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Research Paper

Investigation on Some Morphological and Physiological Characteristics of *Gerbera jamesonii* as Affected by Humic Acid and Nano-Calcium Chelate in Hydroponic Culture Conditions

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Abstract

In order to investigate the effect of humic acid (HA) and nano-calcium chelate on the cut flowers of gerbera cv. Dune, an experiment was performed as a completely randomized design with two factors and three replications in hydroponic conditions. The first factor included humic acid at 4 concentrations of 0 (control), 500, 1000, and 2000 mg L^{-1} as drench and the second factor was assigned to nano-calcium chelate at 4 concentrations of 0 (control), 1, 2 and 3 g L^{-1} as foliar application. The recorded growth traits included the flower and flowering stem diameter of gerbera cv. Dune, leaf and flowering stem length, and leaf fresh and dry weight. The biochemical traits were anthocyanins, carotenoids, total soluble sugars, and phenolic compounds. The results showed that leaf length and fresh and dry weight were influenced by HA so that with the increase in HA concentration, an increase was observed in leaf length and dry weight. The highest flowering stem diameter was observed in the plants exposed to 2000 mg L^{-1} HA and 0 g L^{-1} nano calcium chelate. Flower and flowering stem diameter were increased at higher HA rates. Total soluble sugars and phenolic compounds were affected by the application of HA. Overall, the concurrent application of HA and nano calcium chelate improved the growth and biochemical traits of *Gerbera*, and approximately 2000 mg/l humic acid and 2 g/l nano-calcium chelate were the most effective.

Keywords: Anthocyanin, Carotenoid, Flower diameter, Phenol, Total soluble sugars.

Abbreviations: ANOVA: Analysis of variance, DW: Dry weight, FS: Flowering stem, FW: Fresh weight, HA: Humic acid, NCC: Nano calcium chelate.

INTRODUCTION

Gerbera jamesonii is a permanent, herbaceous, and chilling-sensitive plant species from the family Asteraceae that is native to hot regions (Ghasemi Gahsareh and Kafi, 2012). The species has a high color diversity (Rashidi, 2010) and is among the top ten cut flowers in the world (Danaee *et al*., 2011).

Humic acid is formed under the effect of abiotic catalyzers like primary minerals and silicate layers on the organic matter, including lignin (Muscolo *et al*., 2007). Humic substances derived from different sources have a specific fundamental composition. They usually contain 50% carbon, 35% oxygen, 5% hydrogen, and an equal ratio of nitrogen and sulfur (Melo *et al*., 2015). The main biological effects of humic acid on living organisms include inducing seed germination and growth, stimulating biomass accumulation in plants, stimulating nitrogen accumulation, and inducing the uptake of minerals (Shahsavan Markadeh and Chamani, 2014).

Calcium is an abundant element in the crust. It is a relatively immobile macronutrient that is absorbed as divalent cations (Ca^{2+}). Ca^{2+} in cell walls is in the form of calcium pectate that acts as poly between the proteins of the membrane surface and phospholipid groups. It contributes to protecting membrane structure (Barker and Pilbeam, 2007). Ca positively affects the formation and enhancement of mitochondrial proteins and increases water uptake, fresh weight, and water balance of the flowers (Jing *et al.,* 2004; Seyedi *et al.,* 2013). Through its positive effects on phenylalanine ammonia-lyase, Ca helps the synthesis of anthocyanins (Li and Evans, 2002). Ca is a co-factor for amylase and ATPase, and plays a role in the stability and mechanical resistance of cell walls and stomatal closure (Fageria, 2009).

In a study on *Spathiphyllum wallisii*, the application of 2.5% humic acid increased plant height, leaf number, and flower size (Manda *et al*., 2014). In a study on *Gladiolus communis* L., Hassanpour Asil *et al.* (2017) observed that the application of 250 and 500 mg L^{-1} humic acid increased the number of flower and leaf, plant height and flower dry weight compared to the control. In a study on the effect of humic acid at the rate of 0, 200, 400 or 600 mg L^{-1} and nano-calcium chelate at the rate of 0, 1.5 or 3 g L^{-1} on the growth and flowering of chrysanthemum 'Chinita', Balazadeh and Hassanpour Asil (2014) found that the treatments increased flower diameter, shoot fresh weight, root and stem dry weight, leaf area, stem length, and chlorophyll content significantly. In another study on *Impatiens walleriana* L., the application of 40 mg L^{-1} humic acid increased the number of flowers and plant height versus the control plants (Esringu *et al.,* 2015). Humic acid improved α-amylase, soluble sugars, and anthocyanin content in Lilium. Besides, it's enhanced antioxidant capacity (Parandian and Samavat, 2012). In a soilless growth of tulips, when Ca was applied in the soluble form at the rate of 5 mM from Ca $(NO_3)_2$ source, stem grew to as long as 40 cm and the symptoms of Ca deficiency did not appear (Nelson and Niedziela, 1998). Shams *et al.* (2012) stated that the Ca application (2.5, 5 or 7.5 mM) to cut rose flowers improved their length, root fresh and dry weight, flower number, and flowering stem length. According to Allahvirdizadeh and Nazari Deljou (2014), the application of humic acid increased Ca uptake, total phenol content as an antioxidant agent, and carotenoid content of marigold. In a study on LA hybrid lilies, the application of 20 g m^{-2} calcium nitrate increased flower diameter, the number of leaves, flower size, and fresh weight of bulblet (Bala *et al.,* 2019). The effect of growth medium and foliar application of calcium nano-fertilizer on the quality of cut gerbera flowers was investigated by Mohammadbagheri and Naderi (2017). The results demonstrated that the application of nano-calcium influenced flower diameter, height, and fresh weight compared to the control.

