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ORIGINAL ARTICLE

Accelerating the growth and flowering of *Eustoma grandiflorum* by foliar application of nano-ZnO and nano-$CaCO_3$

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Abstract One of the important goals of lisianthus (cut flower) producers is to reduce the growth period and increase the quality of the flower. The current research aimed to investigate the effects of foliar application of nano ZnO and nano $CaCO_3$ on growth and flowering of lisianthus (*Eustoma grandiflorum* cv. Mariachi Blue). The experiment was carried out in greenhouse condition by spraying various concentrations of nano ZnO (3, 6 and 9 mg L^{-1}) and nano $CaCO_3$ (250, 500 and 750 mg L^{-1}) every 20 days. According to the results, foliar spraying of nano ZnO (6 mg L^{-1}) on lisianthus increased number of leaf and lateral branches, leaf chlorophyll content and petal anthocyanin content. Nano ZnO spray also increased number of flowers. The results demonstrated that the plants sprayed with 500 mg L^{-1} nano $CaCO_3$, entered the flowering stage earlier and flowered about 15 days earlier than the control plants, while foliar spraying of nano ZnO delayed the flowering time. Foliar spraying during the growth period with nano $CaCO_3$ (500 mg L^{-1}) increased the number of flowers per plant. Also, the number of flowers was 56.3% higher than the control treatment. An increase in plant size was observed with the use of nano $CaCO_3$. Highest flower diameter, plant height and leaf length in lisianthus were obtained by foliar spraying of nano $CaCO_3$. The present study showed that calcium carbonate and zinc oxide nano fertilizers have a significant effect on growth characteristics and flowering quality of lisianthus.

Keywords Flower size · Flowering time · Nano-fertilizer · Nutrition · Photosynthetic pigments

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Introduction

Eustoma grandiflorum is one of the most beautiful cut flowers, which is native to the central and southern regions of the United States (Ohkawa et al. 1991). This plant has several types including annual, biennial or perennial plant, and also, short and tall forms. Tall forms are used to produce cut flowers and shorter forms are used for planting in pot or garden. Lisianthus is a slow-growing plant which is one of the main problems with its production. It takes 50–140 days from seed germination to the stage where seedlings grow 2–3 pair of leaves (Harbaugh 1995). It takes 22–28 weeks to reach the flowering stage (Halevy 1984). The long period from planting of seed to flowering is a major problem for growers of lisianthus.

The development of nanotechnology and production of nanoparticles at the nanoscale (≤ 100 nm in at least one dimension) enhanced delivery of agrochemicals and agricultural productivity (White and Gardea-Torresdey 2018; Patle et al. 2018). With the use of nano fertilizers instead of conventional fertilizers, nutrient elements would be released and controlled gradually in the soil. Therefore, the plant would be able to absorb the highest amount of nutrient, while reducing the elements leaching. Also, the yield of the product would be increased (De Rosa et al. 2010). Tremendous achievements such as reducing energy consumption, saving production costs and avoiding environmental problems could be achieved through the use of nanotechnology to optimize the formulation of chemical fertilizers (Shehata et al. 2016). The conversion of materials to nano-scales would change their physical, chemical, biological and catalytic activities (da Silva et al. 2011). Proper fertilization and nutrition would accelerate the growth and thus increase the quality of plant. Each element has a distinct role in the physiological processes of the plant. Plants are able to absorb nutrients through their leaves. The amount of adsorption through foliar application depends on the type of leaf tissue, the composition and thickness of the cuticle, stomatal densities and trichrome type at the leaf surface (Sturikova et al. 2018).

