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Research Paper

Morphological and Biochemical Responses of Gerbera (*Gerbera jamesonii* L.) to Application of Silica Nanoparticles and Calcium Chelate under Hydroponic State

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Abstract

The purpose of this study was to investigate the response of physicochemical properties of gerbera (*Gerbera jamesonii* L.) to silica nanoparticles (nanoparticle-SiO_2) and calcium chelate (Ca-Chelate) in nutrient solutions. A factorial experiment based on completely randomized design with two factors was conducted in four replicates. The first factor the concentration of nanoparticle-SiO_2 in nutrient solutions with four levels (0, 20, 40, 80 mg L^{-1}), and the second factor was the concentration of Ca-chelate in the nutrient solution with four levels (0, 60, 120 and 240 mg L^{-1}). Flower diameter, peduncle length, leaf area, fresh and dry weights of root, leaf and flower, stem bending, leaf ionic leakage, chlorophyll index, maximum efficiency of Photosystem II (F_v/F_m), percentages of cellulose, hemicellulose, and holocellulose in stem, and phenylalanine ammonia-lyase activity in leaves were measured. According to the results, in comparison to the control, application of 240 mg L^{-1} Ca-chelate together with 80 mg L^{-1} nanoparticle-SiO_2, resulted in 1.5 times less stem bending and decreased leaf ionic leakage by 28%. The highest activity of phenylalanine ammonia-lyase in leaves was observed in the treatment with 60 mg L^{-1} Ca-chelate (2.91 unit mg fresh leaves^{-1}). The best treatment in terms of the chlorophyll index, leaf area, percentages of cellulose and the maximum efficiency of Photosystem II (F_v/F_m) was the treatment with 120 mg L^{-1} Ca-chelate and 80 mg L^{-1} nanoparticle-SiO_2. Therefore, application of 40 mg L^{-1} nanoparticle-SiO_2 and 60 mg L^{-1} Ca-chelate in the hydroponic nutrition solution can be suggested for gerbera plants.

Keywords: Chlorophyll index, Ionic leakage, Phenylalanine ammonia-lyase enzyme, Stem bending.

INTRODUCTION

Gerbera with the scientific name of *Gerbera jamesonii* belongs to the Asteraceae family (Danaee *et al*., 2010), which is believed to be one of the cut flowers grown in the world and is native to Africa and tropical Asia (Soad *et al*., 2011). This plant has attractive flowers with various colors, which are raised as cut flowers, pot flowers and garden flowers in natural (soil) and artificial (pit, perlite, pumice or mixture of these materials) beds (Rahman *et al*., 2014). Nutrition is reported to be an effective and important factor in the production and growth of cut flowers (Asrar, 2012). To maintain the optimal crop yield, management of environmental conditions and the use of appropriate nutrition solutions are crucial (Tiwari *et al*., 2019).

After oxygen, silicon is the second most abundant element in soil, which is a useful and semi-essential element for plants (Jones, 2016). Silicon is highly useful for the improvement of crop yield and quality (Geerdink *et al*., 2020; Soundharya *et al*., 2019; Snyder *et al*., 2016), it also enhances plant resistance against pests and diseases (Ranjbar *et al*., 2017; Sarma *et al*., 2019), and tolerates abiotic stresses (Snyder *et al*., 2016). One of the important nutrition problems regarding the significance of silicon for plants despite its abundance in soil is its limited application in soilless agriculture and lack of attention and knowledge of the producers; therefore, it is necessary to utilize silicon in nutrient solutions and soilless agriculture (Soundharya *et al*., 2019; Snyder *et al*., 2016; Suriyaprabha *et al*., 2012). Recently, the application of nanoparticles and nano fertilizers has drawn a great deal of scientific attention, which is owing to their easy and fast penetration into cell membrane (Panpatte *et al*., 2016). Nanoparticles of diameters 1-10 nm have specific characteristics including higher ratio of surface area to volume, better electrical conductivity, higher efficiency and capabilities like higher nutrient uptake efficiency (Mahajan *et al*., 2011). In recent researches, positive effects of applying nanoparticle-SiO_2 on improvement of qualitative and quantitative properties of cut flowers have been reported (Panpatte *et al*., 2016; Rastogi *et al*., 2019; Suriyaprabha *et al*., 2012). Studies have indicated that in comparison to the control, treatment of lisianthus (*Eustoma grandiflora* cv. Echo) with 40 mg L^{-1} nanoparticle-SiO_2 increased flower longevity, total chlorophyll content, and dissolved solids by 12, 23.2 and 15.6%, respectively (Kamiab *et al*., 2017). Ranjbar *et al*. (2017) reported that compared to the control (without nanoparticle-SiO_2), the application of 100 mg L^{-1} nanoparticle-SiO_2 was significantly effective on the activity of antioxidant enzymes such as catalase and peroxidase, flower quality including its diameter, stem length, and longevity of gerbera cut flower. Kalteh *et al*. (2018) suggested that the application of nanoparticle-SiO_2 (on basil) increased the leaf number (10.8%), the flower fresh weight (8.1%), the flower dry weight (5.6%) and the leaf chlorophyll content (14.5%) compared to the control. In another study, the effects of nanoparticle-SiO_2 on rose cut flower were investigated, and it was found that the application of 2 mg m^{-3} nanoparticle-SiO_2 increased fresh and dry weights, stem diameter and antioxidant enzymes activity, compared to the control (El-Serafy, 2019).

Calcium, one of the nutrients of great importance in improving and maintaining the quality of cut flowers, could be observed in plant cell wall. Owing to its functions in plant metabolism, it plays a crucial role in the better quality of horticultural plants, particularly ornamental plants (Mohammadbagheri and Naderi, 2017; Hepler, 2005). Calcium is usually found in plant tissues and its transfer is done by water through xylem during the transpiration process (White and Broadley, 2003; Halevy *et al*., 2001). In plant organs, like flower, which is one of the assimilate sinks and has low transpiration and obtains nutrients from phloem, calcium concentration is relatively low. A change in the nitrogen content of the nutrient solution lowers the calcium concentration (Singh, 2020). Furthermore, the calcium absorbed for cell wall stability and membrane integrity might be lower than its critical level, which is due to the high growth rate, number of stomata and low transpiration (Hepler, 2005; White and Broadley, 2003). Therefore, the side-effects triggered by calcium deficiency can be mostly seen in organs with a low transpiration rate such as flowers.