This study aimed to apply different levels of humic acid as drench and calcium nano chelate as foliar application to evaluate their effects on the growth and physiological parameters of *Gerbera jamesonii* cv. Dune.

MATERIALS AND METHODS

The study was carried out as a factorial experiment based on a complete randomized design with 16 treatments and 3 replications. The growth medium (65% peat moss, 30% perlite, 5% cocopeat) was first prepared. Then, the seedlings of the tissue-cultured gerbera (*Gerbera jamesonii* cv. Dune) (Purchased from Etka Breeding Company in Tehran) were planted in size-20 pots (volume 7 L, height 19 cm, diameter 24 cm) in greenhouse conditions. Day/night temperature regime was set at 20-25/13-16°C and light intensity at 400-500 μmol m^{-2} s^{-1}. The plants were fertilized three times a week based on the composition of the nutrient solution shown in Table 1. It should be noted that microelements were also added to the nutrient solution at a specific concentration. The plants were fed three times a week. Treatments included humic acid at four rates (0 as a control, 500, 1000 and 2000 mg L^{-1}) incorporated into the growth medium and nano-calcium chelate at four rates (0 as a control, 1, 2 or 3 g L^{-1}) as a foliar application. Treatments were applied at 5–6 leaf stage every 15 days.

After the treatments were applied, morphological parameters were subject to measurement. They included flower and flowering stem diameter, leaf and flowering stem length (measured by a ruler). Also, the recorded physiological parameters included carotenoid (grinding 0.1 g of leaf fresh tissue using 5 mL of acetone 100%, centrifuging the extract at 2500 rpm for 10 minutes, diluting the extract to 1:3 with distilled water, and reading absorption at 470 nm with a spectrophotometer), anthocyanin (grinding 0.1 g of fresh leaf tissue with 10 mL of acidic methanol, placing the extract in darkness at room temperature for 24 hours, centrifuging the extract at 4000 rpm for 10 minutes, reading the absorption of supernatant at 550 nm with a spectrophotometer), total phenol content (grinding 0.5 g from leaf fresh tissue with methanol 90%, centrifuging the extract at 10000 rpm and 4°C for 15 minutes, mixing 1 mL of extract with 9 mL of distilled water and 1 mL of Folin-Ciocalteu's reagent, after 5 minutes adding 10 mL of sodium carbonate, reading absorption at 750 nm), total soluble sugars (crushing 0.5 g of leaf tissue with 5 mL of ethanol 95%, separating supernatant and pouring it into a 25-mL tube, adding 5% of ethanol 70% to the solid, separating supernatant and pouring it into a 25-mL tube, separating 10 mL of extract, centrifuging at 3500 rpm for 15 minutes, pouring 0.1 mL of extract with a micropipette to a tube, adding 3 mL of fresh anthrone containing 150 mg of anthrone + 100 mL of sulfuric acid 72%, placing tubes in water bath for 10 minutes in which a colorful substance was formed, cooling down, reading absorption at 625 nm with a spectrophotometer).

Finally, the SAS software package (version 9.1) was used for data analysis, and the means of the tested traits were compared using Duncan's Multiple Range Test at $P < 0.01$ and $P < 0.05$ levels.

Table 1. Nutritional program used for gerbera.

$5Ca(NO_3)_2$-NH_4NO_3. $10H_2O$	Fe chelate 6%	K_2SO_4	MAP	NH_4NO_3	KNO_3	$Mg(NO_3)_2$
75 g	20 g	87 g	115 g	100 g	493 g	210 g

RESULTS

Leaf length

Analysis of variance (ANOVA) showed that the main effect of humic acid was significant ($P < 0.01$) on leaf length. Nevertheless, the main effect of nano calcium chelate and the interaction between humic acid and nano calcium chelate was not significant for this trait (Table 2). According to means comparison, leaf length was increased with the increase in the rate of humic acid. The

highest leaf length of 58.5 cm was obtained from the plants treated with 2000 mg L^{-1} humic acid and the lowest one of 53.75 cm from those treated with 500 mg L^{-1} humic acid differing from control insignificantly (Fig. 1).

