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Effect of Cycocel and Uniconazole on Some Morphological and Biochemical Properties of Zinnia

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Abstract
One of the important objectives during the production cycle of ornamental plants is to regulate and control their growth. Hence, application of some plant growth regulators such as uniconazole (UN) and cycocel (CCC) is considered as an efficient method for reaching to this objective. To investigation the effect of uniconazole and cycocel on some morphological and biochemical characteristics of zinnia, a factorial experiment was conducted in a completely randomized design with five replications. Application of UN (0, 5 and 10 mg L^{-1}) and CCC (0, 1000 and 1500 mg L^{-1}) as growth retardants, was considered as the first and their application method (leaf spraying and soil application) was considered as the second factor. The effect of growth retardants on plant height, Time of flowering, peroxidase activity, endogenous gibberellins and leaf area were significant. The lowest plant height was observed in soil application of 10 mg L^{-1} UN. The tallest plant was observed in the control treatment. Growth retardants had significant effects on endogenous gibberellins and leaf chlorophyll contents. Highest activity of peroxidase enzyme was detected in plants treated by 10 mg L^{-1} UN. The highest and lowest numbers of leaves (21.6 and 16.4 leaves, respectively) were counted in control and 5 mg L^{-1} UN, respectively. According to the obtained results, the use of growth retardants is recommended to reduce the height and improve the qualitative and quantitative characteristics of zinnia flower.

Keywords: Enzyme activity, Gibberellin, Ornamental plants, Plant morphology, Growth retardant

Abbreviations: Cycocel (CCC), Uniconazole (UN)

Introduction

Controlling plant size is one of the most important aspects in ornamental plants production. Plant size can be limited by various methods such as genetic control, environmental conditions and use of plant growth retardants (Cowling, 2010).

Common zinnia (*Zinnia elegans*) from the Asteracea family is one of cold-sensitive flower with a wide variety of colors, shapes and flower size (Dole, 1999; Heidari *et al*., 2016). This plant grows up to 76 cm in height. It has solitary flower heads about five across. Zinnia is one of the most important annual flowers, due to the long flowering period (from late spring to mid fall), as well as drought and heat tolerance (Dole, 1999). Zinnias are popular garden flowers; therefore are interested by many gardeners (Dole, 1999). It is planted as a seasonal flower in most green spaces.

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Usually most of the zinnia bushes have tall height, which reduces their beauty. Therefore controlling vegetative growth and decreasing plant height is an important factor in for the ornamental value of common zinnia.

Growth regulators in small amounts can promote, stop, or change the physiological processes of plants. In the meanwhile growth retardants prevent the cellular splitting and growth of the apical meristem, thus the plant does not grow normally and remains short.

Cycocel (CCC) is a growth retardant with a quaternary ammonium compound that prevents gibberellin biosynthesis (Taiz and Zeiger, 2006; Megersa et al., 2018). It has been reported that CCC (2000 $mg \cdot L^{-1}$) inhibits stem elongation in different hibiscus species including *Hibisus radiatus*, *H. coccineus* and *H. trionum*. Furthermore, elevated leaf chlorophyll content in hibiscus (Warner and Erwin 2003), reduced plant height in black iris (*Iris nigricans* Dinsm.) (AL-Khassawneh et al., 2006), increased essential oil yield, vegetative growth and dry matter percentage, and leaf to stem ratio per plant in geranium (*Pelargonium graveolens* L. cv. Bourbon) (Rabbi Angourani et al., 2013) have been reported by CCC application.

Triazoles are the most important compounds of gibberellin biosynthesis inhibitors, which have plant growth regulating ability. These compounds are considered to be the most active inhibitors of growth that prevent stem elongation of the plants. The most important responses of plant to triazoles are reducing internode length and consequently, restrict height of plants. Also, triazole influences some processes such as hormonal balance, photosynthesis and enzyme activity in plants.

Paclobutrazole, uniconazole (UN) and tryptanol are known as the most important triazoles (Kamoutsis et al., 1999; Megersa et al., 2018). Previous studies showed that, use of UN reduces plant height in corn, tulip, hyacinth and caladium (Barrett, 2001; Krug et al., 2005; Schluttenhofer et al., 2011).