Accordingly, it is recommended that calcium application be included in the work instructions of the producers of ornamental plants, particularly the producers of cut flowers, since calcium improves plant yield and its post-harvest quality (Halevy *et al*., 2001). Nazari Deljou and Gholipour (2013) reported that compared to the control, calcium treatment increased flower diameter (10.5%) and flowering stem (12.3%), and significantly improved flower longevity (34.7%). In the study conducted by Mohammadbagheri and Naderi (2017), the application of calcium nanoparticles increased flower longevity, diameter, and stem length in gerbera cut flower, compared to the control. Samadzadeh and Kamiab (2017) reported that the application of 40 mg L^{-1} calcium increased the longevity of alstroemeria (cv. Konst) by 14 days, compared to the control. In a study conducted on lilium plant, it was indicated that the application of calcium nitrate increased flower diameter, leaf number, flower number and fresh and dry weights of plant (Bala *et al*., 2018). Mirzaee Esgandian *et al*. (2020) stated that the simultaneous application of humic acid (200 mg L^{-1}) and chelated nano calcium (2 g L^{-1}) enhanced the biochemical properties of gerbera cut flower.

This research aimed to investigate the effect of silica nanoparticles (nanoparticle-SiO_2) and calcium chelate (Ca-chelate) on certain vegetative and biochemical characteristics of gerbera (*Gerbera jamesonii* L.) under hydroponic condition. Herein, we could determine the optimal concentrations of nanoparticle-SiO_2 and Ca-chelate in the nutrient solution in order to improve flower yield (quantitatively and qualitatively), and to provide recommendations to producers.

MATERIALS AND METHODS

This study was conducted in the hydroponic greenhouse of Shahed University, Tehran, Iran in 2020. The factorial experiment was performed in the form of completely randomized design with two factors in four replicates at the hydroponic greenhouse of Shahed University. In this experiment the first factor was considered to be the concentration of nanoparticle-SiO_2 in the nutrient solution at 4 levels (0, 20, 40 and 80 mg L^{-1}) from the source material SiO_2, and the second factor was Ca-chelate concentration in the nutrient solution at four levels (0, 60, 120 and 240 mg L^{-1}) from source material Ca-chelate (Ca-EDTA). The greenhouse was located at 31° 36' N, 48° 53' E and 1050 m elevation from the sea level with the mean precipitation of 216 mm. The greenhouse daily temperature was 20-25 °C, its night temperature was 13-16 °C, light intensity was 500- 600 µmol m^{-2} s^{-1}, and the humidity at the greenhouse was adjusted on 60-70% using a humidity controller (pressure fogging system). The plant material used in this experiment was gerbera cultivar Stanza, which is a modified cultivar, and the farming at the greenhouse was carried out similar to other studies.

Preparation of seedbed and nutrient solutions for hydroponic cultivation

To fill the 4-liter pots, perlite and peat moss pot media were used at 50: 50 ratio (V/V). Additionally, 3-4 leafed transplants resulted from the tissue culture of gerbera cultivar Stanza were cultivated individually in the 4-liter plastic pots. The pots were placed on 1-m-high platforms. The pots space in one row was 40×40 cm.

To treat pots with the basic nutrient solution, Hoagland formula (Hoagland and Arnon, 1950), which was achieved at the Agricultural Research Center of California State, USA was employed with certain changes. The changes before and after applying the modification were added in Table 1. Silicium dioxide (SiO_2) from Tecnan Co. England was used to prepare a solution containing different concentrations of silica nanoparticles. To make solutions of 0, 20, 40, 80 mg L^{-1} silica nanoparticles, 0, 1.2, 2.4 and 3.6 g of silica nanoparticles were weighted, respectively. Then they were added to 100-liter barrels containing nutrient solution and distilled water with ratio of 1:100 (V/V) and dissolved in them. Calcium chelated with EDTA from BASF Co. England, which contained 9.5% pure calcium, was used to prepare solutions with various calcium concentrations.

To make solutions with 0, 60, 120 and 240 mg L^{-1} calcium chelate, 0, 37.8, 75.7 and 151.8 g of calcium chelate were weighted, respectively. Then, they were added to 100-liter barrels containing nutrient solution and distilled water with ratio of 1:100 (V/V) and dissolved in them.

The hydroponic system in this experiment was open solution application; nutrients were transferred from nutrient repository through dropping tubes with the capacity of 4 L h^{-1} to 4 L pots with the capacity of 400 ml. The application of the solution for every pot was conducted at specific times, twice a day, and each time around 4 minutes (0.53 L). This practice was conducted using an adjusted digital timer. Furthermore, to prevent salts concentration and salinity stress in cultivation bed, the complete washing of plant root zone was conducted utilizing distilled water every week. The pH of the nutrient solution was 6, and its EC was 2- 2.1 dS m^{-1}.

Measuring some of the plant morphological properties

To measure flower diameter at harvest time, digital caliper (Marcal model, Germany) was used. After harvesting flowering stem, to measure peduncle length, its length was measured utilizing a tape measure from where it was separated from shrub to the point where the flower was attached to the stem. Following the experiment, the plants were completely taken out of their flower beds. Their roots and shoots were separated, and then, fresh weights of roots, flowers and leaves were measured using a digital scale with the precision of 0.001 grams (g). To measure the dry weights of flowers, leaves and roots, they were oven-dried for 72 h at 65 °C, and they were then weighted. At the end of the experiment, leaf area was measured using a device named leaf area meter UT-11 (Winarea co. England). After flower harvest, flowering stem bending (deviation from 90°) was measured using a goniometer.

Measuring some of the plant biochemical properties

To measure leaf ionic leakage, the method conducted in Lutts *et al*. (1996) was followed. Stem holocellulose, hemicellulose and cellulose content were measured using the method proposed by Browning (1967). For the measurement of the activity of phenylalanine ammonia-lyase in leaf, the method suggested by Saunders and McClure (1974) was followed. To measure the chlorophyll content in leaves, three middle leaves of each were selected at the harvest time, and their chlorophyll contents were recorded using SPAD-502 chlorophyll-meter (Konica Minolta Co. Japan). At the plant flowering phase, chlorophyll fluorescence parameters, including minimal fluorescence from dark-adapted leaf (F_0) and the maximal fluorescence from dark-adapted leaf (F_m) were measured utilizing a Handy PEA device (model RS232, Hansatech Instruments Co. England) from three

Table 1. Nutrient concentration in the Hoagland nutrient solution.