Table 2. Analysis of variance of the recorded traits of *Gerbera jamesonii* 'Dune'.

S.o.V.	df	Leaf length	Leaf FW†	Leaf DW†	FS† diameter	FS† length	Anthocyanin	Carotenoid	Phenol	Soluble sugars
Humic acid (a)	3	60.72**	10.78**	0.36**	1.95**	53.19**	33.5.**	0.0293**	0.0079**	23.051**
NCC† (b)	3	15.27ns	2.12ns	0.08ns	0.56ns	180.62**	7.039*	0.0077**	0.0010ns	4.019**
a × b	9	7.74ns	1.192ns	0.067ns	1.02**	18.41*	17.91**	0.0048**	0.0031**	1.36**
Error	32	9.54	0.78	0.057	0.24	7.38	1.83	0.0002	0.00044	0.126
C.V. (%)		5.54	10.19	17.01	6.42	4.38	10.54	6.33	8.75	11.2

*, ** and ns: Significant at $P < 0.05$, $P < 0.01$ and insignificant, respectively. †: NCC: Nano calcium chelate; FW: Fresh weight; DW: Dry weight; FS: Flowering stem.

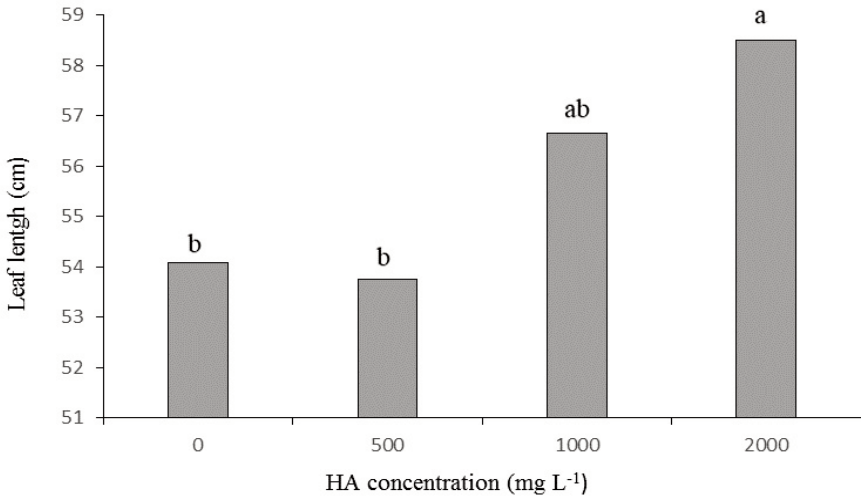

Fig. 1. Effect of different concentrations of humic acid (HA) on leaf length of gerbera 'Dune'. In each column, means with similar letter(s) are not significantly different ($P < 0.01$) using the LSD test.

Leaf fresh and dry weight

ANOVA revealed that only the main effect of humic acid was significant ($P < 0.01$) on leaf fresh weight. However, this trait was not significantly influenced by nano calcium chelate and its interaction with humic acid (Table 2). The highest and lowest leaf fresh weight was obtained from the treatment of 1000 mg L^{-1} humic acid and control, respectively. Also, humic acid rates of 0, 500, and 2000 mg L^{-1} did not bring about statistically significant differences (Fig. 2).

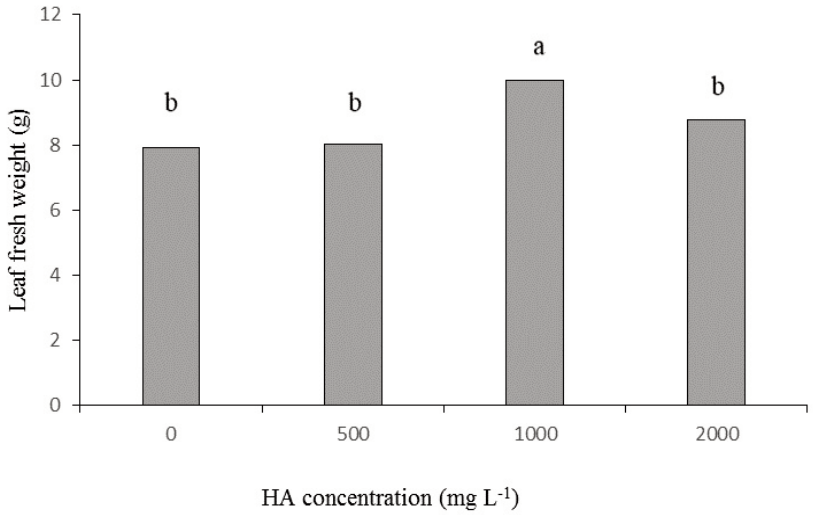

Fig. 2. Effect of different rates of humic acid (HA) on leaf fresh weight (FW) of gerbera 'Dune'. In each column, means with similar letter(s) are not significantly different ($P < 0.01$) using the LSD test.