Some growth retardants such as ethephon and UN considerably influence flower development, plant height and root length as reported in potted *Hibiscus rosa-sinensis* L. and *Narcissus* cv. 'Ice Follies' (Nazarudin, 2012; Demir and Gursel celikel, 2018). Therefore, the present study aimed to investigate the role of CCC and UN treatments to control height and quantitative and qualitative characteristics of Zinnia plant.

Materials and methods

This study was carried out in a factorial experiment based on completely randomized design with five replications. Different concentrations of UN (0, 5 and 10 mg L^{-1}) and CCC (0, 1000 and 1500 mg L^{-1}) were considered as the first factor (at 7 levels and distilled water was used as control), the second factor was consist of different application methods of the above compounds in two levels (soil application and foliar application).

Seeds of zinnia (*Zinnia elegans* cv. swizzle series) were obtained from an (Bazr co, Iran) and were planted in a cocopeat and perlite mixture (in a ratio of 2 to 1) in 15 cm diameter pots. The pots were placed in a greenhouse with an average day/night temperature of 25 C°/18 C°. The plants at four-leaf stage were treated with different concentrations of UN and CCC (16 mL) in two-leaf growth stages. Leaf number, plant height, root dry weight, root fresh weight, total chlorophyll, total phenol, leaf area, flower diameter and time of flowering were measured at the beginning of flowering stage.

Total chlorophyll content

Fresh leaf tissue (0.5 g) was placed in 8 mL of 100% methanol for 24 h in dark place at 25 °C. Then, the absorbance was measured using a spectrophotometer at 652 and 665.2 wavelengths (porra et al., 1989).

Total phenol content

The total phenol content was measured according to the modified Folin- Ciocalteau assay as gallic acid equivalents. After extraction (5 mg in acetone:water (60:40) v/v) 2 mL of the extracts were mixed with 0.4 mL Folin- Ciocalteau`s reagent (1:2 diluted with water) and 2 mL of 2% Na_2CO_3 was added to the mixture. Also reagent without adding extract was used as control. The samples incubated at room temperature for 30 min, the absorbance of the samples was measured at 725 nm. Gallic acid was used for calibration curve (Tunc-Ozdemir et al., 2009).

Peroxidase activity (POD, EC 1.11.1.7)

For determining of POD activity, 0.5 g of fresh tissue samples were frozen in liquid nitrogen and then ground with 10 mL of extraction buffer [50 mM phosphate buffer, pH 7 containing 0.5 mM EDTA and 2% PVPP (w/v)]. The homogenated samples were centrifuged for 20 min at 15000 × g, and the supernatant was used to determine enzymatic activity. Spectrophotometrically measurement was used to assay POD activity by guaiacol formation in 1 mL of a reaction mixture consisting of 450 µL of 25 mM guaiacol, 450 µL of 225 mM H_2O_2 and 100 µL of crude enzyme (Ghanati et al., 2002).

Endogenous gibberellins

Endogenous gibberellins were quantitatively determined in *Zinnia elegans* leaves using High- Performance Liquid Chromato-graphy (HPLC) according to Koshioka et al. (1983).

Statistical Analysis

Analysis of variance was performed using SAS software and mean comparisons were analyzed by the least significant difference test (LSD). Also for data record and drawing graphs the Microsoft excel software was used.

Results

Time of flowering

The results showed that the effect of growth retardant, application method and their interactions was significant on time of flowering. The shortest time to flowering with the average of 47 d was related to 1000 and 1500 mg L^{-1} CCC in soil application. The longest time to flowering with 61 d was belonged to 5 mg L^{-1} UN in soil application method. In the control treatment, it took 53 d from sowing to the appearance of the first flower bud (Fig. 1).