Nutrient element	Chemical formula	Content (mg L^{-1})	
		Before modifications	After modifications
Nitrogen	$N\text{-}NO_3$	200	242
Phosphorous	P	60	31
Potassium	K	300	232
Calcium	Ca	170	224
Magnesium	Mg	50	49
Sulfur	S	33	113
Boron	B	1.5	0.45
Copper	Cu	0.1	0.02
Iron	Fe	12	3
Manganese	Mn	2	0.50
Molybdenum	Mo	0.1	0.0106
Zinc	Zn	3	0.48

leaves developed from the middle part of the plant, and the average value was recorded. The maximum quantum efficiency of PSII photochemistry (F_V/F_m) was obtained using Eq.1 (Baker, 2008).

$$\text{Eq.1: } F_V/F_m = (F_m - F_0)/F_m$$

RESULTS

Effects of the treatments on the morphological properties of gerbera

Flower diameter

The results of the comparison between the means indicated that the treatments with nanoparticle-SiO_2 and Ca-chelate increased flower diameter. The highest flower diameter (100.67 mm) was observed in the treatment with 60 mg L^{-1} Ca-chelate and 40 mg L^{-1} nanoparticle-SiO_2, which was significantly different from other treatments and resulted in 29% of increase in flower diameter compared to the control (without nanoparticle-SiO_2 and Ca-chelate) (Table 2). The increase in the application rate of nanoparticle-SiO_2 to 40 mg L^{-1} increased flower diameter by 22% compared to the control, but in higher concentrations (80 mg L^{-1}), there was a decreasing trend. Among the treatments including only Ca-chelate, without adding nanoparticle-SiO_2, the highest flower diameter (96.19 mm) was observed when 120 mg L^{-1} Ca-chelate was applied, and in higher concentrations, (240 mg L^{-1}), there was a decreasing trend.

Peduncle length

According to the comparison between the means, the longest peduncle (64.34 cm) belonged to the treatment with 40 mg L^{-1} nanoparticle- SiO_2 and 60 mg L^{-1} Ca-chelate, which was significantly different from other treatments (Table 2). The shortest peduncle (47.56 cm) was observed in the control. With the increase in the application rate of nanoparticle-SiO_2 without applying Ca-chelate, the peduncle length increased; accordingly, the longest peduncle (59.38 cm) was obtained in the treatment with 80 mg L^{-1} nanoparticle-SiO_2, which was not significantly different from the treatment having 40 mg L^{-1} nanoparticle-SiO_2. Among the treatments only with the application of Ca-chelate, the longest peduncle (60.54 cm) was seen in the treatment with 60 mg L^{-1} Ca-chelate, and higher application rates (120 and 240 mg L^{-1} Ca-chelate without nanoparticle-SiO_2 application) decreased the peduncle length.

Flower fresh and dry weights

The comparison between the means indicated that the highest flower fresh (31.30 g) and dry (3.80 g) weights belonged to the treatment with 40 mg L^{-1} nanoparticle-SiO_2 and 60 mg L^{-1} Ca-chelate (Table 2). Furthermore, the lowest flower fresh weight (18.95 g) and dry weight (2.82 g) were obtained in the control. Once nanopartile-SiO_2 was used individually (without applying Ca-chelate), the treatment with 40 mg L^{-1} nanoparticle-SiO_2 produced the highest flower fresh (27.59 g) and dry (3.51 g) weights. Among the treatments including only Ca-chelate, without the application of nanoparticle-SiO_2, the heaviest fresh and dry flowers were observed when applying 120 mg L^{-1} Ca-chelate, and higher application rates (240 mg L^{-1}) decreased flower fresh and dry weights.

Root fresh and dry weights

According to the mean comparison of the data, the highest fresh weight (102.50 g) of roots was observed in the treatment with 60 mg L^{-1} Ca-chelate and 0 mg L^{-1} nanoparticle-SiO_2, which was not significantly different from the treatments having 20 mg L^{-1} nanoparticle-SiO_2 and 0, 60 or 120 mg L^{-1} Ca-chelate (Table 2). The lowest root fresh weight (80.65 g) belonged to the control. The highest root dry weight (25.29 g) and the lowest root dry weight (14.43 g) was seen in the treatment with 60 mg L^{-1} Ca-chelate and no nanoparticle-SiO_2, and in the control, respectively. Among the treatments including only nanoparticle-SiO_2 without applying Ca-chelate, the highest root dry weight (18.48 g) was obtained by applying 20 mg L^{-1} nanoparticle-SiO_2, which increased the root dry weight by 28% compared to the control.

Leaves fresh and dry weights

The findings of the mean comparison indicated that the application of nanoparticle-SiO_2 and Ca-chelate increased leaf fresh and dry weights, so the highest fresh (314.60 g) and dry (51.43 g) weights of the roots were observed in the treatment with 20 mg L^{-1} nanoparticle-SiO_2 and 60 mg L^{-1} Ca-chelate. In addition, the control had the lowest fresh (196.11 g) and dry (25.52 g) weights (Table 2). The increases in the application rate of nanoparticle-SiO_2 with addition of no Ca-chelate resulted in heavier fresh and dry leaves; therefore, compared to the control, the application of 20, 40 and 80 mg L^{-1} nanoparticle-SiO_2 increased the leaf fresh weight by 34%, 40%, 42%, and its dry weight by 54%, 61%, and 67%, respectively. Among the treatments having only Ca-chelate, the highest leaf fresh (253.17 g) and dry (41.10 g) weight were obtained applying 60 mg L^{-1} Ca-chelate, and in higher concentrations, a decreasing trend can be observed.

Leaf area

As a result of applying the nutrient solution, leaf area revealed an increasing trend, according to which the largest leaf area (6808.82 cm^2) was observed in the treatment with 20 mg L^{-1} nanoparticle-SiO_2 and 60 mg L^{-1} Ca-chelate, which was not statistically different with the treatment having 80 mg L^{-1} nanoparticle-SiO_2 and 120 and 240 mg L^{-1} Ca-chelate and other treatments (Fig. 1). The lowest leaf area (4284.51 cm^2) belonged to the control. Among the treatments including only nanoparticle-SiO_2 doses, without applying Ca-chelate, the highest leaf area (5828.25 cm^2)

Table 2. The mean comparison of the interaction between different concentrations of nanoparticle-SiO_2 and Ca-chelate on some of the morphological characteristics of gerbera (*Gerbera jamesonii* L.).