ANOVA indicated that merely humic acid could change leaf dry weight significantly ($P < 0.01$), and the effect of nano calcium chelate and its interaction with humic acid was not significant for this trait (Table 2). Means comparison revealed that the plants treated with 2000 mg L^{-1} humic acid produced the maximum leaf dry weight of 1.56 g, and the control was associated with the lowest one of 1.17 g. In other words, higher humic acid was related to higher leaf dry weight, but the difference between humic acid rates of 1000 and 2000 mg L^{-1} was not statistically significant (Fig. 3).

Flowering stem diameter

According to the results of ANOVA, flowering stem diameter was significantly ($P < 0.01$) influenced by the main effect of humic acid and its interaction with nano calcium chelate, but the main effect of nano calcium chelate was statistically insignificant (Table 2). Means comparison of data revealed the desirable impact of simultaneous application of humic acid and nano calcium chelate on flowering stem diameter so that with the increase in nano calcium chelate (rates of 2 and 3 g L^{-1}), the increase in the humic acid rate improved flowering stem diameter although, at nano calcium chelate rate of 3 g L^{-1}, this increase was not statistically significant. However, at nano calcium chelate rates of 0 and 1 g L^{-1}, no specific trend was discovered in flowering stem diameter variations with the increase in humic acid rate.

Flowering stem length

The results of ANOVA indicated that the flower stem diameter was significantly influenced by the main effects of humic acid and nano calcium chelate ($P < 0.01$) and their interaction ($P < 0.05$). According to means comparison, the longest flower stems were 69.66 cm observed in plants exposed to 2000 mg L^{-1} humic acid and 3 g L^{-1} nano calcium chelate and the shortest ones were 51.4 cm observed in control (not exposed to humic acid or nano calcium chelate) (Fig. 5). As is evident in Fig. 5, at nano calcium chelate levels of 0 and 3 g L^{-1}, the increase in the humic acid rate improved flowering stem length significantly as compared to control, whereas the increase in humic acid rate had no significant impact on flowering stem length in plants exposed to 1 or 2 g L^{-1} nano calcium chelate.

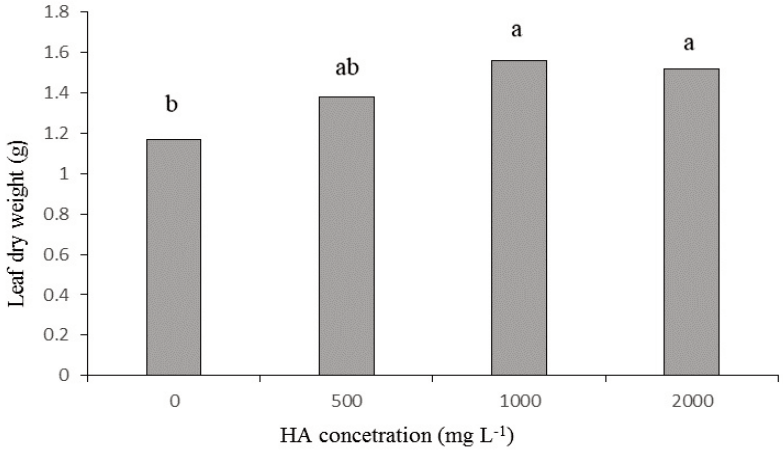

Fig. 3. Effect of different rates of humic acid (HA) on leaf dry weight of gerbera 'Dune'. In each column, means with similar letter(s) are not significantly different ($P < 0.01$) using the LSD test.

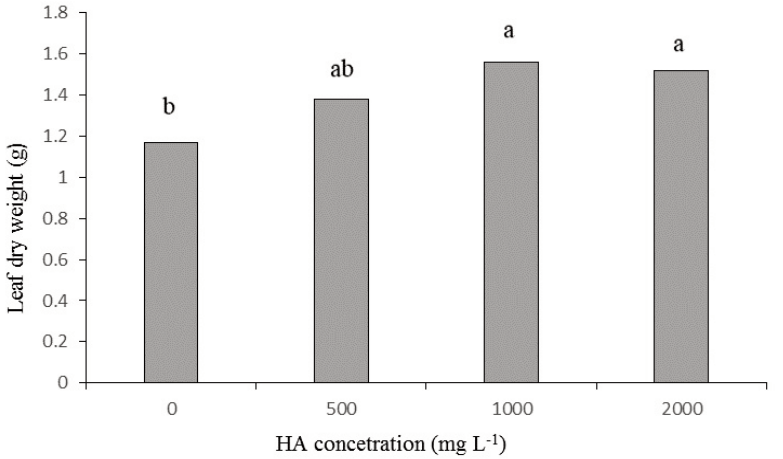

Fig. 4. Effect of different rates of humic acid (HA) and nano calcium chelate on flowering stem diameter of gerbera 'Dune'. In each column, means with similar letter(s) are not significantly different ($P < 0.01$) using the LSD test.