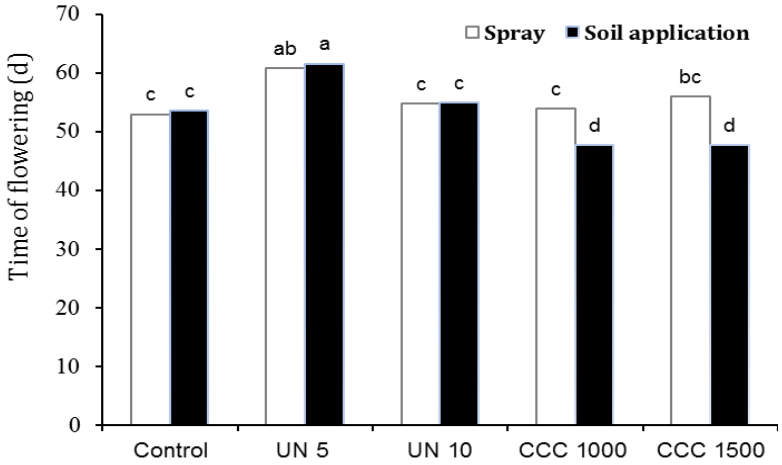

Fig. 1. Interaction of cycocel (CCC; 1000 and 1500 mg L^{-1}) and uniconazole (UN; 5 and 10 mg L^{-1}) with their application method on time of flowering; the columns with the same letters are not significantly different at the 5% probability level.

Number of leaves

The effect of growth retardant on number of leaves was significant at 1% probability level. The application method and their interaction were not statistically significant. The highest and lowest numbers of leaves with 21.6 and 16.4 leaves were observed in control and 5 mg L^{-1} UN, respectively. There was no significant difference between 5 and 10 mg L^{-1} UN (Fig. 2).

Leaf area

The effect of growth retardants, application method and their interactions were significant on the leaf area. The highest leaf area of plants (17 cm^2) was observed in foliar application of 1500 mg L^{-1} CCC. Also, there was no significant difference between leaf area of mentioned treatment and 1000 mg L^{-1} CCC. The lowest leaf area (12 cm^2) was observed in 5 and 10 mg L^{-1} UN (Fig. 3).

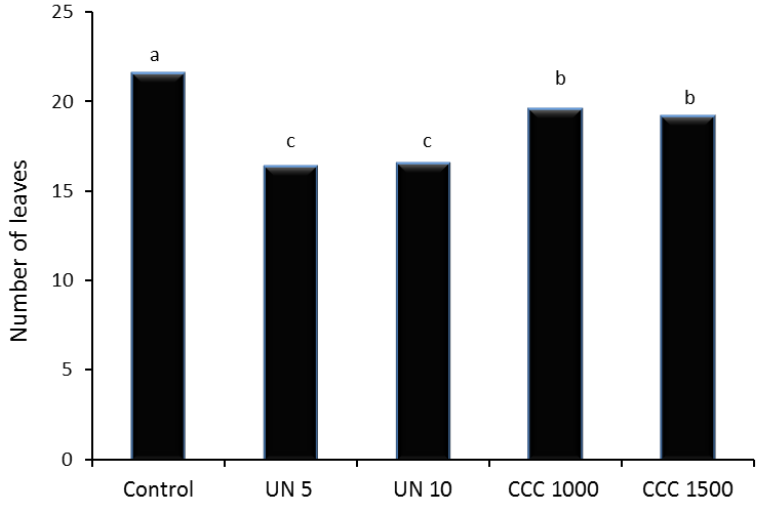

Fig. 2. The effect of different concentration of CCC; 1000 and 1500 mg L^{-1}) and uniconazole (UN; 5 and 10 mg L^{-1}) on number of leaves; the columns with the same letters are not significantly different at the 5% probability level.

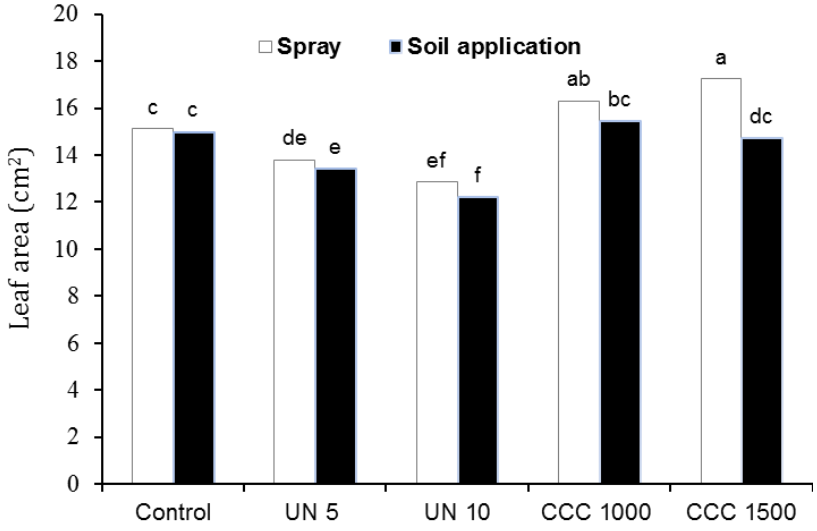

Fig. 3. Interaction of CCC; 1000 and 1500 mg L^{-1}) and uniconazole (UN; 5 and 10 mg L^{-1}) with their application method on leaf area; the columns with the same letters are not significantly different at the 5% probability level.

