to boost crop yields, or it can have an indirect effect by altering soil structure (Memon *et al*., 2014). Humic acid is a natural polymer with carboxyl and phenolic groups and is derived from various sources, such as humus, peat, oxidized lignite and coal (Adam, 2021). Humic acid forms stable and soluble complexes with micronutrients as a result of chemical and biological interactions in the soil, known as humification. Humic acid has a molecular weight of 3,000 to 30,000 Daltons and forms stable and soluble complexes with micronutrients (Boogar *et al*., 2014). It has also proven to be useful in mineral nutrient absorption. Humic substances provide support to growing plants, increase soil fertility and productivity, increase the water holding capacity of soil, and assist plants in drought resistance and seed germination. These advantages are attributed to humic acid. It improves nutrient accessibility, enhances root system expansion, enhances soil aeration and drainage, boosts protein and mineral contents in most crops, and creates a favourable environment for microorganism development (Memon *et al*., 2014; Rasouli *et al*., 2022). Previous studies have revealed the beneficial effects of humic acid fertilizer on yield increases in ornamental plants (Mirzaee *et al*., 2020; Adam, 2021), vegetables (Noroozisharaf

and Kaviani, 2018; Abbass *et al*., 2020; Kamali Omidi *et al*., 2022), medicinal plants (Hourani, 2022; Rasouli *et al*., 2022), fruits (Mohamed *et al*., 2020) and nuts (Li, 2019).

This study is the first to report the effects of humic acid, a biofertilizer, an iron chelate and iron sulfate, which are chemical fertilizers, on the morphological, physiological and biochemical characteristics of cineraria (*P. hybrida*). Additionally, the antioxidant enzyme activity of the fertilized plants was evaluated under alkaline soil conditions. The findings of this study could be useful in the floriculture industry for cineraria production under unfavourable high-pH conditions in the soil and for alleviating iron deficiency.

MATERIALS AND METHODS

Plant materials and treatments

The authors confirm that the present study complies with national regulations and international guidelines and legislations for research on plants. This study adheres to the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora. The seeds of cineraria (*Pericallus x hybrida* cv. Early perfection Red) were purchased from Takii Seed Company (Takii, Japan). The plants were subsequently grown for 15 days in seed trays containing germination peat substrate, after which the plants were transplanted into plastic pots containing a mixture of leaf mold, sand, and soil (2:1:1). The study was carried out in a greenhouse with an average day/night temperature of 28 ± 2/20 ± 2°C and a relative humidity of 55±5%.

This research was conducted as a factorial experiment based on a completely randomized design (CRD) with three replications and 4 sub replicates. As the plants reached the six fully expanded leaf stage, the soil was treated with humic acid (0, 0.5, or 1 g/kg) and iron fertilizers (5 mg/kg iron sulfate, 10 mg/kg iron sulfate, 5 mg/kg iron chelate, or 10 mg/kg iron chelate). It is worthy to note that as the content of the iron in the tested soil was 4 (mg/kg), by adding 1 (mg/kg) iron sulfate and 6 (mg/kg) iron chelate, the final iron concentration of the soil was reached to 5 (mg/kg) and 10 (mg/kg), respectively. The treatments were applied monthly, and soil fertilization continued for six months.

Plant height and stem diameter

At the end of the experiment, the length of the stem was measured from the crown to the end of the last meristem. The stem diameter was measured using a digital calliper.

Flowering characteristics

The flower diameter was measured using a digital calliper. The petal, flower and inflorescence numbers were counted in each pot. The number of days from the beginning of flowering of the first flower to the full opening of the last flower on each plant was calculated as the flowering duration.

Root fresh weight

At the end of the experiment, the roots were weighed using a digital balance.

Leaf area

Leaf area was measured (Delta-T-Devices LTD England) on 3 leaves that had been collected from the 3rd, 5th and 7th leaves from the bottom to the top of the plant. The results are presented as the means of three leaves.

Chlorophyll determination

Chlorophyll and carotenoid contents were measured according to the methods described by Hiscox and Israelstam (1979). Chlorophyll contents were determined by a spectrophotometer (Epoch Microplate Spectrophotometer, BioTek Instruments, Inc., USA) at wavelengths of 645 and 663 nm for chlorophylls (a, b and total). DMSO was used as the extraction solvent for the pigments. Chlorophyll concentrations were calculated by the following equations, and the results are expressed as mg/g FW:

$$\text{Chlorophyll a (mg/g FW)} = \frac{12.7(A_{663}) - 2.69(A_{645}) \times \text{Volume made}}{\text{Wt of the sample}}$$

$$\text{Chlorophyll b (mg/g FW)} = \frac{22.9(A_{645}) - 4.68(A_{663}) \times \text{Volume made}}{\text{Wt of the sample}}$$

$$\text{Total Chlorophyll (mg/gFW)} = \frac{20.2(A_{645}) + 8.02(A_{663}) \times \text{Volume made}}{\text{Wt of the sample}}$$

where Wt is the weight of the sample and A λ is the absorption at wavelength λ (nm).

Total soluble solids and starch

To determine the soluble solids content, 0.1 g of leaf powder was extracted with ethanol (80%). The samples were centrifuged at 5000 rpm for 20 min, after which the volume of the supernatant was adjusted to 10 ml. The soluble solid content was measured according to the method described by Fox and Robyt (1991). The starch content of the samples was determined according to the method described by McCready *et al*. (1950) using anthrone reagent. The starch content was read by a spectrophotometer (Epoch Microplate Spectrophotometer, BioTek Instruments, Inc., USA) at a wavelength of 630 nm.