The presence of zinc (Zn) as one of the important micronutrients in the plant results in protein synthesis, photosynthesis process improvement, chloroplast development and cell division (Sarwar et al. 2013; Sturikova et al. 2018). It also plays a very important role in regulating ion balance in plant (Vazin 2012). The role of Zn in the synthesis of plant hormones, such as Auxin, Abscisic acid, Gibberellin and Cytokinin, is vital. Also, one of the other effects of Zn is increase in the levels of antioxidants in plant tissues (Saeed et al. 2013). Therefore, Zn deficiency prevents the proper functioning of enzymes and photosynthesis. In order to improve the quality of *Lilium* flower, the effects of Zn at four levels: 0, 3, 6 and 9 mg L^{-1}, were investigated. The results showed that Zn could regulate antioxidant activity and increase the postharvest life of lilium cut flower. Furthermore, the results demonstrated that the best level of Zn used, was 6 mg L^{-1} which had the highest effect on flower characteristics such as flower diameter, number of flowers per plant, stem length and stem diameter (Shaheen et al. 2015). With foliar application of zinc sulfate ($ZnSO_4$) on Iris (Iridaceae) plant, similar effects were observed (Khalifa et al. 2011). Foliar application of $ZnSO_4$ on *Pisum sativum* leaves led to increase in the number of flowers, pods and seeds (Pandey et al. 2013). Increase in flower number and flower yield in *Rosa damascena* was observed after foliar spray with $ZnSO_4$ (Kumar et al. 2016). Favorable effects of nano-form of ZnO on growth of rice seedlings was reported (Pavithra et al. 2017; Singh et al. 2018). Definite concentration of nano-form of ZnO (5 g L^{-1}) can improve the growth and yield characteristics of *Oryza sativa* plants (Bala et al. 2019). Also, Zinc oxide nanoparticles reduced the oxidative stress in wheat (Rizwan et al. 2019) and promote yield in sorghum under drought stress (Dimkpa et al. 2019). Calcium (Ca^{2+}) is an essential macronutrient that controls the growth and development of plants (Hepler 2005; Sharma et al. 2017). Its role in improving and increasing yields in plants, nitrate absorption (usable nitrogen form), transpiration rates, photosynthetic parameters, stomatal conductance and chlorophyll content has been confirmed (Savithramma 2004; Savithramma et al. 2007; Liu et al. 2005; Dolatabadian et al. 2013). Calcium accelerates plant growth simultaneously to strengthen the structure of the flower stem. In addition, it increases the resistance of plants to bacterial and viral diseases (Ustun et al. 2007). Little research has been reported on nano-calcium carbonate in plants (Hua et al. 2015). The effect of nano calcium carbonate at four levels (0.5, 1, 2 and 3 g L^{-1}) on tomato was investigated. The results demonstrated that nano calcium carbonate at a concentration of 0.5 g L^{-1} significantly reduced salinity effect, increased fruit yield and improved nutritional status (Tantawy et al. 2014). Foliar spraying of calcium on sugarcane leaves delayed flowering and reduced the number of flowers (Endres et al. 2016). Likewise, the use of calcium played a role in increasing the diameter and plant height, and increased the number of flower buds and the flower diameter of lisianthus (Hernández-Pérez et al. 2016). Little is known about effects of nano fertilizers on growth characteristics and flower quality in ornamental plants. Therefore, the aim of this study is to investigate the effects of nano fertilizers on the growth and flowering of lisianthus. Also, to determine the best concentration of nano $CaCO_3$ and nano ZnO for improvement of the growth parameters, as well as increase in the flowering quality and longevity of lisianthus flower.

Materials and methods

Seed germination conditions

For the present study, F_1 seed of *Eustoma grandiflorum* cv. Mariachi Blue was used. The seeds were cultivated in trays containing peat and perlite in a ratio of 1:1. Due to the fact that lisianthus seeds are sensitive to drought stress and high humidity, irrigation was done with extra care. The seeds germinated after 15 days under controlled greenhouse conditions.

Nano ZnO preparation

Nano ZnO was purchased from Mehregan Chemistry Company in Iran. To prepare the required concentrations, the desired amount of nano ZnO was weighted and dissolved in 1000 mL of distilled water. The obtained solution was placed in ultrasonic apparatus (40 kHz, 100 W) for 40 min. Also, to prepare non-nano zinc oxide, due to its insolubility in water, first, it was dissolved in few drops of HCl as solvent to reach the volume by distilled water.