Treatments(mg L^{-1})		Flower diameter (mm)	Peduncle length (cm)	Flower fresh weight (g)	Flower dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Leaf fresh weight (g)	Leaf dry weight (g)
Nanoparticle-SiO_2	Ca-chelate								
0	0	78.08 i	47.56 j	18.95 h	2.82 i	80.65 g	14.43 j	196.11 m	25.52 h
0	60	94.11 cde	60.54 c	26.26 ef	3.37 ef	102.50 a	25.29 a	253.17 h	41.10 cd
0	120	96.19 bc	52.46 hi	27.54 cd	3.51 bc	89.00 ef	20.00 cde	217.51 k	38.17 e
0	240	89.67 h	53.43 ghi	24.27 g	2.45 j	90.00 def	20.49 cd	204.00 l	34.01 g
20	0	93.30 def	53.54 gh	25.93 f	3.34 f	98.67 abc	18.48 ef	262.96 g	39.21 de
20	60	91.32 fgh	55.32 f	26.39 ef	3.46 cd	99.51 ab	21.23 bc	314.60 a	51.43 a
20	120	90.28 gh	52.30 i	25.74 f	3.11 g	101.23 ab	17.03 fgh	283.77 d	41.06 cd
20	240	98.47 ab	53.74 g	25.78 f	3.45 cde	95.54 bcd	17.39 fg	229.43 j	37.64 ef
40	0	95.57 cd	59.15 d	27.59 cd	3.38 def	96.33 bcd	18.48 ef	275.16 f	41.15 cd
40	60	100.67 a	64.34 a	31.30 a	3.80 a	98.02 bc	14.54 ij	256.48 h	42.54 c
40	120	95.02 cd	56.45 e	28.24 bc	3.43 cde	97.50 bc	18.06 ef	290.22 c	45.51 b
40	240	91.58 fgh	56.62 e	23.27 g	2.90 hi	91.48 def	22.49 b	241.31 i	35.51 fg
80	0	92.47 efg	59.38 d	26.92 de	3.33 f	90.59 def	17.06 fgh	278.13 ef	42.68 c
80	60	90.66 gh	58.43 d	27.79 cd	3.44 cde	87.49 f	16.27 ghi	252.53 h	37.53 ef
80	120	94.20 cde	52.27 b	29.11 b	3.58 b	90.95 def	19.36 de	291.51 b	46.52 b
80	240	92.28 efgh	56.31 ef	23.83 g	2.98 h	93.39 cde	15.31 hij	281.77 de	45.55 b

*In each column, means with the similar letters are not significantly different ($P<0.05$) using the LSD test.

was observed in the treatment with 20 mg L^{-1} nanoprticle-SiO_2, which was not significantly different with the treatments having 40 and 80 mg L^{-1} nanoprticle-SiO_2. Among the treatments with only Ca-chelate doses, without applying nanoparticle-SiO_2, the highest leaf area belonged to the treatment with 60 mg L^{-1} Ca-chelate. In higher concentrations (120 and 240 mg L^{-1} Ca-chelate without nanoparticle-SiO_2 application), there was a decrease in leaf area.

Stem bending

According to Fig. 2, the highest stem bending (12.35 deviation from 90°) belonged to the control. The lowest stem bending (8.16 deviation from 90°) was observed in the treatment with 40 mg L^{-1} nanoparticle-SiO_2 and 240 mg L^{-1} Ca-chelate. The increases in the application of nanoparticle-SiO_2 resulted in lower stem bending, such that compared to the control, applying 20, 40, and 80 mg L^{-1} nanoparticle-SiO_2 decreased stem building by 10%, 21% and 25%, respectively. Furthermore, compared to the control, applying 60, 120 and 240 mg L^{-1} Ca-chelate without applying nanoparticle-SiO_2 decreased stem bending by 19%, 21% and 28%, respectively.

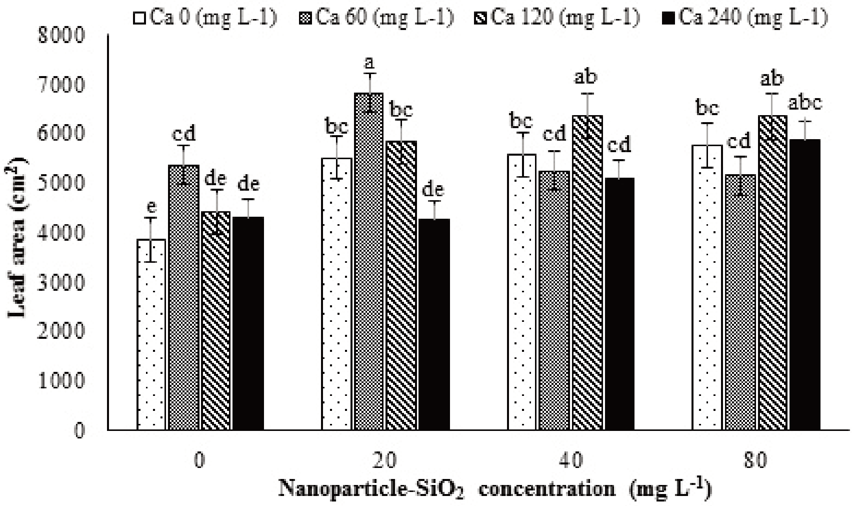

Fig. 1. Interaction of different concentrations of nanoparticle-SiO_2 and Ca-chelate on the leaf area of gerbera (*Gerbera jamesonii* L.). In each column, means with the similar letters are not significantly different (P < 0.05) using the LSD test.