Anthocyanin content

The anthocyanin content of the petals was measured according to the method of Wang *et al*. (2017). One hundred milligrams of fresh petals were extracted with 1 ml of methanol containing 1% HCl and placed on a shaker at 150 rpm at 4°C overnight. Then, the extract was centrifuged at 10500 rpm for 10 min. The content of anthocyanin was measured according to the following formula. The results are expressed as micrograms per gram of fresh weight.

$$\text{Anthocyanin content (mg/L)} = (A530\text{-}0.25) \times A\ 657$$

$$\mu\ g/(g\ F.W.) = ((\text{Anthocyanin content (mg/L)} \times \text{Volume of solvent})/(\text{Sample weight})) \times 1000$$

Phosphorous, nitrogen and potassium contents

The dry ash method was used to measure phosphorous (P) and potassium (K). Leaf samples were dried at 70°C for 48 hrs in an oven. The dried samples were ashed at 500 °C for 4 hr and subsequently dissolved in 10 ml of 2 N hydrochloric acid. The phosphorous concentration was determined using spectrophotometry according to the method of Murphy and Riley (1962). Potassium was measured via flame emission spectroscopy. Nitrogen (N) uptake was determined according to the Kjeldahl method, described by Bremner (1996).

Ion leakage

Ion leakage from the leaves was measured using the method of Gulen and Eris (2004). A total of 0.1 g of fresh leaf disc was washed with distilled water and placed in a test tube filled with 15 ml of distilled water. After shaking for 24 h, the electrolytic conductivity (EC1) of the samples was measured using a digital conductivity meter. The samples were then autoclaved at 115°C for 20 min. Then, the solutions were cooled, and the conductance was measured (EC2). The extent of ionic leakage (EC%) was calculated as the percentage of the final reading via the following equation:

$$EC\% = EC1/EC2 \times 100$$

Antioxidant enzyme activity

Leaf samples were subjected to enzyme extraction according to the protocol described by Ozden *et al.* (2009). The CAT activity was measured as described previously (Dhindsa *et al*., 1981). and the absorbance of 1 mL of the reaction mixture (50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2 and 50 µL of extracted enzyme) was read at 240 nm. CAT activity was assessed as the reduction in H_2O_2 with an extinction coefficient of 39.4 mM^{-1} cm^{-1} and is expressed as units/mg FW. For the SOD assay, the decrease in light absorbance of the nitrobuterazolium chloride (NBT) superoxide complex under the influence of enzyme activity was measured at 560 nm. The activity of the SOD enzyme was measured using the method of Giannopolitis and Ries (1977) and is expressed as units/mg FW. For the POD assay, 50 µl of enzyme extract, 2.9 ml of 10 mM potassium phosphate buffer (pH = 7) and 0.05 ml of 20 mM guaiacol were used as the reaction mixture. The reaction was started by adding 20 µL of 40 mM H_2O_2. Using the extinction coefficient of 26.6 mM^{-1} cm^{-1}, the activity of the enzyme was calculated and reported as micromoles of oxidized guaiacol in one minute per gram of fresh weight of the sample.

Statistical analysis

The data were analysed using SAS software (ver. 9.4), and the means were compared using the least significant difference (LSD) test at $P < 5\%$.

RESULTS

Shoot and root growth

The application of humic acid increased the leaf area (60%), stem diameter (61%), plant height (81%) and fresh weight of roots (92%) of the cineraria plants compared with those of the control (Fig. 1). When humic acid was used in combination with iron fertilizer, shoot and root growth nearly doubled. Combining 0.5 g/kg humic acid with 10 mg/kg iron chelate increased the leaf area by 140%. Treatment of plants with 1 g/kg humic acid in combination with 10 mg/kg iron chelate increased the plant height, stem diameter and fresh weight of roots by 86%, 100%, and 170%, respectively (Fig. 1).

Flowering characteristics

Treatment of *P.* × *hybrida* with humic acid increased the flower diameter (35%), flowering duration (86%), flower number (115%) and inflorescence number (155%) of cineraria plants compared with those of the control (Fig. 2). When humic acid was used together with iron fertilizer, the flowering characteristics exhibited a multifold increase. The combination of 1 g/kg humic acid and 10 mg/kg iron chelate increased the flower diameter, flowering duration, flower number and inflorescence number by 59%, 166%, 182% and 252%, respectively (Fig. 2).

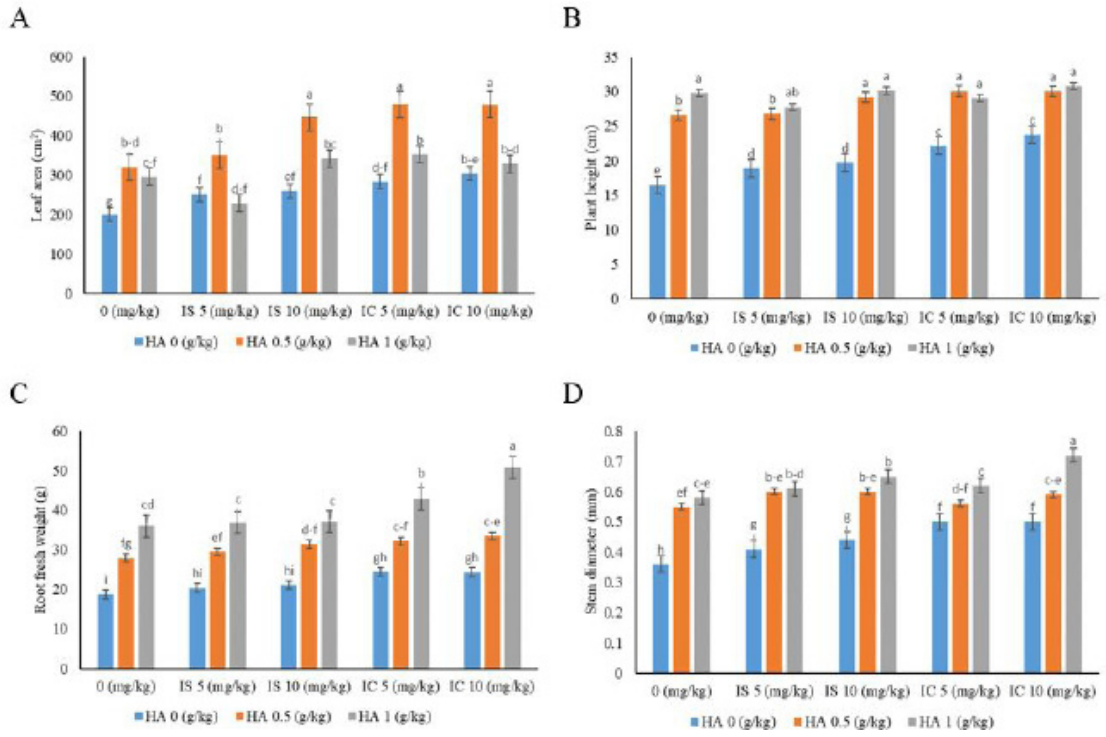

Fig. 1. Shoot and root growth enhancement of *P.* × *hybrida* by application of humic acid and iron fertilizers. HA= Humic acid, IS= Iron sulfate, IC= Iron chelate.