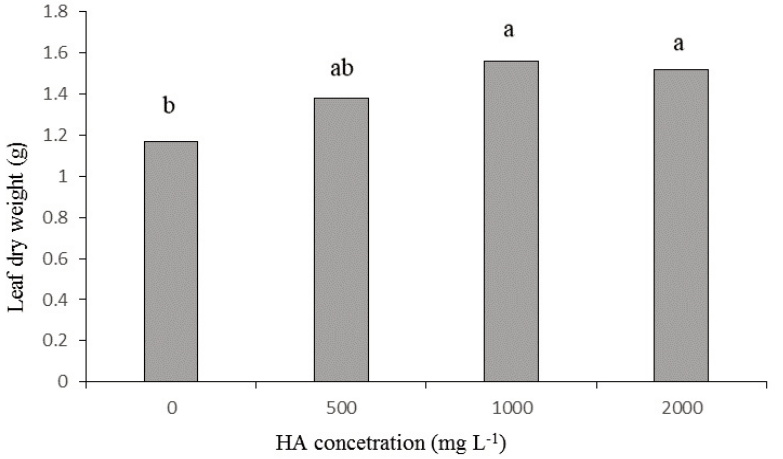

Fig. 5. Effect of different rates of humic acid (HA) and nano calcium chelate on flowering stem length of gerbera 'Dune'. In each column, means with similar letter(s) are not significantly different ($P < 0.01$) using the LSD test.

Leaf carotenoid and anthocyanin

According to the results of ANOVA, the main effects of humic acid and nano calcium chelate, as well as their interaction, were significant ($P < 0.01$) for carotenoid content (Table 2). The highest carotenoid content of 0.37 mg g^{-1} FW was observed in plants exposed to 500 mg L^{-1} humic acid and 2 g L^{-1} nano calcium chelate and the lowest was 0.136 mg g^{-1} FW observed in control, i.e. the untreated plants (Fig. 6). When humic acid was applied alone, carotenoid content showed an increasing trend. When humic acid and nano calcium chelate were applied together, humic acid gave more optimal results at the rate of 500 mg L^{-1}.

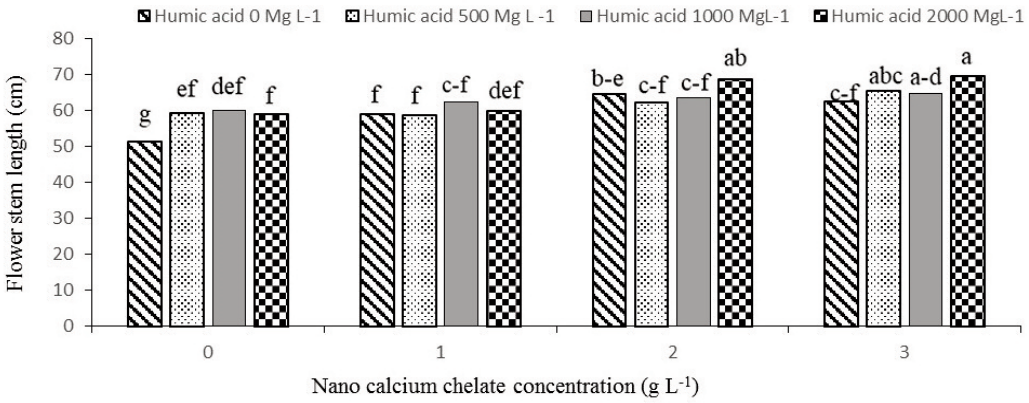

Fig. 6. Effect of different rates of humic acid (HA) and nano calcium chelate on carotenoid content of Gerbera 'Dune'. In each column, means with similar letter(s) are not significantly different ($P < 0.01$) using the LSD test.