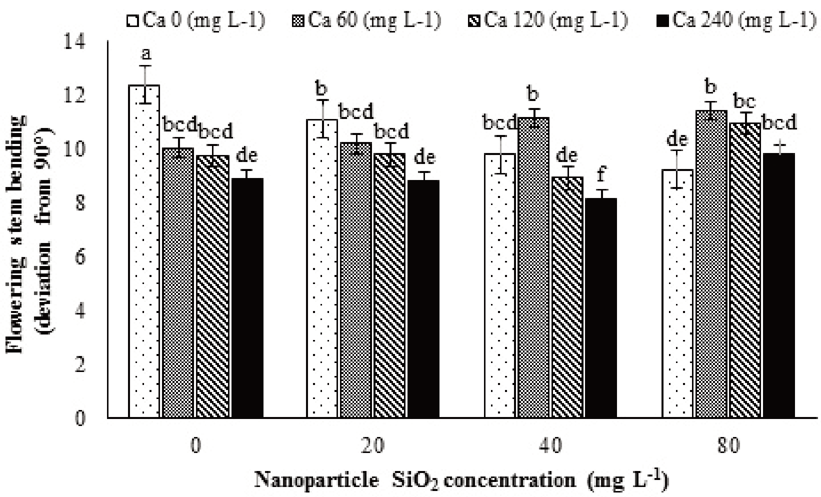

Fig. 2. Interaction of different concentrations of nanoparticle-SiO_2 and Ca-chelate on stem bending in the flowering stems of gerbera (*Gerbera jamesonii* L.). In each column, means with the similar letters are not significantly different (P<0.05) using the LSD test.

Effects of treatments on physiological and biochemical characteristics of gerbera

Leaf ionic leakage

The results of the mean comparison indicated that applying nanoparticle-SiO_2 and Ca-chelate decreased leaf ionic leakage, in a way that the highest (46.03 %) and lowest (32.96 %) ionic leakage were observed in the control and the treatment with 40 mg L^{-1} nanoparticle-SiO_2 and 240 mg L^{-1} Ca-chelate, respectively. Among the treatments including only nanoparticle-SiO_2, the treatment with 80 mg L^{-1} nanoparticle-SiO_2, resulted in 15% less ionic leakage compared to the control. Furthermore, the increases in the application rate of Ca-chelate without adding nanoparticle-SiO_2 led to less ionic leakage. Compared to the control, applying 60, 120 and 240 mg L^{-1} Ca-chelate resulted in 5%, 16%, 20% and 22% less ionic leakage, respectively.

Cellulose, hemicellulose and holocellulose contents

According to the mean comparison, the highest cellulose (44.98%), hemicellulose (24.57%) and holocellulose (68.75%) contents belonged to the treatment with 80 mg L^{-1} nanoparticle-SiO_2 and 60 mg L^{-1} Ca-chelate, which was not significantly different from the treatments with 80 mg L^{-1} nanoparticle-SiO_2 and 120 or 240 mg L^{-1} Ca-chelate (Table 3). The lowest cellulose (39.01%), hemicellulose (18.54%) and holocellulose (59.62%) contents were observed in the control. Among the treatments having only nanoparticle-SiO_2 without any Ca-chelate, the highest cellulose (43.11%), hemicellulose (23.54%) and holocellulose (66.28%) contents were obtained in the treatment having 80 mg L^{-1} nanoparticle-SiO_2. In the absence of nanoparticle-SiO_2, the increases in the application rate of Ca-chelate led to lower cellulose, hemicellulose and holocellulose contents, and the highest contents of cellulose (41.10%), hemicellulose (21.12%) and holocellulose (61.43%) were observed in the treatment with 60 mg L^{-1} Ca-chelate.

Chlorophyll index

The findings concerning the mean comparison implied that applying nanoparticle-SiO_2 and Ca-chelate increased the chlorophyll index; accordingly, the highest chlorophyll index (48.54) was obtained in the treatment with 80 mg L^{-1} nanoparticle-SiO_2 and 60 mg L^{-1} Ca-chelate, which was not significantly different with the treatment with 20 mg L^{-1} nanoparticle-SiO_2 and 60 mg L^{-1} Ca-chelate (Table 3). The lowest chlorophyll index (34.01) belonged to the control. In the treatments only with nanoparticle-SiO_2, the chlorophyll index had an increasing application rate, in a way that the highest (41.98) index was seen in the treatment with 80 mg L^{-1} nanoparticle-SiO_2. In addition, increasing the application rate of Ca-chelate to 120 mg L^{-1} without applying nanoparticle-SiO_2 increased chlorophyll index (23.55% compared to the control). Meanwhile, in higher concentrations (240 mg L^{-1} Ca-chelate), we observed a decreasing trend.

Maximum efficiency of photosystem II (F_V/F_m)

Our results of the mean comparison indicated that the maximum efficiency of photosystem II (F_V/F_m) (0.60) was obtained in the treatment with 80 mg L^{-1} nanoparticle-SiO_2 and 120 mg L^{-1} Ca-chelate, which was not significantly different with the treatment with 80 mg L^{-1} nanoparticle-SiO_2 and 60 or 240 mg L^{-1} Ca-chelate (Table 3). The lowest maximum efficiency of photosystem II (0.46) was obtained in the control. In the treatments only with nanoparticle-SiO_2 application, the highest maximum efficiency of photosystem II (F_V/F_m) (0.55) was seen in the treatment with 80 mg L^{-1} nanoparticle-SiO_2. Furthermore, with the increasing application rate of Ca-chelate to 120 mg L^{-1} without applying nanoparticle-SiO_2, the maximum efficiency of photosystem II (F_V/F_m) had an increasing trend (24% compared to the control), but in higher concentrations (240 mg L^{-1} Ca-chelate), a decreasing trend was observed.

Table 3. The mean comparison of the interaction between different concentrations of nanoparticle-SiO_2 and Ca-chelate on some of the biochemical characteristics of gerbera (*Gerbera jamesonii* L.).

Treatments($mg\ L^{-1}$)		Leaf ionic leakage (٪)	Cellulose (٪)	Hemicellu-lose (٪)	Holocellulose (٪)	Chlorophyll index	Maximum efficiency of photosystem II (F_v/F_m)
Nanoparti-cle-SiO_2	Ca-chelate						
0	0	46.03 a	39.01 i	18.54 g	59.62 f	34.01 e	0.46 e
0	60	39.51 gh	41.10 ef	21.12 d	61.43 e	39.04 bc	0.52 d
0	120	38.49 h	40.72 efg	20.26 def	61.23 e	42.02 b	0.57 bc
0	240	35.82 i	39.89 gh	20.28 def	60.63 e	37.96 cd	0.50 d
20	0	43.20 cd	40.76 efg	20.04 ef	62.45 d	40.12 bc	0.50 d
20	60	45.02 abc	41.52 de	20.61 de	62.77 cd	46.49 a	0.52 d
20	120	40.97 efg	42.69 bc	19.31 f	62.50 d	39.12 bc	0.51 d
20	240	43.71 bcd	42.73 bc	19.53 f	63.38 c	41.33 bc	0.50 d
40	0	42.89 cde	42.37 cd	23.23 bc	64.52 b	41.02 bc	0.50 d
40	60	38.03 hi	40.27 fgh	23.45 bc	62.75 cd	40.95 bc	0.49 d
40	120	37.63 hi	41.52 de	22.68 c	64.51 b	41.99 b	0.51 d
40	240	32.96 j	41.33 ef	23.42 bc	64.63 b	40.29 bc	0.50 d
80	0	38.98 gh	43.11 bc	23.54 bc	66.28 b	41.98 b	0.55 c
80	60	36.68 hi	44.98 a	24.57 a	68.75 a	48.54 a	0.59 ab
80	120	38.98 gh	44.05 a	24.33 ab	68.46 a	42.20 b	0.60 a
80	240	39.05 gh	44.13 a	24.11 ab	68.25 a	35.08 d	0.58 ab