Nano $CaCO_3$ preparation

Nano $CaCO_3$ and non-nano $CaCO_3$ were purchased from Mehregan Chemistry Company in Iran. To prepare the required concentrations, the desired amount of substances was weighted and suspended in 1000 mL of distilled water.

Plant growth conditions and application of treatments

At first, pots with 14 cm diameter were prepared. The pots were filled with a ratio of 1:1:1 peat, perlite and coco-peat. Then the seedlings, which reached the stage of 2–3 pairs of leaves, were carefully transferred to the pots so that the roots are not damaged. All the plants were exposed to the same lighting and temperature in the greenhouse. In order to eliminate the possible environmental effects, pots were randomly swapped during the growth period. Foliar spraying with nano-fertilizers continued every 20 days for 80 days on all aerial parts of the plants until the vegetative stage was completed (Fig. 1). The treatments consisted of nano ZnO at three concentrations of 3, 6 and 9 mg L^{-1} and nano $CaCO_3$ with three concentrations of 250, 500 and 750 mg L^{-1}. Non-nano zinc oxide, 6 mg L^{-1}; non-nano calcium carbonate, 500 mg L^{-1} and distilled water were used for the control treatments. It should be noted that Zn is one of the essential micronutrients and needed in small amounts for plants, while Ca as a macronutrient required in greater quantities.

Evaluated characteristics

During the experiment, different traits including number of leaves, number of flowers, flower diameter, flowering time (number of days from transplanting to opening of first flower), number of branching of the stem, plant height, leaf length, longevity of first flower (from flower opening to petals fading) and fresh weight of the plant were measured. The contents of chlorophyll a, and chlorophyll b were measured according to the method of Lichtenthaler and Buschmann (2001). All operations were carried out at 4 °C and acetone with 20% water selected as the solvent. Briefly, 0.2 g of leaf samples were ground to a fine powder in liquid nitrogen. 3 mL 80% acetone was added and the mixture was incubated on ice for 30 min with the dark condition. After centrifugation (4 °C, 6000 g, 10 min), the absorbance of the supernatant at 663 and 647 nm were measured by spectrophotometer (Analytik Jena AG-Specord 50). The amount of chlorophyll content was calculated using the following equations: Chlorophyll ‘a’ = (12.25 A_{663}) − (2.79 A_{647}) and Chlorophyll ‘b’ = (21.50 A_{647}) − (5.10 A_{663}). The final concentrations of each chlorophyll were expressed in µg mg^{-1} leaf fresh weight.

The content of anthocyanin pigment in petal measured by acidic solvent (1% HCl in methanol) as described by Giusti and Wrolstad (2001). Briefly, 0.2 g of petal samples were ground to a fine powder in liquid nitrogen in 3 mL 1% HCl-methanol and incubated for 24 h at 4 °C in the dark. After centrifugation (4 °C, 10000 g, 15 min) the supernatant was collected for the measurement of anthocyanin. Two dilutions of the supernatant prepared, one for potassium chloride buffer, pH 1.0, and the other for sodium acetate buffer, pH 4.5. After a 15 min equilibrates of solutions, the absorbance of each dilution at 530 and 700 nm were measured by spectrophotometer. The absorbance of the diluted sample (A) calculated using the following equations: A = ($A_{\lambda\ vis\text{-}max}$ − A_{700}) pH $_{1.0}$ − ($A_{\lambda\ vis\text{-}max}$ − A_{700}) pH $_{4.5}$. Finally, the anthocyanin content was calculated in mg g^{-1} of petal fresh weight.

Statistical analysis

The experiment was completely randomized design with nine treatments and three replications. Each replication included 8 pots. One-way analysis of variance (ANOVA) was performed using SAS (9.2) software. To compare means, Tukey’s test at $P \leq 0.05$ level was used. Graphs were plotted in Excel.