ANOVA revealed that anthocyanin content was significantly influenced by humic acid ($P < 0.01$) and nano calcium chelate ($P < 0.05$) (Table 2). According to the means comparison, the highest anthocyanin content of 17.72 mg g^{-1} FW was obtained in the application of 2000 mg L^{-1} humic acid and 2 g L^{-1} nano calcium chelate and the lowest one of 9.25 mg g^{-1} FW was obtained from the plants exposed to no humic acid and 3 g L^{-1} nano calcium chelate (Fig. 7). When applied alone, humic acid at the rates of 0, 500, or 2000 mg L^{-1} did not cause significant differences, but its 1000 mg L^{-1} rate resulted in the highest anthocyanin content. At nano calcium chelate rates of 1 and 2 g L^{-1}, anthocyanin content was enhanced by the increase in humic acid rate. Nevertheless, at the nano calcium chelate rate of 3 g L^{-1}, no specific trend was revealed in anthocyanin content as humic acid was increased.

Total phenol

The results of ANOVA showed that the main effect of humic acid and the interaction between humic acid and nano calcium chelate were significant ($P < 0.01$) for total phenol, but the main effect of nano calcium chelate was statistically insignificant (Table 2). Means comparison indicated that the highest total phenol content (0.297 mg gallic acid per g FW) was observed in plants treated with 2000 mg L^{-1} humic acid and 2 g L^{-1} nano calcium chelate and the lowest one (0.147 mg gallic acid per g FW) was obtained from the control plants (Fig. 8). Total phenol content was increased when humic acid was applied alone, although different rates exhibited statistically insignificant differences. At nano calcium chelate rates of 0, 1, and 2 g L^{-1}, higher humic acid resulted in higher total phenol content, but the effect of humic acid did not follow an inevitable trend a nano chelate rate of 3 g L^{-1}.

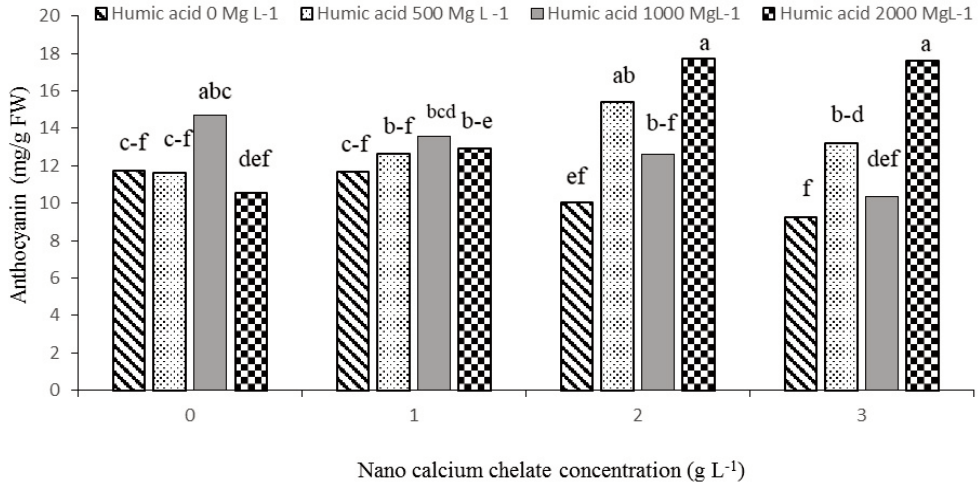

Fig. 7. Effect of different rates of humic acid (HA) and nano calcium chelate on anthocyanin content of gerbera 'Dune'. In each column, means with similar letter(s) are not significantly different ($P < 0.01$) using the LSD test.

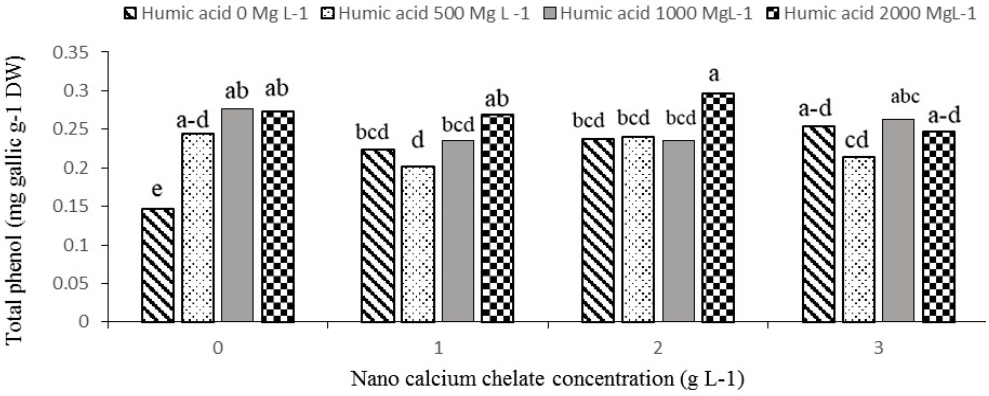

Fig. 8. Effect of different rates of humic acid (HA) and nano calcium chelate on total phenol of gerbera 'Dune'. In each column, means with similar letter(s) are not significantly different ($P < 0.01$) using the LSD test.