* In each column, means with the similar letters are not significantly different ($P<0.05$) using the LSD test.

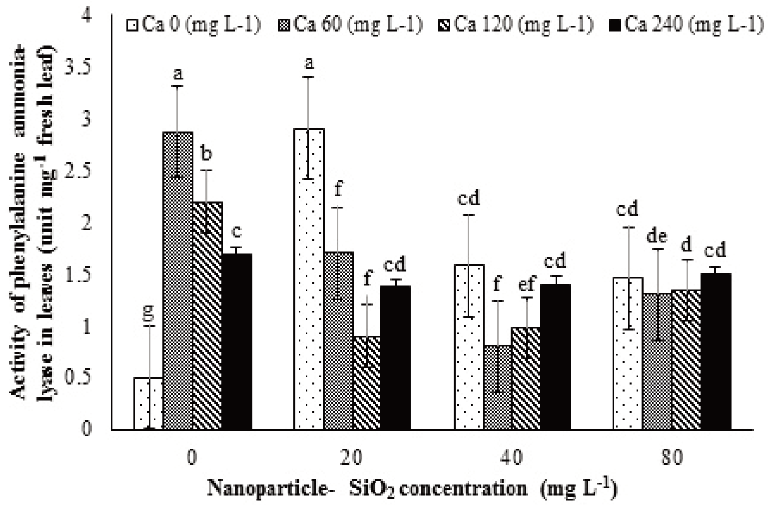

Fig. 3. Interaction of the different concentrations of nanoparticle-SiO_2 and Ca-chelate on the activity of phenylalanine ammonia-lyase enzyme in gerbera leaves (*Gerbera jamesonii* L.). In each column, means with the similar letters are not significantly different ($P<0.05$) using the LSD test.

Activity of phenylalanine ammonia-lyase enzyme

According to Fig. 3, applying nanoparticle-SiO_2 and Ca-chelate increased the activity of phenylalanine ammonia-lyase in leaves; the highest activity (2.91 unit mg^{-1} fresh leaf) was observed in the treatment with 60 $mg\ L^{-1}$ Ca-chelate with no nanoparticle-SiO_2 application, which was not significantly different with the treatment having 20 $mg\ L^{-1}$ nanoparticle-SiO_2 without addition of Ca-chelate. The lowest activity of phenylalanine ammonia-lyase in leaves (0.51 units mg^{-1} fresh leaf) belonged to the control. With the increasing application rate of Ca-chelate (more than 60 mg

L^{-1}) without silica, and nanoparticle-SiO_2 (over 20 mg L^{-1}) without Ca-chelate addition, there was a decreasing trend in the activity of this enzyme. In the combined treatments, the highest activity of phenylalanine ammonia-lyase in leaf (1.7 units mg^{-1} fresh leaf) was observed in the treatment with 20 mg L^{-1} nanoparticle-SiO_2 and 60 mg L^{-1} Ca-chelate.

DISCUSSION

Regarding the study results, the highest flower diameter belonged to the combined applications of nanoparticle-SiO_2 and Ca-chelate (Table 2). Silica enhances the movement of water and nutrient toward cells which results into their better growth and development. Moreover, researches have indicated that silica induces cytokinin biosynthesis, and the cytokinin in plant branches, which increases flower bud development. Therefore, it could be expected that larger buds create larger flowers (Markovich *et al*., 2017). In several studies, increases in flower diameter due to silica application were reported in gerberas (Moyer *et al*., 2008; da Silva *et al*., 2020). In addition, calcium plays a direct role in photosynthetic processes, and since calcium efficiency reduces the efficiencies of carboxylation and photosynthesis, its efficiency can lead to significant decreases in plant biomass content (Kokabi and Tabatabaei, 2011). Therefore, improvements in plant vegetative growth, including flower diameter due to the application of Ca-chelate can be explained. In a study, compared to the control, the addition of 2.5 and 5 mM $CaSO_4$ to the nutrient solution increased rose flower diameter by 14% and 19%, respectively (Hosseini Farahi and Aboutalebi Jahromi, 2018).

Peduncle length is highly important for gerbera as an ornamental plant. According to the study results, the longest peduncle was observed in the treatment with 40 mg L^{-1} nanoparticle-SiO_2 and 60 mg L^{-1} Ca-chelate (Table 2). Silica increases RuBisCo enzyme activity and leaf chlorophyll content, thereby improving photosynthesis and solids accumulation in plant. Accumulation of photosynthates in plant induces plant cell division and ultimately its growth. Furthermore, one of the positive effects of nanoparticles in plants is adsorbing nutrients on their surface. Nanoparticles with a wide specific surface area, have high potential and capacity to retain plant nutrients (Cui *et al*., 2010; Mohammadi *et al*., 2019; Mohammadi *et al*., 2020). Finally, all the factors resulted in better growth of vegetative organs like peduncle (Bayat *et al*., 2012). da Silva *et al*. (2020) demonstrated that the application of 0.25 mg L^{-1} potassium silicate significantly increased peduncle length, leaf number, flower bud number, and complete flower of gerberas. The improvement in these characteristics might be owing to calcium effect on photosynthesis efficiency and photosynthate production in leaves (Tan *et al*., 2011). Mirzaee Esgandian *et al*. (2020) investigated Ca-chelate effect on gerbera stem length, and found that compared to the control, the addition of 1 and 2 mg L^{-1} calcium nanochelate to the nutrient solution increased rose stem length by 20% and 28%, respectively.