Phenol and Chlorophyll Contents

Growth retardant treatments had a significant effect on the leaf chlorophyll content at 5% probability level. The highest chlorophyll content (9 µg mL^{-1}) was observed in 10 mg L^{-1} UN, but there was no significant difference with 10 mg L^{-1} UN and 1000 mg L^{-1} CCC (Fig. 4).

Plant height

The effect of growth retardant, the type of application and their interaction was significant on plant height. The tallest plants were observed at control with 57 cm. While the shortest plants (17.6 cm) were observed in soil application of 10 mg L^{-1} UN. There was no significant difference between soil application and foliar application for plant height (Figs. 5, 6).

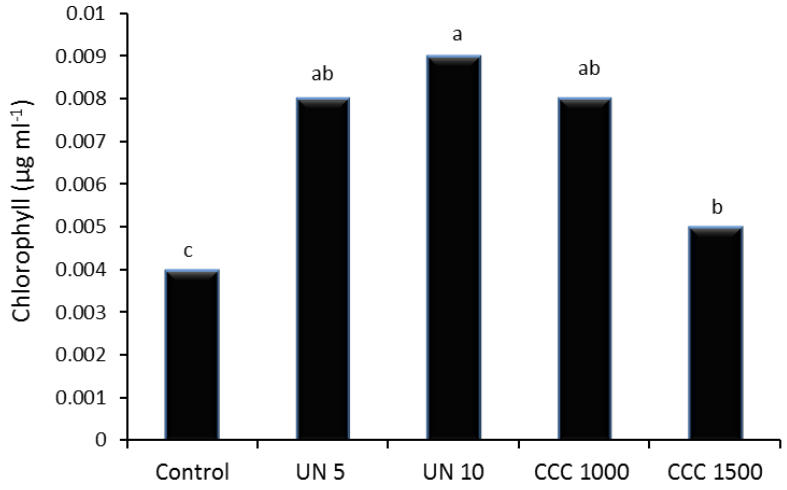

Fig. 4. Effect of different concentrations of cycocel (CCC; 1000 and 1500 mg L^{-1}) and uniconazole (UN; 5 and 10 mg L^{-1}) on chlorophyll content; the columns with the same letters are not significantly different at the 5% probability level.

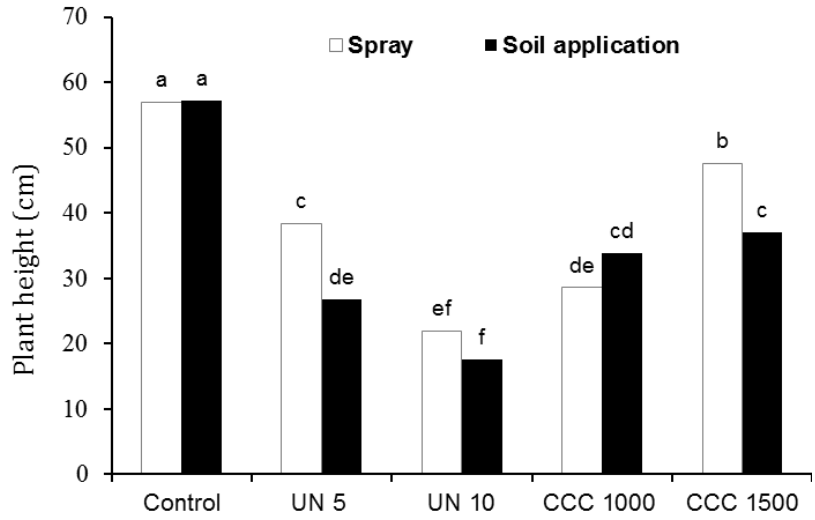

Fig. 5. Interaction of cycocel (CCC; 1000 and 1500 mg L^{-1}) and uniconazole (UN; 5 and 10 mg L^{-1}) with their application method on plant height. The columns with the same letters are not significantly different at the 5% probability level.