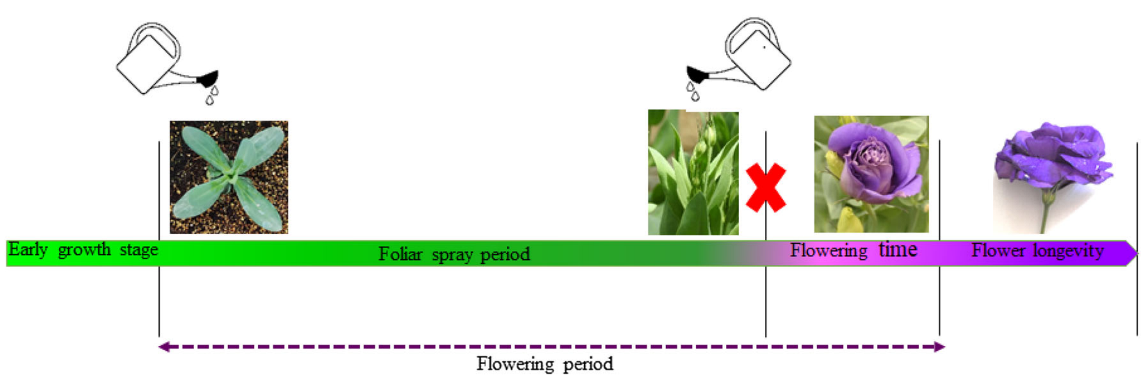

Fig. 1 Time line of experiment process that providing a schematic diagram to describe different stages of foliar spraying and flowering of *Eustoma grandiflorum* cv. Mariachi Blue

Results and discussion

Flowering time

The results showed that zinc and calcium carbonate as nano-form have a significant effect on the flowering time of lisianthus (Table 1). Based on the results, the minimum time required from transplanting to flowering stage was observed for treatment of 500 mg L^{-1} nano $CaCO_3$ with a mean of 277.4 days. Therefore, using this concentration during the growth period made the lisianthus to enter flowering stage about 15 days earlier. With increase in concentration of nano $CaCO_3$ to 750 mg L^{-1}, no acceleration was observed at flowering time (Fig. 2). On the other hand, the longest flowering time was observed in the treatment of 6 mg L^{-1} non-nano zinc oxide with a mean of 297.3 days, which entered the flowering stage 14.5 days later than the control plants. In fact, zinc oxide (whether nano or non-nano) caused a delay in flowering. The period of growth and planting in the greenhouse is an important commercial and economic issue. Identifying the factors that contribute to the quality of plant in reducing the period of planting time to flowering is accepted by the growers of ornamental plants. Calcium carbonate acts as a reservoir for CO_2 storage in plants. Generally, the increase in plant growth due to application of calcium fertilizer during the growth period is due to the effect of this element on photosynthesis. Therefore, the mechanism of calcium carbonate for accelerating the flowering of lisianthus can be related to CO_2 storage and its role in photosynthesis. Due to the increase in the rate of photosynthesis and the shortening of the vegetative growth period, the plant quickly enters the reproductive phase and flowering stage. The findings of the current study demonstrated that plant access to calcium during the growth period accelerates the flowering of lisianthus (about 15 days earlier). Dissimilarity, Enders et al. (2016) found that foliar application of calcium sulfate would lead to delay of flowering time of sugarcane up to 3 weeks and a 55% decrease in flowering. On the other hand, the positive effect of calcium spray on the reduction of time to flowering of *Origanum vulgare* was reported and results similar to the current study were obtained (Dordas 2009).