Total soluble sugars

The results of ANOVA showed that the main effects of humic acid and nano calcium chelate and their interaction were significant ($P < 0.01$) for total soluble sugar. According to means comparison, the highest total soluble sugars of 5.49 mg g^{-1} FW were obtained from the plants exposed to 500 mg L^{-1} humic acid and 2 g L^{-1} nano calcium chelate although it did not differ significantly from that of plants exposed to 500 mg L^{-1} humic acid and 1 or 3 g L^{-1} nano chelate. The lowest sugar content was observed when no humic acid was applied (Fig. 9). In the treatments in which no nano calcium chelate was applied, humic acid rates of 500 and 1000 mg L^{-1} increased soluble sugar equally, but when nano calcium chelate and humic acid were applied concurrently, humic acid at the rate of 500 mg L^{-1} was related to the highest increase in the soluble sugar, but its higher rates resulted in the loss of the increase in this trait.

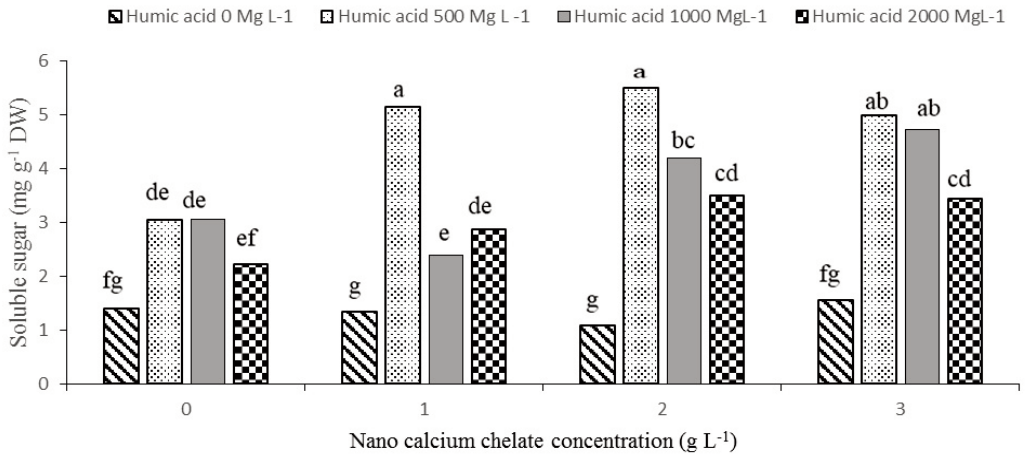

Fig.9. Effect of different rates of humic acid (HA) and nano calcium chelate on soluble sugar content of gerbera 'Dune'. In each column, means with similar letter(s) are not significantly different ($P < 0.01$) using the LSD test.

DISCUSSION

Figs. 2 and 3 show that humic acid enhanced leaf fresh and dry weight versus control. The formation of complexes between humic acid and mineral ions, the impact of humic acid on respiration and photosynthesis, the stimulation of nucleic acid metabolism, and the hormonal activity of humic acid are some hypotheses to explain the effect of humic acid on growth traits of the plants (Fahramand *et al*., 2014). The effect of humic acid on improving growth characteristics in the first stage is probably related to its impact on the activity of H-ATPase in roots and the distribution of root nitrate in plants (Rubio *et al*., 2009). The other mechanism to explain the desirable effect of humic acid on elongation is related to its semi-auxin compounds (Canellas and Olivares, 2014). These results are in accordance with the findings of Manda *et al.* (2014) on *Spathiphyllum wallisii,* who observed that the soil application of HA improved growth parameters.

As was observed in the present study; humic acid is beneficial for the improvement of growth parameters like leaf length (Fig. 1) and leaf fresh and dry weight (Fig. 2 and 3). It is likely that the role of humic acid in the uptake of nutrients, especially nitrogen, contributes to increasing photosynthesis rate and energy generation in plants. Consequently, the plants enjoy ideal growth conditions. Also, it may be related to the action of humic acid as a plant growth regulator, so the increase in these hormones improves plant growth. Thus, it can be said that the effect of humic acid on plant growth is associated with the improvement of other plant factors.

The figs. 4 and 5 illustrate that humic acid and nano calcium chelate improved the flower characteristics of *G. jamesonii*. The positive effect of humic acid can be attributed to its direct quasi-hormonal impact or its indirect impact on Ca uptake, which enhances the mechanical resistance of cell walls and the stability of cell membranes (Nikbakht *et al*., 2008). As was mentioned, humic acid has a semi-auxin effect that can be a reason for the increase in flowering stem diameter and length. Also, humic acid can bolster the uptake of N, K, P, and Mg. Ca is useful in the formation and enhancement of mitochondrial protein and improves water uptake, fresh weight, and water balance in flowers (Jing *et al*., 2004). Ca is a cofactor of amylase and ATPase and plays a role in the stability and mechanical resistance of cell walls and stomata closure (Fageria, 2009). The improvement of nutrients uptake by humic acid and the maintenance and balance of water by Ca are the possible reasons to enhance flower fresh weight and flowering stem diameter in the present

study. Ca is also involved in the synthesis of mitochondrial proteins. Mitochondria have a role to play in aerobic respiration and the active mobilization of several nutrients. It can be concluded that there is a positive relationship between nutrient uptake and Ca. Ca enhances the growth and dry matter by inhibiting the decomposition of chlorophyll and proteins.