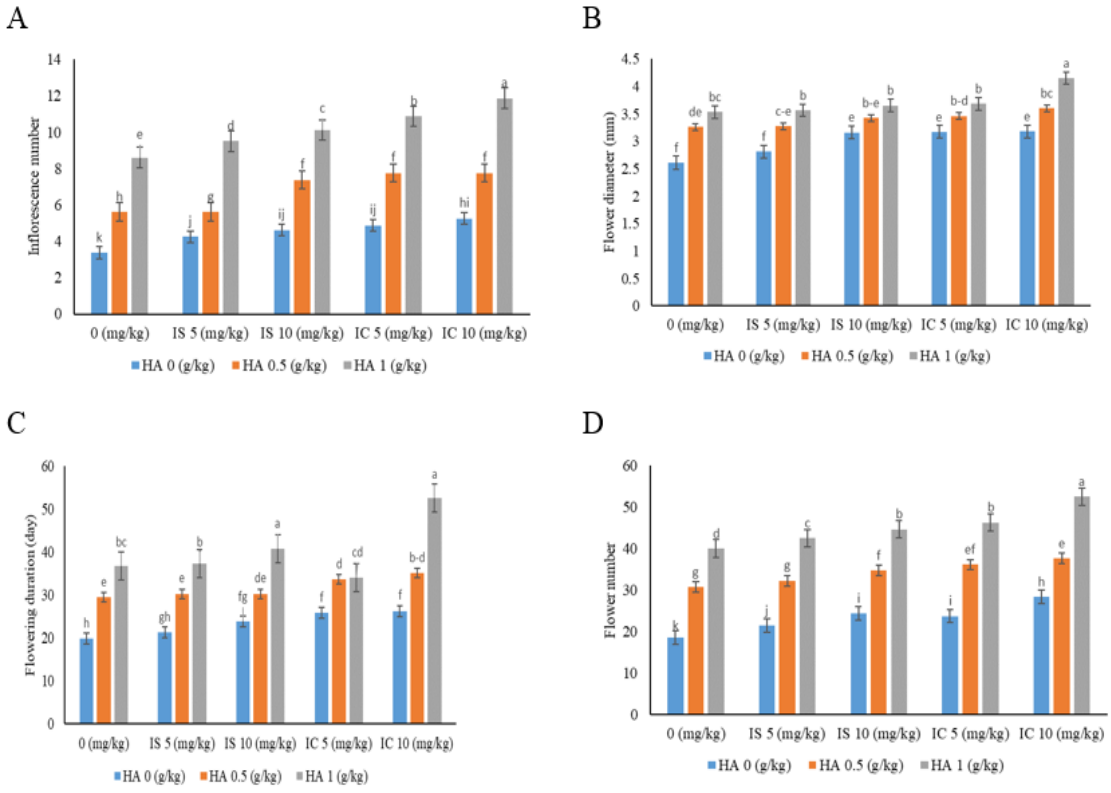

Fig. 2. Improvement of cineraria flowering by application of humic acid and iron fertilizers. HA= Humic acid, IS= Iron sulfate, IC= Iron chelate.

Chlorophyll content

Compared with those of the control, the fertilization of cineraria plants with humic acid doubled the chlorophyll content of the leaves (Fig. 3). When humic acid was used in combination with iron fertilizer, the chlorophyll content tripled. The combination of 1 g/kg humic acid and 10 mg/kg iron chelate improved the chlorophyll content by 3-fold (Fig. 3).

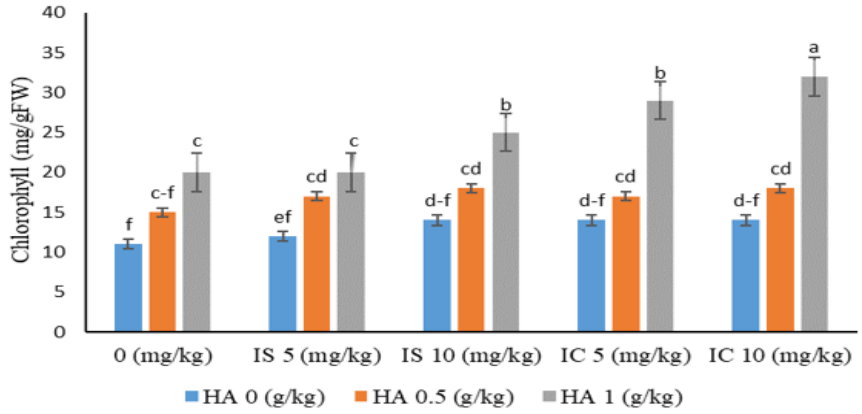

Fig. 3. The effect of humic acid and iron fertilizers on the chlorophyll content of cineraria leaves. HA= Humic acid, IS=Iron sulfate, IC=Iron chelate.

Total soluble solids, starch and anthocyanin content

Humic acid increased the anthocyanin (26%), total soluble solids (68%) and starch (190%) contents of the cineraria leaves compared with those of the control (Fig. 4). When humic acid was used in combination with iron fertilizer, the anthocyanin, total soluble solids and starch contents greatly increased. Coapplication of 0.5 g/kg humic acid with 10 mg/kg iron chelate amplified the amount of anthocyanin, total soluble solids and starch content by 131%, 332% and 642%, respectively (Fig. 4).