Joint application of nanoparticle-SiO_2 and Ca-chelate significantly increased flower fresh and dry weights (Table 3). The presence of assimilates, like carbohydrates, is essential for water penetration and cell development (Ichimura *et al*., 1999); higher carbohydrate content leads to better water penetration, better cell development and therefore, heavier petals (O'Donoghue *et al*., 2002). Furthermore, the increases in the dry and fresh weights of flower and petals can be due to silica's effect on the contents of carbohydrates, sugars and photosynthates (Fanourakis *et al*., 2007). de Silva (2020), Dissanayake *et al*. (2017) and Kamenidou *et al*. (2010) reported increases in gerbera fresh and dry weights due to silica application. Moreover, calcium is involved in producing mitochondrial proteins. Mitochondrion is involved in aerobic respiration and active transport of many nutrients (Chen *et al*., 2004). It can be concluded that a positive relationship exists between calcium and nutrient absorption. Calcium prevents chlorophyll and protein breakdown and can enhance plant growth and its fresh and dry weights. The role of calcium in carbohydrate transfer is another reason for increasing leaf fresh and dry weights (Chen *et al*., 2004). Studies on lilium (Bala *et al*., 2018), and gerbera (Mirzaei *et al*., 2019; Mirzaee Esgandian *et al*., 2020) demonstrated that calcium increased flower fresh and dry weights, which is consistent with the results of the present study.

Treatments including both nanoparticle-SiO_2 and Ca-chelate had higher effects on leaf fresh and dry weights (Table 2). In fact, silica increases photosynthesis efficiency, exchangeable CO_2, and chlorophyll and carbohydrate contents and thus, increases leaf storage and products (Vasanthi *et al*., 2012); therefore, it probably increases leaf dry and fresh weights. Additionally, silica, by precipitating in epidermal leaf tissues, increases leaf thickness and thus enhances its fresh and dry weights (Ma and Takahashi, 2002). Silica plays a direct role in increasing leaf fresh and dry weights through increasing leaf area; silica increases leaf fresh and dry weights through its effect on nitrogen uptake and carbon assimilation (Anser *et al*., 2012). In the present work, the increases in fresh and dry weights in gerbera leaves due to silica application are consistent with other researchers conducted on gerbera (da Silva *et al*., 2020; Kamenidou *et al*., 2010). In one study, Mohammed and Abood (2020) reported increases in fresh (36%) and dry (34%) weights of gerbera leaves due to Ca-chelate application in the nutrient solution.

Results indicated that even though applying silica significantly increased root fresh and dry weights, Ca-chelate application was more effective on these characteristics; accordingly, the heaviest fresh and dry leaves were observed in the treatment with 60 mg L^{-1} Ca-chelate (Table 2). The addition of silica to the root zone or its foliar application increases hairy root area (Ma *et al*., 2001). Thus, higher fresh and dry weights can be expected. Furthermore, plants treated with silica are of a higher photosynthesis rate, fresh weight of stem and leaf and higher efficiency in shoots. Lux *et al*. (2002) and Hattori *et al*. (2005) demonstrated in their studies that silica played a crucial role in root growth and water movement from rhizosphere to roots. Our results regarding the increases in root fresh weight were in accordance with those obtained in *Lisianthus* (Kamiab *et al*., 2017) and gerbera (da Silva *et al*., 2020; Dissanayake *et al*., 2017). Concerning the calcium effect on root growth, it is reported that calcium is indirectly essential for root growth, and its role is developing the primary and lateral meristems increasing plant fresh and dry weights (Schulze, 1983). Abdolmaleki *et al*., (2015) investigated the effect of calcium in the nutrient solution on dry and fresh weights of rose roots and indicated that compared to the control, applying calcium chloride increased root fresh (12%) and dry (13%) weights. El-Serafy (2015) stated that the joint application of 100 mg L^{-1} silicon and 150 mg L^{-1} calcium in the nutrient solution improved vegetative traits, such as peduncle length, stem length, flower diameter, stem diameter, fresh and dry weights of leaf, root and petal, and flower longevity in cloves.

Treatments with naoparticle-SiO_2 and Ca-chelate increased leaf area, so that the widest leaf area was observed in the treatment including 20 mg L^{-1} nanoparticle-SiO_2 and 60 mg L^{-1} Ca-chelate (Fig. 1.). Increasing the RuBisCo activity and the leaf chlorophyll content, silica leads to higher photosynthesis rates and photosynthate accumulation in plants, thereby stimulating plant cell division and increasing leaf area (Cui *et al*., 2010; Mohammadi *et al*., 2019). Kamenidou *et al*. (2010) reported that the use of silica in the hydroponic production of ornamental sunflower and gerbera resulted in wider leaf area. In some ways, calcium is an essential nutrient playing a key role in cell division and elongation and therefore in leaf area expansion. Mohammed and Abood (2020) reported that the addition of 500 mg L^{-1} Ca-chelate to the nutrient solution of gerbera increased leaf area (437 cm^2), which was 87% more than the leaf area in the control.

A reduction was observed in leaf electrolyte leakage in all the treatments. In the joint treatments of nanoparticle-SiO_2 and Ca-chelate, treatments with a higher amount of Ca-chelate showed lower leaf electrolyte leakage rates (Table 3). Calcium accumulation in plant tissues enhances a polymeric connection between middle lamella of pectocellulose membrane, which strengths cell wall network and results in higher mechanical strengths of tissues and less electrolyte leakage (Mortazavi *et al*., 2007). The reduction in leaf ionic leakage has been reported in the leaves of wheat (Tian *et al*., 2015) and barely (Kabiri and Naghizadeh, 2015). On top of that, the addition of silica to the nutrient solution mitigates the abovementioned stress and improves membrane permeability through precipitating Si on cell wall, thereby reducing cell wall destruction and ionic leakage (Kaya *et al*., 2006). Results of several studies are consistent with our findings and demonstrate a reduction in leaf ionic leakage owing to the application of silica (Kaya *et al*., 2006; Reezi *et al*., 2009).