Fig. 6. Comparison of plant height in control and uniconazole (UN) (left: 10 mg L^{-1} soil application; middle: 10 mg L^{-1} leaf spraying) treatments

Activity of peroxidase enzyme

Peroxidase enzyme activity was significantly affected by growth retardant at 1% level. The highest activity of peroxidase enzyme (2.5 unit mg^{-1} protein) was related to UN at 10 mg L^{-1} concentration, but there is no significant difference between above treatment with CCC. Also, the lowest enzyme activity (2 unit mg^{-1} protein) was belonged to control treatment (Fig. 7).

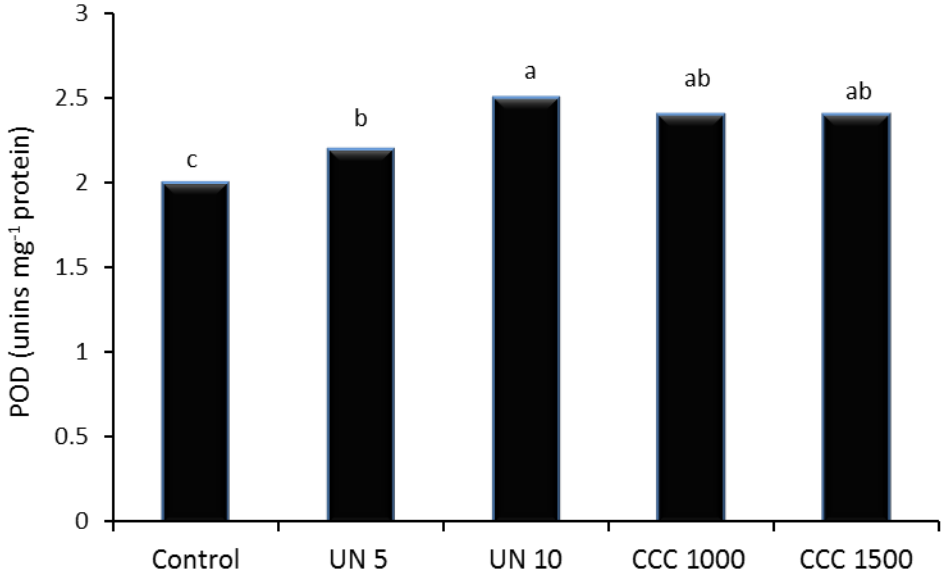

Fig. 7. Effect of cycocel (CCC; 1000 and 1500 mg L^{-1}) and uniconazole (UN; 5 and 10 mg L^{-1}) treatments on POD activity; the columns with the same letters are not significantly different at the 5% probability level

Fresh weight, dry weight and the length of the longest root

The results showed that the effect of application method of growth retardant on fresh and dry weights and length of the longest root was significant at 5% probability level. The highest fresh weight (2.8 g) and dry weight (0.17 g) of roots were related to foliar application of growth retardant treatments on leaves. The longest root (13.4 cm) was detected in 5 mg L^{-1} UN; however, there was no significant difference with 10 mg L^{-1} UN and CCC treatments. Shortest root (8 cm) was detected in control treatment (Fig. 8).

Flower diameter

The effect of growth retardant was significant on flower diameter. The largest diameter of flowers (46 mm) was observed in 1000 mg L^{-1} of CCC. The smallest flower diameter (40.2 mm) was achieved in the control treatment (Fig. 9).