Flower parameters

The results showed that the effect of nano ZnO and nano $CaCO_3$ on flower number, flowers diameter and flower longevity was significant (Table 1). According to the results, foliar application of 500 mg L^{-1} nano $CaCO_3$ caused to highest number of flowers (10.2) and maximum flower diameter (76.49 mm) The results showed that non-fertilized plants had the lowest number of flowers per plant (4.46 flower), which had no significant difference with non-nano zinc oxide at 6 mg L^{-1} and nano ZnO at 3 and 9 mg L^{-1}. Also the smallest flowers (57.86 mm) were observed in the control treatment (Table 1). According to the results, nano $CaCO_3$ played a more effective role in increasing the longevity of flowers per plant than nano ZnO. The highest longevity of flowers was shown by two treatments: 500 and 750 mg L^{-1} nano $CaCO_3$ with a mean of 12.83 and 12.2 days, respectively. The plants that were foliar sprayed with 6 mg L^{-1} non-nano zinc oxides faded much earlier than other treatments and only survived for 6.75 days (Table 1). Due to the small size and high level of mobility of calcium nanoparticles, they can be moved in apoplastic and symplastic pathways and upward movement through the xylem and provide uniformity in leaves, flowers and

Table 1 Effects of different concentrations of nano $CaCO_3$ and nano ZnO on flowering time and flower properties of *Eustoma grandiflorum* cv. Mariachi Blue

Treatments	Flowering time (day)	Flower number	Flower diameter (mm)	Flower longevity (day)
Control	292.2bc	4.46d	57.806e	8.83 cd
500 mg L^{-1} $CaCO_3$	281.80f	6.65bc	68.22bc	10.34b
250 mg L^{-1} nano $CaCO_3$	289.66 cd	6.84bc	69.39b	9.63bc
500 mg L^{-1} nano $CaCO_3$	277.38g	10.20a	76.49a	12.83a
750 mg L^{-1} nano $CaCO_3$	285.35f	7.20b	70.80b	12.20a
6 mg L^{-1} ZnO	297.34a	4.18d	63.22d	6.75e
3 mg L^{-1} nano ZnO	294.74ab	5.29d	62.47d	8.17d
6 mg L^{-1} nano ZnO	288.78d	9.09a	71.70b	10.76b
9 mg L^{-1} nano ZnO	294.16b	5.77bcd	65.14cd	7.87de
Significance	**	**	**	**
CV (%)	0.29	7.36	1.53	3.77

Values within column that followed by the different letters indicates statistical difference at $P < 0.01$. **, showed probability level of 1%

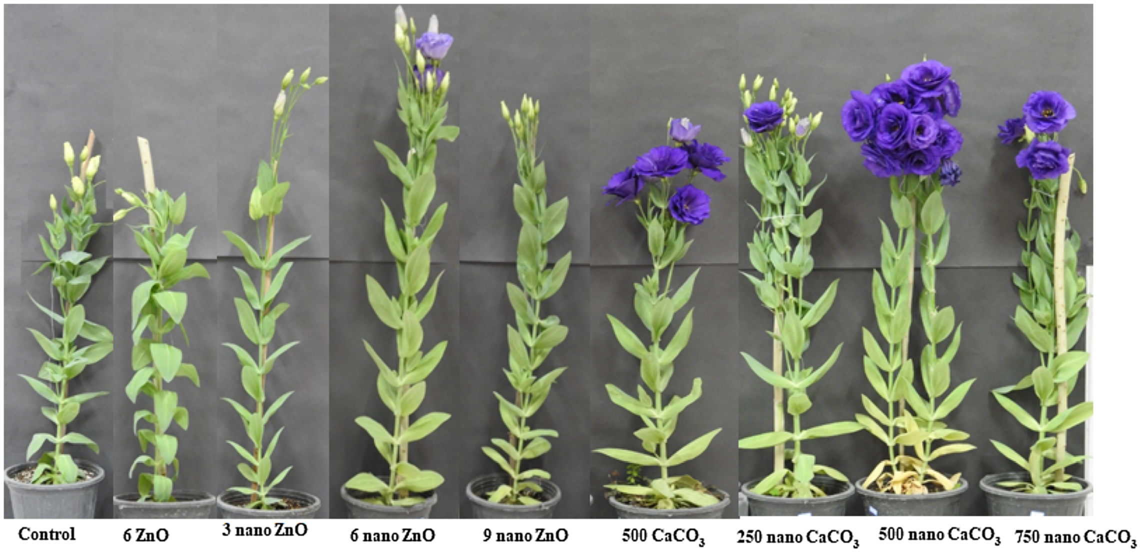

Fig. 2 Effect of foliar application of nano zinc oxide and nano calcium carbonate with various levels during vegetative phase on flowering time, number flower and plant height in *Eustoma grandiflorum* cv. Mariachi Blue