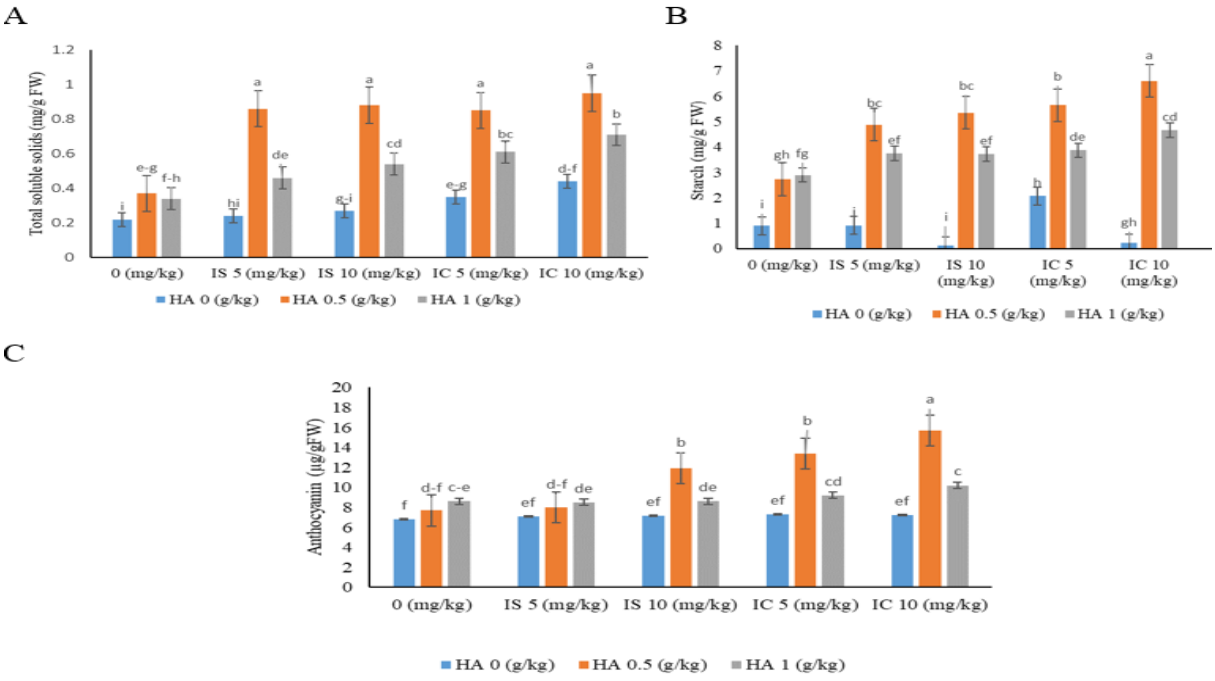

Fig. 4. The effect of humic acid and iron fertilizers on total soluble solids, starch and anthocyanin content of cineraria. HA= Humic acid, IS= Iron sulfate, IC= Iron chelate.

Phosphorous, nitrogen and potassium contents

Treatment of cineraria with humic acid increased the potassium (13%), nitrogen (101%) and phosphorous (308%) contents of the leaves compared with those of the control (Fig. 5). When humic acid was used together with iron fertilizer, slight increases in the phosphorous, nitrogen and potassium contents of the plants were observed. The combination of 1 g/kg humic acid and 10 mg/kg iron chelate increased the potassium, nitrogen and phosphorous contents of the plants by 179%, 193% and 675%, respectively (Fig. 5).

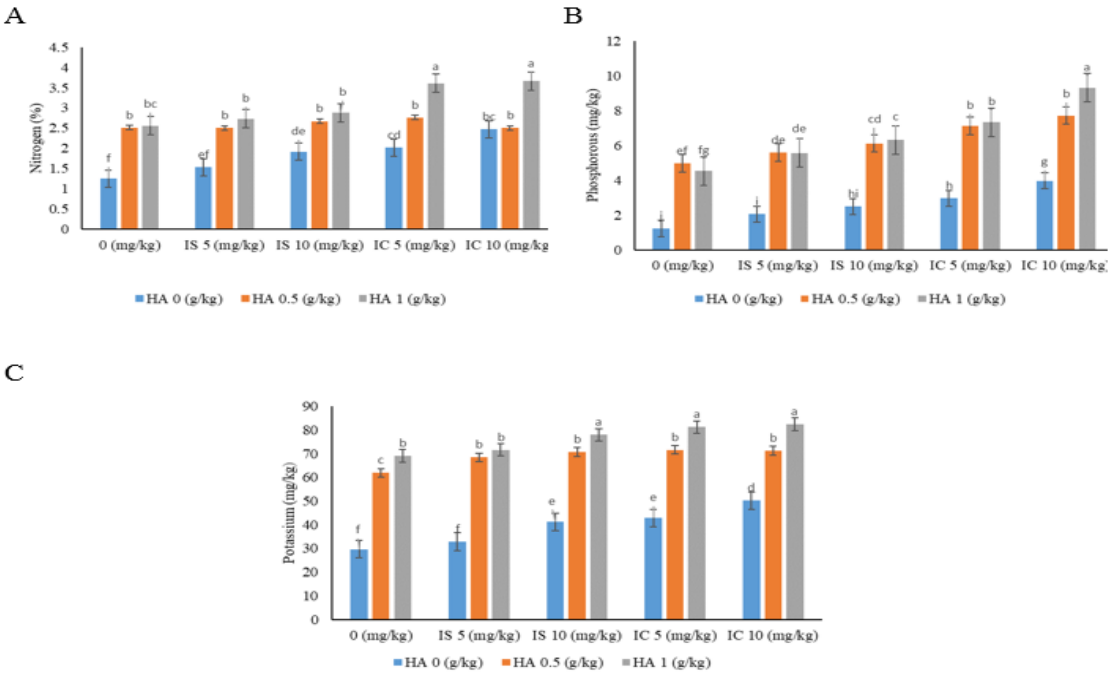

Fig. 5. Effect of different levels of iron and humic acid on phosphorous, nitrogen and potassium content of *Pericallis* × *hybrida*. HA= Humic acid, IS= Iron sulfate, IC= Iron chelate.

Ion leakage

Compared with that in the control treatment, humic acid application to the cineraria plants decreased the ion leakage of the plants by 13% (Fig. 6). When humic acid was used in combination with iron fertilizer, the ion leakage drastically diminished. The combination of 0.5 g/kg humic acid and 5 mg/kg iron chelate had the least ion leakage and a 60% reduction (Fig. 6).