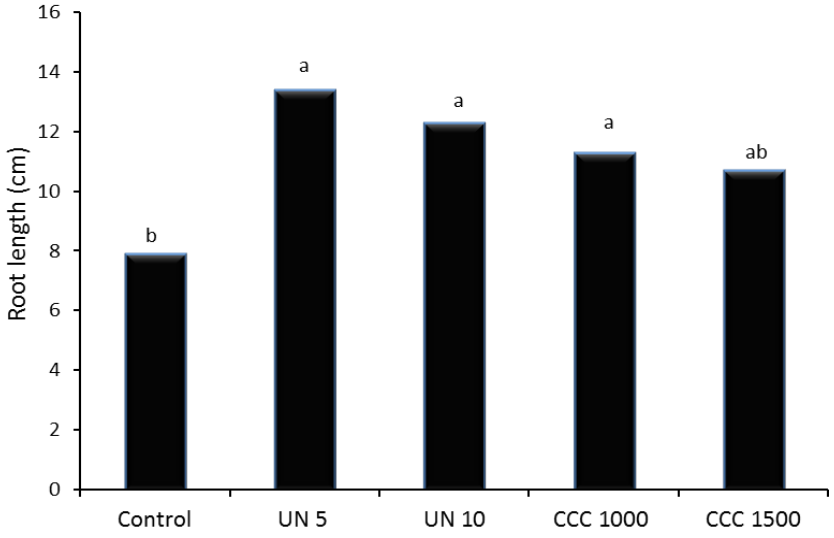

Fig. 8. Effect of cycocel (CCC; 1000 and 1500 mg L^{-1}) and uniconazole (UN; 5 and 10 mg L^{-1}) treatments on root length; the columns with the same letters are not significantly different at the 5% probability level.

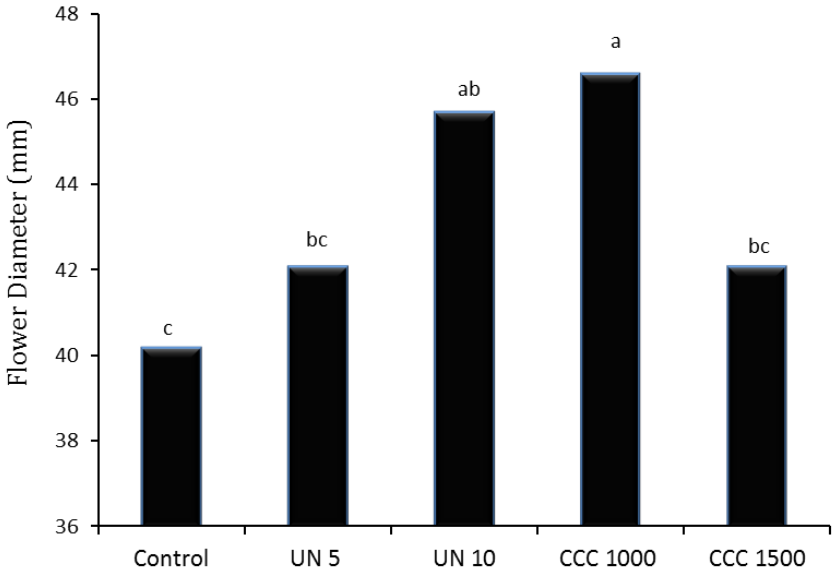

Fig. 9 Effect of cycocel (CCC; 1000 and 1500 mg L^{-1}) and uniconazole (UN; 5 and 10 mg L^{-1}) treatments on flower diameter. The columns with the same letters are not significantly different at the 5% probability level.

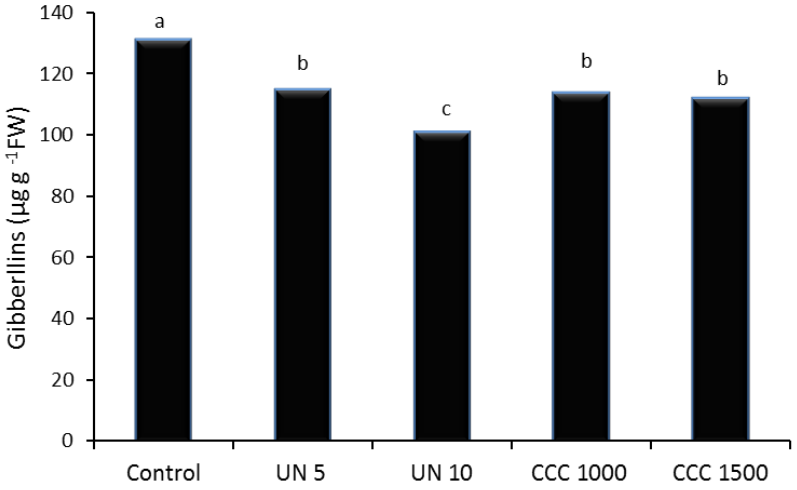

Fig. 10 Effect of cycocel (CCC; 1000 and 1500 mg L^{-1}) and uniconazole (UN; 5 and 10 mg L^{-1}) treatments on endogenous gibberellins in Zinnia leaves; the columns with the same letters are not significantly different at the 5% probability level.