other tissues (Yang and Jie 2005). Therefore, this can justify the effective role of nano $CaCO_3$ in increasing flower and plant size. Based on the results, longevity of flowers in plants which were foliar sprayed by nano $CaCO_3$, were higher. Calcium is an important element in increasing and improving the quality of cut flowers and plays an important role in delaying the senescence process and increasing the longevity of cut flowers. Some of these mechanisms have an effect on the protection of membrane proteins and phospholipids from degradation, preserving the integrity of the membranes, reducing ethylene production, and delay petal senescence (Torre et al. 1999). Based on the findings of the present study, the presence of Zn in the form of nano in concentration of 6 mg L^{-1} played a favorable role in increasing the number of flowers and doubling the number of flower on plant. On the other hand, the results showed that increase in the concentration of nano ZnO did not increase the number of flowers per plant. The results of the study on the effect of Zn fertilizer on the number of *Lilium* flowers showed that this element had a favorable effect on the number of flowers, which is consistent with the results of the present study (Shaheen et al. 2015).

Growth characteristics

According to the results, all applied fertilizers positively affected on the plant height, leaf number, leaf length and branches number of lisianthus plants (Table 2). The highest plant height and leaf length were obtained with nano $CaCO_3$ at a concentration of 500 mg L^{-1}, which respectively were about 18 cm higher and 18 mm larger than the control plant (Table 2). Plants sprayed with 6 mg L^{-1} nano ZnO were in the next rank (Fig. 2). All the treatments led to increase in the number of leaves. The highest number of leaves and branches were related to 6 mg L^{-1} nano ZnO treatment (40.64 and 7.35 leaf, respectively). Generally, foliar application of nano $CaCO_3$ also had an increasing effect on leaves number but less than that of nano ZnO spray. Also nano $CaCO_3$ treatment, except in 500 mg L^{-1} concentration, had no significant effect on number of branches (Table 2). Zinc and calcium carbonate nano-fertilizers had different effects on fresh weight of the plant. Fresh weight of the plants that were foliar sprayed with calcium carbonate in all concentrations (nano and non-nano) increased (Table 2). The maximum fresh weight of plant belonged to 6 mg L^{-1} nano ZnO treatment (71.56 g) but other zinc treatments didn't have significant effect on fresh weight (Table 2). One of the main criteria for the expression of plant quality, especially cut flowers, is stem length. The stem strength should be sufficient to withstand the weight of the leaves and flowers in inflorescence when flower is placed in a pot or vase, and to support them well. In fact, increase in the photosynthesis process due to the use of calcium carbonate nano-fertilizers is associated with available higher levels of CO_2. Therefore, photosynthetic materials are introduced into different plant sites, including the stems, and ultimately, the plant height increases (Rebbeck and Scherzar 2002; Kumar and Haripriya 2010).

Zn is an essential element for the plant, which plays an important role in the stability of the membrane structure. Protein synthesis, elongation of the cell and tolerance of environmental stresses are the effect of this element on the plant (Marreiro et al. 2017; Rosrami Fard et al. 2012).

Table 2 Effects of different concentrations of nano $CaCO_3$ and nano ZnO on growth characteristics of *Eustoma grandiflorum* cv. Mariachi Blue