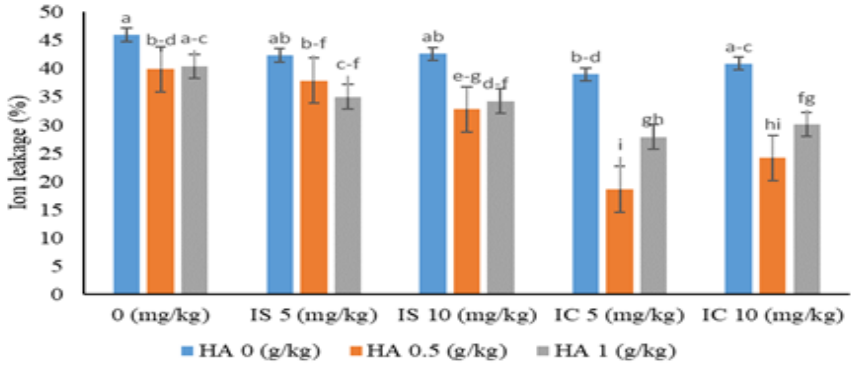

Fig. 6. Effects of different levels of iron and humic acid on the ion leakage of *Pericallis* × *hybrida*. HA= Humic acid, IS=Iron sulfate, IC=Iron chelate.

Antioxidant enzyme activity

Humic acid increased the antioxidant enzyme activity of SOD (59%), POD (172%) and CAT (267%) in *Pericallis* × *hybrida* plants compared with those in the control group (Fig. 7). When humic acid was used in combination with iron fertilizers, the antioxidant enzyme activity strongly increased. Cotreatment with 0.5 g/kg humic acid in combination with 5 mg/kg iron chelate increased the activity of the antioxidant enzymes SOD, POD and CAT by 238%, 324% and 667%, respectively (Fig. 7).

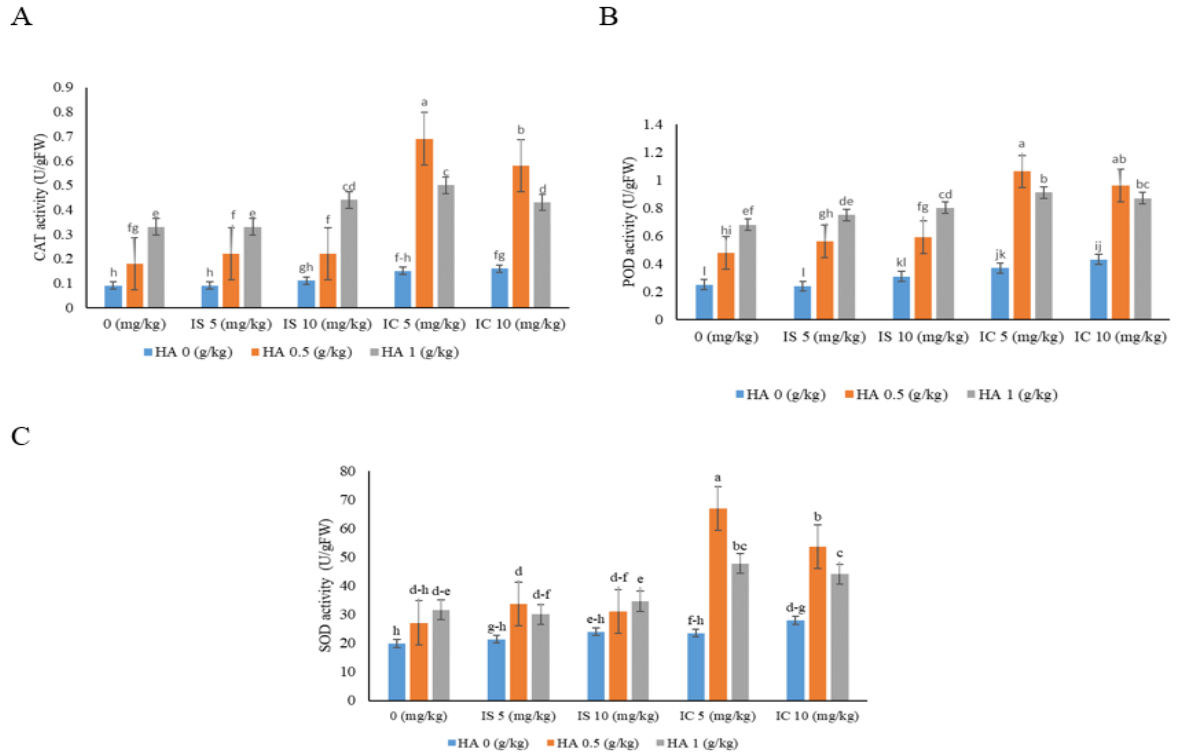

Fig. 7. Effect of different levels of iron and humic acid on antioxidant enzymes activity of cineraria. HA= Humic acid, IS= Iron sulfate, IC= Iron chelate.

DISCUSSION

Iron is required for enzyme systems, chlorophyll synthesis, enzyme activity, and the regulation of respiration, photosynthesis, nitrate and sulfate assimilation, which are important for plant development and reproduction (Salem *et al*., 2019). When the cineraria plants in the present study were treated with the highest concentrations of humic acid and iron chelate fertilizers, the plants presented the most photosynthetic pigments, which is consistent with previous observations (Haghighi *et al*., 2012; Fan *et al*., 2014; Salem *et al*., 2019). Iron is involved in the synthesis of chlorophyll and is required for the maintenance of chloroplast structure and function; therefore, utilizing appropriate treatments will improve chlorophyll synthesis in leaves (Salem *et al*., 2019). On the other hand, humic acid application could enhance NO_3 uptake, N metabolism, and protein production and increase chlorophyll content (Haghighi *et al.*, 2012).