As figs. 6 and 7 show, humic acid and nano calcium chelate improved leaf carotenoid and anthocyanin contents versus control. Anthocyanin synthesis in plants is controlled by various internal and external factors, including light, temperature, carbohydrates, plant hormones, and water stress (Kim *et al*., 2006). The increase in anthocyanin content in the present study was likely to relate to the effect of humic acid on increasing carbohydrates. In a study on lilium; Parandian and Samavat (2012) reported that the application of humic acid bolstered soluble sugar content and anthocyanin content of the immersed plants, which they related to the effect of humic acid on increasing α-amylase activity and soluble sugar. According to Fei *et al*. (2010), calcium is among the factors inducing the expression of some genes that are involved in anthocyanin synthesis pathway, so Ca increases anthocyanin content. According to what stated, humic acid increases Ca content, Ca positively affects phenylalanine ammonia-lyase, and thereby it ameliorates anthocyanin synthesis. Overall, we observed that anthocyanin content was increased with the increase in the rate of humic acid and nano calcium chelate, and this can be attributed to semi-gibberellic properties of humic substances, which stimulates α-amylase and other hydrolyzing enzymes and increases plant carbohydrates and anthocyanin.

It is evident in figure 8 that humic acid and nano calcium chelate increased total phenol content as compared to control. Phenyl propanoate pathway is a significant pathway of plant secondary metabolites. This pathway generates different phenol compounds with defensive structure and roles, such as lignin, phenolic acids, and flavonoids in plants. Phenolic and lignin compounds in plants are biosynthesized by catalyzing enzymes like phenylalanine ammonia-lyase, peroxidase, and cinnamyl-alcohol dehydrogenase. They convert phenylalanine to hydroxyl and methoxy cinnamyl alcohol. Lower activity of these enzymes in reducing lignins of the sclerenchyma cell wall impairs the mechanism of stem integrity (Nazarideljou and Azizi, 2015). With the increase in carbohydrates, more phenolic compounds are accumulated with the balance in the consumption sites of carbohydrates (Mullera *et al*., 2013). The application of humic acid contributes to the accumulation of more Ca in stems (Hadavi *et al*., 2011). Calcium facilitates the transverse linkage of polymers; especially in the middle layer; it forms a grid of cell walls to increase its mechanical resistance (Haghighi *et al*., 2014). So, it can be concluded that the effect of humic acid and nano calcium chelate in increasing the accumulation of phenolic compounds in *G. jamesonii* in the present study can be attributed to their impact on photosynthesis and the accumulation of carbohydrates and calcium. It is likely that since phenolic compounds and lignin use a shared synthesis pathway, the increase in phenol results in the lignification of stems and the mitigation of the bending neck.

As is illustrated in fig. 9, humic acid can improve soluble sugar content as compared to control. Soluble sugar act as osmotic protectors in the osmotic adjustment of cells, and they are accumulated in response to environmental stresses (Pagter *et al*., 2005). It seems that the effect of humic acid in increasing the uptake of nutrients, like N, P and K, in plants is related to its semi-hormonal activity (Abbas and Hammad, 2017), which improves photosynthesis and increases the synthesis of soluble sugar (Thi Lua and Bohme, 2001). Also, it is likely that humic acid could hinder the increasing rate of total soluble sugar by maintaining antioxidant capacity and increasing the activity of antioxidant enzymes, as well as by alleviating the damages to cells and inhibiting ion leakage.

In the present study, the application of nano calcium chelate enhanced total soluble sugar and total phenol. Shin *et al*. (2013) report that the calcium connection of the cells causes the uptake of sugar to cells and their accumulation, and finally, they are converted to anthocyanins. Calcium

induces the synthesis of phenolic compounds and related anthocyanins in the presence of glucose and hinders their decomposition.

CONCLUSION

In total, it can be concluded that the application of humic acid as an organic matter can remarkably influence the growth characteristics of the plants in various ways. In the present study, humic acid has improved the growth and biochemical parameters of *G. jamesonii*. Between all treatments, humic acid at the rate of 2000 mg L^{-1} and nano calcium chelate at the rate of 3 g L^{-1} were the most effective.

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