Treatments	Plant height (cm)	Leaf number	Leaf length (mm)	Branches number	Plant fresh weight
Control	47.29e	23.35e	72.45e	3.52d	39.4cd
500 mg L^{-1} $CaCO_3$	52.72d	27.75c	79.77c	4.68bcd	56.59abc
250 mg L^{-1} nano $CaCO_3$	54.46cd	26.6cd	85.73b	4.1cd	53.86abc
500 mg L^{-1} nano $CaCO_3$	65.43a	32.93b	90.64a	5.95ab	66.42ab
750 mg L^{-1} nano $CaCO_3$	57.05c	25.78cde	86.64ab	4.61bcd	61.51abc
6 mg L^{-1} ZnO	52.4d	23.69e	72.73e	3.54d	29d
3 mg L^{-1} nano ZnO	53.16d	33.4b	78.29cd	5.85ab	43.46bcd
6 mg L^{-1} nano ZnO	60.85b	40.64a	87.02ab	7.35a	71.56a
9 mg L^{-1} nano ZnO	56.76c	35.18b	75.34de	5.52bc	42.04cd
Significance	**	**	**	**	**
CV (%)	1.82	2.85	1.51	9.46	12.95

Values within column that followed by the different letters indicates statistical difference at $P < 0.01$. **, showed probability level of 1%

The enzymes that are activated by Zn are mainly involved in the metabolism of carbohydrates, maintaining and integrating cell membrane structure, protein synthesis, regulation of the synthesis of auxin and the formation of pollen and increased fertilization (Hafeez et al. 2013; Moreira et al. 2018). Also, Zn plays a role in nitrogen metabolism and stimulates plant growth (Maurya and Kumar 2014). In fact, Zn led to an increase in vegetative growth and ultimately improved the size, weight and quality of the plant. The study results are in accordance with the results of foliar application of zinc sulfate and zinc nanoparticles that positively affected the fresh weight of *Coffea arabica* (Rossi et al. 2019). Based on the results, nano ZnO at a specific concentration of 6 mg L^{-1} had a favorable effect on plant height, which could be due to Zn application and its effect on increasing cell division and elongation. The number of branches in lisianthus in addition to marketability is a good criterion for full flower of stem. Improvement of the appearance and marketability of this flower would be obtained through increase in the number of flowering branches which increases the number of flowers per plant. Based on the results of this study, foliar application of 6 mg L^{-1} nano ZnO increased the number of branches in comparison with the control plants. Zn is one of the important factors that involved in tryptophan biosynthesis. The tryptophan is an amino acid acts as a precursor of indole-3-acetic acid (IAA) (Moreira et al. 2018). Thus increasing the auxin formation, as a result, increasing longitudinal growth and number of shoots. The presence of Zn in the meristem areas increases plant vegetative growth due to its efficacy in producing auxin hormone, cell division and elongation of the cell. All these factors are effective in increasing growth parameters such as number and size of leaves, plant height and number of lateral branches, thereby increasing the yield of the plant (Broadley et al. 2007; Moreira et al. 2018).

Pigments of leaf and flower

All fertilizers significantly affected photosynthetic pigments as well ($P \leq 0.01$). Based on the results, all treatments increased chlorophyll 'a' and total chlorophyll compered to control. Among $CaCO_3$ treatments, 500 and 750 mg L^{-1} nano $CaCO_3$ resulted in marked reduction in chlorophyll 'b' while 6 mg L^{-1} nano ZnO showed highest chlorophyll 'a' and 'b' levels in comparison to other treatments (Table 3). According to the results, foliar application of nano $CaCO_3$ and nano ZnO at vegetative growth stage significantly affected the total anthocyanin content in lisianthus petal (Table 3). In 6 mg L^{-1} nano ZnO (99.6 mg g Fw^{-1}) and 500 mg L^{-1} nano $CaCO_3$ (91.67 mg g Fw^{-1}) treatments, anthocyanin content was almost doubled while it was significantly lower in 9 mg L^{-1} nano ZnO (39.3 mg g Fw^{-1}) and was similar to control (39.75 mg g Fw^{-1}).