The impact of iron on increasing plant growth and leaf area has been confirmed in previous reports, including for safflower plants (Ibrahim, 2019). In addition, humic acid is absorbed by plant roots and translocated to shoots, which in turn enhances plant growth responses. The current research revealed that humic acid and iron fertilizer improved vegetative

growth in cineraria plants, which was in agreement with the findings of Ahmad *et al*. (2013) in *Gladiolus* and Hembrom and Singh (2015) in *Lilium.* The increase in fresh weight of cineraria roots due to humic acid consumption can be attributed to the important role of humic acid in the formation of lateral roots through enhancing the root surface and subsequently increasing nutrient absorption. Similar results were found by Cordeiro *et al.* (2011) in *Zea mays* plants. In accordance with the results of the present study, humic acid application prominently improved gladiolus growth due to its impact on photosynthetic activity, nitrogen metabolism and protein synthesis (Baldotto and Baldotto, 2013). The use of humic acid by plants enhances photosynthetic activity and increases leaf area. Given the role of humic acid in hastening protein synthesis, enhancing water and nutrient absorption, and increasing fertilizer use efficiency (Rasouli *et al*., 2022), increasing plant growth is expected.

The use of humid acid and iron chelate fertilizer increased cineraria height, which could be related to the role of iron in activating the vegetative growth rate, cell division, and elongation of ornamental plants, resulting in greater stem height (Salem *et al*., 2019). Cineraria stem length also greatly increased when various doses of humic acid were used. These findings validated the significance of humic acid in increasing stem length and overall plant quality by enhancing nitrogen uptake. Ahmad *et al.* (2013) reported similar findings of improved nutrient absorption (i.e., N, P, and S) as a result of humic acid uptake. Additionally, the increase in flowering stem diameter and length can be attributed to the auxin-like action of humic acid (Mirzaee Esgandian *et al*., 2020).

Moreover, an investigation of humic acid and iron chelate fertilizers applied to *Cineraria* plants in this study revealed increases in the total soluble solids (TSS), starch and anthocyanin contents of the plants, which directly impact flower quality. These findings are in accordance with those of Abbass *et al*. (2020), where the application of humic acid and iron chelate increased the total soluble solids in *Freesia* plants by 51%, although cineraria plants exhibited a much greater increase (by 332%). A previous study in strawberry plants showed that humic acid and iron chelate could increase leaf area, dry matter, carbohydrate and anthocyanin content (Mohamed *et al*., 2020). Moreover, similar results were found for *Hibiscus sabdariffa* (Ibrahim, 2019) and *Cymbopogon citrus* (Ghatas and Mohamed, 2018).

Iron has been shown to increase the formation of healthy green leaves, resulting in more assimilates being distributed to floral parts (Hembrom and Singh, 2015; Salem *et al*., 2019). In the present research, the flower diameter, flower number, inflorescence number, and flowering period of cineraria increased following coapplication of iron chelates and humic acid fertilizers. Other findings were also reported in roses, where iron use enhanced metabolic activity, cell wall loosening, cell elongation, and cell expansion, resulting in an increased flower diameter (Poornima *et al.*, 2018; Salem *et al.,* 2019). Similarly, humic acid exerts a positive effect on flower characteristics through direct quasihormonal activity or indirect influence on Ca uptake, which improves cell wall mechanical resistance and cell membrane integrity (Nikbakht *et al.*, 2008).

The application of iron and humic acids to ornamental plants results in increased flowering duration by enhancing protein synthesis and further chlorophyll formation. The results of the current study in cineraria showed that flowering time was prolonged. Previous studies have shown that iron application effectively leads to extended flowering in *Rosa* plants (Salem *et al.,* 2019). Iron treatment has been shown to prolong flowering time in both *Calendula officinalis* (Izadi *et al.,* 2020) and chrysanthemum (Bhute *et al*., 2017). Ahmad *et al.* (2013) previously showed that humic acid application at relatively high concentrations resulted in increased duration of gladiolus flowering.

The cineraria plants in this study fertilized with iron chelate and humic acid exhibited a great increase in potassium (179%), nitrogen (193%), and phosphorus (675%) absorption. Ngan *et al*. (2020) also demonstrated that iron fertilization could enhance mineral uptake in carnation plants, although the present research showed greater absorption of essential elements. Previous studies on rose (Nikbakht *et al*., 2008) and pumpkin (Kamali Omidi *et al.*, 2022) plants confirmed the findings in cineraria, where humic acid treatments increased the absorption of macronutrients (i.e., nitrogen, phosphorous, and potassium).

Humic acid and iron chelate fertilizers decreased electrolyte leakage in cineraria plants by 60%. These findings showed that their coapplication could help maintain cell membrane integrity. The positive effect of these compounds could be partially explained by the strong antioxidant capacity induced by their use. Earlier reports on canola plants under environmental stress indicated that cell membrane damage and subsequent ion leakage are relieved by iron application (Shokri-Gharelo and Ghader, 2017).

This study revealed that coapplication of humic acid with iron chelate in cineraria plants exponentially increased the activity of the antioxidant enzymes SOD, POD and CAT by 238%, 324% and 667%, respectively. Researchers have previously shown that the application of humic acid and iron chelate fertilizers enhances the activity of antioxidant enzymes in azalea (Elmongy *et al.,* 2018) and pot marigold (Izadi *et al.,* 2020), respectively. This could be attributed to the fact that humic acid acts as an antioxidant, auxin activator and scavenger of reactive oxygen species (Elmongy *et al.,* 2018). Iron is a vital element that is well known as a main structural component of many enzymes and plays an important role as a functional and regulatory cofactor of enzyme activity (Ribeiro *et al.*, 2015; Ibrahim, 2019). Okra plants grown in low-iron media had lower catalase activity than control plants (Kabir *et al.*, 2015), which demonstrated that iron deficiency results in reduced catalase activity. Increased antioxidant enzyme activity has been proven to be a plant adaptation strategy for reducing ROS damage (Ribeiro *et al.*, 2017).

CONCLUSION

In conclusion, the application of humic acid as a biofertilizer in soil is an effective substitute and a financially sustainable solution for enhancing plant growth and production. Although the application of humic acid combined with iron chelate in soil has been explored for other crops, this is the first study on this topic in cineraria plants. The combined application of humic acid with iron chelate improved the vegetative and reproductive growth of cineraria. Therefore, the use of humic acid and iron chelate fertilizers could be recommended for the production of cineraria and possibly other ornamental plants, especially in calcareous soils and challenging soil pH conditions.