Cut flowers are among the flowers sensitive to gravity; during preserving cut flowers, flower buds bend due to gravity and block water path to buds and semi-open flowers and accelerates their wilting. Resistance to geotropism depends on stem strength and turgor due to solution absorption rate (Li *et al*., 2012; Zhang *et al*., 2013; Nazaralian *et al*., 2017). The application of nanoparticle-SiO_2 and Ca-Chelate increases water uptake, resulting in heavier flowers and leaves and less electrolyte leakage from leaves. Furthermore, it improves stem strength and lignification through increasing cellulose and lignin in stems, leading to less stem bending. Results indicated that compared to the control, the interaction of nanoparticle-SiO_2 and Ca-chelate significantly increased cellulose, hemicellulose and holocellulose contents and decreased stem bending (Table 3, Fig. 2).

In fact, silica precipitates on cell walls and increases the synthesis of phenolic compounds and lignin and their precipitation on cell wall. Cell wall strength in account of silica might be attributed to the biosynthesis of phenolic compounds (Zhang *et al*., 2013). Plant cell wall is composed of polysaccharides, such as cellulose, pectin, and hemicellulose. Silica significantly increases cellulose, hemicellulose and finally lignin content. In cell wall, silica serves as a ring between lignin and carbohydrates. Therefore, utilizing silica creates more lignin-carbohydrate complexes on epidermal cell walls. Thus, in addition to Si precipitation on lignified cell walls, silica influences plant stem bending through regulating lignification (Li *et al*., 2012). Nazaralian *et al*. (2017) suggested that silica was effective in lignification, creating cellulose, hemicellulose and antioxidant enzymes. Additionally, calcium accumulation in plant tissues enhances a polymeric connection between middle lamella of pectocellulose membrane strengthening cell wall network and result in higher mechanical strengths of tissues and less stem bending (Gerasopoulos and Chebli, 1999). Aghdam *et al*. (2019) stated that employing calcium significantly increased lignin, cellulose and hemicellulose contents in two gerbera cultivars (Intense and Rosaline), which decreased stem bending compared to the control.

The highest activity of phenylalanine ammonia-lyase in leaf belonged to the treatment with 60 mg L^{-1} Ca-chelate and the treatment with 20 mg L^{-1} nanoparticle-SiO_2. However, combined applications of Ca-chelate and nanoparticle-SiO_2 were significantly effective (Fig. 3). A possible reason of higher PAL activity (which is the most important enzyme in the phenyl-propanoid cycle) due to Si can be owing to positive effects of Si on plant resistance, which is the result of increasing antioxidant power and capacity. This is in line with the results of Edrisi *et al*. (2019) and Ranjbar *et al*. (2017) in gerberas indicating the positive effect of silicon on the activities of antioxidant enzymes, including catalase, peroxidase and superoxide dismutase. Additionally, calcium is significantly effective on some of the plant physiological processes, like enzymatic activities. Free radicals are composites or free and unpaired unstable electrons, and calcium as a cation can provide the positive charges needed to neutralize free radicals, reduce the negative effects of free radicals and contribute to cell antioxidant activity (Jiang and Huang, 2001). Consistent with these results, Siddiqui *et al*. (2012) reported increases in the activities of antioxidant enzymes, including catalase as a result of calcium application. Calcium serves as a secondary messenger of environmental stimuli and stimulates pseudo-calmodulin proteins reacting to calcium. The changes in the structure of calmodulin proteins, as a result of binding with calcium ions, provide a series of mechanisms, for instance ion transport, gene expression, cell motility, cell growth, cell proliferation and its stress resistance through activating antioxidant systems (Jiang and Huang, 2001). Therefore, applying appropriate amounts of calcium, plant resistance to environmental stress, like stem bending, could be improved. Nazari Deljou and Gholipour (2013) exhibited that phenylalanine ammonia-lyase was an effective factor on lignification, increasing stem strength.

The highest chlorophyll index was observed in the combined treatment with 80 mg L^{-1} nanoparticle-SiO_2 and 60 mg L^{-1} Ca-chelate followed by the treatment with 20 mg L^{-1} nanoparticle-SiO_2 and 60 mg L^{-1} Ca-chelate (Table 3). Regarding the importance of silicon for the RuBisCo function in plant leaves, this enzyme increases the plant efficiency in carbon dioxide fixation, and thus improves plant chlorophyll content and photosynthesis (Mohaghegh *et al*., 2010). In this regard, it has been reported that calcium can increase plant chlorophyll content and photosynthesis through enhancing stomatal conductivity and RuBisCo activity in the treated plants (Tan *et al*., 2011). Mohammed and Abood (2020) studied the effects of Ca-chelate in the nutrient solution on the total chlorophyll content of gerbera and found that compared to the control, applying 500 and 1000 mg L^{-1} Ca-chelate increased the total chlorophyll content by 17 and 52%, respectively. Abdolmaleki *et al*. (2015) suggested that compared to the control, the addition of 0.75% and 1.5% calcium chloride to the nutrient solutions of rose cut flowers increased the total chlorophyll content by 12% and 17%, respectively.

According to the results of the current research, the highest maximum efficiency of photosystem II (F_V/F_m) was observed in the treatments having both Ca-chelate and nanoparticle-SiO_2 (Table 3). Feng *et al*. (2009) reported that the application of silica in the nutrient solution for cucumber production increased the photosynthesis and maximum efficiency of photosystem II (F_V/F_m). Thus far, there is no clear evidence on how silicon and efficiency of photosystem II are related, but silicon is probably capable of facilitating the exchange between PS I and PS II by increasing the absorption of some nutrients like iron, thereby enhancing maximum efficiency of photosystem II (F_V/F_m) (Al-aghabary *et al*., 2005). The effects of calcium application on improving the maximum efficiency of photosystem II (F_V/F_m) in tomato (Sakhonwasee and Phingkasan, 2017) and *Eustoma* (Chen *et al*., 2018) were reported.

CONCLUSION

It can be concluded that the application of nanoparticle-SiO_2 and Ca-chelate can be useful for plant growth properties in different ways. Herein, the treatments including nanoparticle-SiO_2 and Ca-chelate improved the vegetative and biochemical properties of gerbera (*Gerbera jamesonii* L.). In the treatments only with one of Ca-chelate or nanoparticle-SiO_2, the treatment with 80 mg L^{-1} nanoparticle-SiO_2 and the treatment with 60 mg L^{-1} Ca-chelate were among the most effective treatments. It can be noticed that among all the treatments, combined treatments of nanoparticle-SiO_2 and Ca-chelate were more effective than their individual applications were. The treatment with 40 mg L^{-1} nanoparticle-SiO_2 and 60 mg L^{-1} Ca-chelate could be recommended in hydroponic nutrient solutions to raise gerbera.

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