Chloroplasts participate in the uptake of Ca^{2+} for regulation of NAD kinase (Hepler 2005). The effect of calcium on leaf number, as mentioned earlier, is related to increased access to CO_2, which increases the chlorophyll content, improves plant yield and increases leaf length and number. In this research the highest content of chlorophyll was obtained with 6 mg L^{-1} nano ZnO treatment (Table 3). Zn, due to the increase in photochemical reduction rates, has an effect on the activity of the chloroplast structure, increase in the transfer of photosynthetic electrons, and, eventually, increase in the amount of photosynthesis. With increase in photosynthesis, chlorophyll concentration and stomatal conduction in the leaf would be affected, which delays the aging process and

Table 3 Effects of different concentrations of nano $CaCO_3$ and nano ZnO on pigments of leaf and flower of *Eustoma grandiflorum* cv. Mariachi Blue

Treatments	Chlorophyll a (μg mg Fw^{-1})	Chlorophyll b (μg mg Fw^{-1})	Total chlorophyll (μg mg Fw^{-1})	Anthocyanin (mg g Fw^{-1})
Control	4.34c	3.17c	7.40e	39.75e
500 mg L^{-1} $CaCO_3$	5.79bc	5.63b	11.95ab	62.02c
250 mg L^{-1} nano $CaCO_3$	5.10bc	5.39b	10.40bcd	62.43c
500 mg L^{-1} nano $CaCO_3$	7.91ab	2.09d	8.25de	91.67b
750 mg L^{-1} nano $CaCO_3$	6.24bc	2.59cd	8.83cde	53.60d
6 mg L^{-1} ZnO	4.64c	7.43a	11.40ab	44.22e
3 mg L^{-1} nano ZnO	5.58bc	3.02c	10.82abc	54.24d
6 mg L^{-1} nano ZnO	9.34a	7.53a	13.27a	99.63a
9 mg L^{-1} nano ZnO	6.73b	3.83c	10.57bcd	39.34e
Significance	**	**	**	**
CV (%)	8.90	8.72	6.87	3.28

Values within column that followed by the different letters indicates statistical difference at $P < 0.01$. **, showed probability level of 1%

increases the leaf area (Farahat et al. 2007). The results are consistent with those of nano ZnO effect on spinach leaf length (Kisan et al. 2015). Also, Singh et al. (2018) reported that nano ZnO (at 50 ppm) was the best treatment for increasing rice seedling growth and improving physiological processes. The level of anthocyanin of lisianthus flowers increases from budding to full flower opening when petals are completely colored (Hashimoto et al. 2002). As before reported, treatment with nano calcium carbonate had a significant effect on the anthocyanin content of lisianthus and increased it compered to control. In fact, accumulation of Ca^{2+} in cells leads to the biosynthesis of anthocyanins (Zhang et al. 2018). The results showed that the use of Zn as nano oxide increases the anthocyanin content and longevity of flowers in lisianthus. The Zn as metal could regulate the anti-oxidative activity of plants which leads to membrane stability and prolongation of the flower longevity (Saeed et al. 2013).

Conclusions

In conclusion, foliar application of nano ZnO and $CaCO_3$ could increase the growth of lisianthus. The highest number of flowers, flower diameter and leaf length were obtained in $CaCO_3$ 500 mg L^{-1} treatment; it also accelerated flowering time. The nano ZnO treatments could not reduce the flowering period but could increase the number of leaves, the photosynthetic pigments, number of branches and the fresh weight of the plant. In fact, spraying of nano ZnO increased the vegetative growth duration of lisianthus. Also, the treatments of 6 mg L^{-1} nano ZnO and $CaCO_3$ 500 mg L^{-1} were the best as compared to other treatments. Generally, due to the gradual release of nano materials and its adaptability with the environment, as well as the importance of lisianthus as a valuable cut flower, foliar application of zinc and calcium nano-fertilizer is a good strategy for plant growth and improve the flower's appearance. More studies are required to investigate the role of these nano-fertilizers in physiological processes of plant and their effect on the activity of enzymes during the growth period.

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Author contributions Conception and design: [Zeynab Roein]; Material preparation, data collection: [Zeynab Seyedmohamadi]; Data analysis [Shirin Rezvanipour]; Writing-original draft preparation: [Zeynab Roein]; Reading and editing the final manuscript: [Zeynab Roein; Shirin Rezvanipour].

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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