ACKNOWLEDGMENT

The authors sincerely appreciate the scientific and research support provided by the department of horticultural sciences and the vice chancellor for researvh at shiraz university.

Literature Cited

Abbass, J. A., Al-Zurf, M. T. H. and Ajami, A. T. 2020. Response of freesia (*Freesia hybrida*) to spraying with an organic acid (Laq-Humus) and chelated iron and their effect on growth and flowering indicators. Euphrates Journal of Agriculture Science, 12(1): 31-41.

Adam, A. 2021. Studies the response of gladiolus plants to humic acid, potassium and water irrigation intervals. Scientific Journal of Agricultural Sciences, 3(1): 23-37.

Ahmad, I., Usman Saquib, R., Qasim, M., Saleem, M., Sattar Khan, A. and Yaseen, M. 2013. Humic acid and cultivar effects on growth, yield, vase life, and corm characteristics of gladiolus. Chilean Journal of Agricultural Research, 73(4): 339-344.

Ali, A. F., Salim, H. A., Bader, B. R. and Abed, A. H. 2021. Response of Swiss chard cultivars (*Beta vulgaris* L.) to chelated iron, nano iron and *Glomus mosseae*. *In*: IOP Conference Series: Earth and Environmental Science. IOP Publishing, 735(1): 012057.

Baldotto, M. A. and Baldotto, L. E. B. 2013. *Gladiolus* development in response to bulb treatment with different concentrations of humic acids. Revista Ceres, 60: 138-142.

Bhute, P., Panchbhai, D., Raut, V., Neha, C. and Hemlata, K. 2017. Studies on flower production in annual chrysanthemum in response to iron and zinc. Plant Arch, 17: 1017-1019.

Boogar, A. R., Shirmohammadi, E. and Geikloo, A. 2014. Effect of humic acid application on qualitative characteristic and micronutrient status in (*Petunia hybrida* L.). Bulletin of Environment, Pharmacology and Life Sciences, 3(9): 15-19.

Bremner, J. M. 1996. Nitrogen total. Methods of soil analysis: Part 3 Chemical methods, 5:1085-1121.

Cordeiro, F. C., Santa-Catarina, C., Silveira, V. and de Souza, S. R. 2011. Humic acid effect on catalase activity and the generation of reactive oxygen species in corn (*Zea mays*). Bioscience, Biotechnology, and Biochemistry, 75(1): 70-74.

Dhindsa, R. S., Plumb-Dhindsa, P. A. M. E. L. A. and Thorpe, T. A. 1981. Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. Journal of Experimental Botany, 32(1): 93-101.

Dole, J. and Wilkins, H. 1995. Floriculture, principles and species. Pearson/Prentice Hall. 613 Page.

Elmongy, M. S., Zhou, H., Cao, Y., Liu, B. and Xia, Y. 2018. The effect of humic acid on endogenous hormone levels and antioxidant enzyme activity during *in vitro* rooting of evergreen azalea. Scientia Horticulturae, 227: 234-243.

Fan, H. M., Wang, X. W., Sun, X., Li, Y. Y., Sun, X. Z. and Zheng, C. S. 2014. Effects of humic acid derived from sediments on growth, photosynthesis and chloroplast ultrastructure in chrysanthemum. Scientia Horticulturae, 177: 118-123.

Fox, J. D. and Robyt, J. F. 1991. Miniaturization of three carbohydrate analyses using a microsample plate reader. Analytical Biochemistry, 195(1): 93-96.

Gabra, G. W. R. 2021. Response of *Narcissus* constantinople 'Double Roman' plants of some natural and chemical fertilizers. Egyptian Academic Journal of Biological Sciences, H. Botany, 12(1):147-160.

Ghafari, H. and Razmjoo, J. 2013. Effect of foliar application of nanoiron oxidase, iron chelate and iron sulfate rates on yield and quality of wheat. International Journal of Agronomy and Plant Production, 4(11): 2997-3003.

Ghatas, Y. A. A. and Mohamed, Y. F. Y. 2018. Influence of mineral, micronutrients and lithovit on growth, oil productivity and volatile oil constituents of *Cymbopogon citrus* L. plants. Middle East Journal of Agricultural Research, 7(1):162-174.

Giannopolitis, C. N. and Ries, S. K. 1977. Superoxide dismutases I. Occurrence in higher plants. Plant Physiology, 59(2): 309-314.

Gulen, H. and Eris, A. 2004. Effect of heat stress on peroxidase activity and total protein content in strawberry plants. Plant Science, 166(3): 739-744.

Haghighi, M., Kafi, M. and Fang, P. 2012. Photosynthetic activity and N metabolism of lettuce as affected by humic acid. International Journal of Vegetable Science, 18(2):182-189.

Hembrom, R. and Singh, A. K. 2015. Effect of iron and zinc on growth, flowering and bulb yield in lilium. International Journal of Agriculture, Environment and Biotechnology, 8(1): 61-64.

Hiscox, J. T. and Israelstam, G. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Canadian Journal of Botany, 57(12): 1332-1334.

Hourani, W.2022. Effect of fertilizers on growth and productivity of saffron: A review. Agronomy Research, 20:1-18.

Ibrahim, O. H. M. 2019. Chelated iron and magnesium boost productivity and anthocyanins content in calyces of *Hibiscus sabdariffa* L. Assiut Journal of Agricultural Sciences, 50(2): 93-108.

Izadi, Z., Nejad, A. R. and Abadía, J. 2020. Physio-morphological and biochemical responses of pot marigold (*Calendula officinalis* L.) to split iron nutrition. Acta Physiologiae Plantarum, 42(1): 1-14.

Jin-gang, W., Wei, L., Hong-wei, R., Yuan-da, L., Ding, B. and Jing, X. 2014. Cloning and expression analysis of MIO gene from *Pericallis hybrida* B. Nord. Journal of Northeast Agricultural University, 21(1): 10-15.

Kabir, A. H., Rahman, M. M., Haider, S. A. and Paul, N. K. 2015. Mechanisms associated with differential tolerance to Fe deficiency in okra (*Abelmoschus esculentus* Moench). Environmental and Experimental Botany, 112: 16-26.

Kamali Omidi, T., Khorgami, A. and Taleshi, K. 2022. Effect of foliar application of humic acid levels and nanofertilizer application on some quantitative and qualitative traits of pumpkin (*Cucurbita pepo* L.) in climatic conditions of Khorramabad area, Iran. Caspian Journal of Environmental Sciences, 20 (3): 467-476.

Kasem, M. M. and El-baset, A. 2016. Allelopathic effect of the foliar spray by the aqueous seeds extract of moringa (*Moringa oleifera*) and fenugreek (*Trigonella foenum* graecum) on cineraria (*Pericallis x hybrida*) plant. Journal of Plant Production, 7(11): 1209-1214.

Li, Y. 2019. Humic acid fertilizer improved soil properties and soil microbial diversity of continuous cropping peanut: A three-year experiment. Scientific Reports, 9(1): 1-9.

McCready, R. M., Guggolz, J., Silviera, V. and Owens, H. S. 1950. Determination of starch and amylose in vegetables. Application to peas. Analytical Chemistry, 22(1):1156-1158.

Memon, S. A., Bangulzai, F. M., Keerio, M. İ., Baloch, M. A. and Buriri, M. 2014. Effect of humic acid and iron sulfate on growth and yield of zinnia (*Zinnia elegans*). Journal of Agricultural Technology, 10(6): 1517-152.

Mirzaee Esgandian, N., Jabbarzadeh, Z. and Rasouli-Sadaghiani, M. H. 2020. Investigation on some morphological and physiological characteristics of *Gerbera jamesonii* as affected by humic acid and nanocalcium chelate in hydroponic culture conditions. Journal of Ornamental Plants, 10(1):1-13.

Mohamed, A. A. O., Mohammed, B. K. and Taha, S. M. 2020. Effect of humic acid and chelated iron on yield and quality of two strawberry cultivars (*Fragaria x ananassa* Duch.). Euphrates Journal of Agriculture Science, 12(2): 405-422.

Mousa, G. T., Abdul-Hafeez, E. Y. and Ibrahim, O. H. M. 2015. Response of gardenia plants grown under various growth media and ferrous sulfate application. Pakistan Journal of Agricultural Sciences, 52 (3):651-658.

Murphy, J. and Riley, J. P. A. 1962. Modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta, 21(1): 31-36.

Nemati Lafmejani, Z., Jafari, A. A., Moradi, P. and Ladan Moghadam, A. 2018. Impact of foliar application of iron-chelate and iron nano particles on some morpho-physiological traits and essential oil composition of peppermint (*Mentha piperita* L.). Journal of Essential Oil Bearing Plants, 21(5):1374-1384.

Ngan, H. T. M., Tung, H. T., van Le, B. and Nhut, D. T. 2020. Evaluation of root growth, antioxidant enzyme activity and mineral absorbability of carnation (*Dianthus caryophyllus* 'Express Golem') plantlets cultured in two culture systems supplemented with iron nanoparticles. Scientia Horticulturae, 272: 109612.

Nikbakht, A., Kafi, M., Babalar, M., Xia, Y. P., Luo, A. and Etemadi, N. A. 2008. Effect of humic acid on plant growth, nutrient uptake, and postharvest life of gerbera. Journal of Plant Nutrition, 31(12): 2155-2167.

Noroozisharaf, A. and Kaviani, M. 2018. Effect of soil application of humic acid on nutrients uptake, essential oil and chemical compositions of garden thyme (*Thymus vulgaris* L.) under greenhouse conditions. Physiology and Molecular Biology of Plants, 24(3):423-431.

Ozden, M., Demirel, U. and Kahraman, A. 2009. Effects of proline on antioxidant system in leaves of grapevine (*Vitis vinifera* L.) exposed to oxidative stress by H_2O_2. Scientia Horticulturae, 119(2):163-168.

Pirzad, A. and Shokrani, F. 2012. Effects of iron application on growth characters and flower yield of *Calendula officinalis* L. under water stress. World Applied Sciences Journal, 18(9):1203-1208.

Poornima, S., Munikrishnappa, P. M., Kumar, S. A., Seetharamu, G. K. and Kumar, R. 2018. Effect of foliar application of micronutrients on growth and flowering of floribunda rose under open air condition. International Journal of Current Microbiology and Applied Sciences, 7(10): 1873-1878.

Rasouli, F., Nasiri, Y., Asadi, M., Hassanpouraghdam, M. B., Golestaneh, S. and Pirsarandib, Y. 2022. Fertilizer type and humic acid improve the growth responses, nutrient uptake, and essential oil content on *Coriandrum sativum* L. Scientific Reports, 12(1): 7437.

Ribeiro, T. P., Fernandes, C., Melo, K. V., Ferreira, S. S., Lessa, J. A., Franco, R. W., and Horn, A. 2015. Iron, copper, and manganese complexes with *in vitro* superoxide dismutase and/or catalase activities that keep *Saccharomyces cerevisiae* cells alive under severe oxidative stress. Free Radical Biology and Medicine, 80: 67-76.

Salem, H., Khattab, M. and Yacout, M. 2019. Effect of levels and application methods of iron and zinc on growth and flowering of *Rosa hybrida* cv. Dallas. Alexandria Journal of Agricultural Sciences, 64(2): 63-73.

Shokri-Gharelo, D. D. M. and Ghader, R. 2017. Effects of nanoiron spraying on the antioxidant activities of canola leaf under drought stress. Journal of Biodiversity and Environmental Sciences, 11: 304-311.

Wang, S., Chu, Z., Ren, M., Jia, R., Zhao, C., Fei, D. and Ding, X. 2017. Identification of anthocyanin composition and functional analysis of an anthocyanin activator in *Solanum nigrum* fruits. Molecules, 22 (6): 1-14.

How to cite this article:

Fatehnezhad, L. and Jowkar, A. (2025). Humic Acid and Iron Fertilizers Enhance the Growth Responses and Antioxidant Enzyme Activity of Cineraria (*Pericallis* × *hybrida* L.). Journal of Ornamental Plants, 15(1), 11-24.

https://sanad.iau.ir/en/Journal/jornamental/Article/1185929