Endogenous Gibberellins

The effect of treatments on leaf gibberellin content was significant at 1% probability level. The highest amount of this phytohormone was detected in control (131 µg g^{-1} FW). Plants treated with UN and CCC had lower endogenous gibberellin levels than the control (Fig. 10).

Discussion

One of the most important criteria determining ornamental plants marketing is their height. Nowadays, plant growth regulators are considered as an effective method to control the height of plants. In addition to the application time, the type of substance, concentration, number of applications and type of plant species and their variety can affect the plant height (Cowling, 2010). The mechanism of growth retardants is to block the biosynthesis of gibberellins; therefore they are acting as anti-gibberellin to reduce the plant height. Triazoles inhibit the production of gibberellic acid and reduce the length of the internodes and eliminate the apical dominance (Megersa et al., 2018).

In the present study, growth retardant showed a significant reducing effect on the height of zinnia plants in comparison with the control. The most effective treatment in height reduction was related to 10 mg L^{-1} UN. UN has been known to inhibit the biosynthesis of gibberellins by blocking kaurene oxidase, a P450 enzyme (Srivastava, 2002). That inhibits the oxidation of *ent*-kaurene to ent-kaurenoic acid by P450 monooxygenases. UN has been used to elucidate the involvement of brassinostroeids in the initiation of the final stage of tracheary-element differentiation in zinnia mesophyll cells. The chlormequat chloride blocks the synthesis of ent-kaurene from GGPP. Chlormequat chloride specifically inhibits the activity of copalyl diphosphate synthase (Srivastava, 2002).

In the present study, the amount of gibberellin in short plants was less than the control. It has been reported that UN application in *Ligustrum vulgare* produced short internodes (Steinberg et al., 1991). In potted *Arundina graminifolia* orchid's CCC had no effect on the height of plants (Da Silva Wanderley et al., 2014). The findings have shown that growth retardants increased leaf chlorophyll content (Rossini pinto, 2005; Bosch et al., 2016). The use of growth retardants increased total chlorophyll content and POD enzyme activity compared to the

control treatment. It seems that the increase in chlorophyll content is influenced by growth retardants due to the increase in cytokines levels (Gopi et al., 2005; Zhang et al., 2007). UN modifies the biosynthesis or metabolism of IAA, abscisic acid, ethylene, cytokinins and polyamines (Yokota, 1999; Srivastava, 2002).

It is reported that some growth retardants elevate the chlorophyll content in *Physalis angulata* L (Megersa et al., 2018). UN improved antioxidative defense mechanisms with higher activities of superoxide dismutase (SOD) and peroxidase (POD) enzymes that retarded lipid peroxidation and membrane deterioration (Zeng et al., 1994; Leul and Zhou, 1999)**.** The effect of growth retardant on the diameter of flowers, the frequency of their use, environmental conditions, and the sensitivity of the species and variety depend on these materials and the method of their application (Rossini pinto et al. 2005).

In the present study, the highest flower diameter was observed in 1000 mg L^{-1} CCC treatment. In an experiment, foliar application of the CCC (285 mg L^{-1}) increased the diameter of the flower in *Osteospermum ecklonis* (Olsen and Andersen, 1995). Growth retardants can increase the levels of endogenous cytokinin, resulting in more cell division, improved flower development and flower diameter (Yokota, 1999; Srivastava, 2002).

It has been reported that, application of growth retardants reduces the leaf area, which is attributed to the prevention of gibberellin synthesis and elevation in the abscisic acid content, which ultimately leads to the leaf cells elongation (Gopi e al., 2005). In the present study, UN reduced leaf area compared to the control treatment, and the highest leaf area was observed in plants with 1000 and 1500 mg L^{-1} CCC. The effect of triazole compounds on leaf production depends on the concentration of these compounds, so that in higher concentrations the leaf production is reduced and in low amounts, the number of leaves is not affected (Rossini pinto et al., 2005). In plants treated with UN, gibberellin content was decreased as compared to the plants treated with the CCC. Therefore, vegetative growth, leaf number and leaf area were lower in UN- than in the CCC-treated plants.

In this study, growth retardants reduced the number of leaves compared to the control. It is reported that the number of leaves in potted *Zinnia elegans* 'Liliput' was not affected by CCC application (Rossini pinto et al., 2005). The application of CCC at concentrations of 1000 and 1500 mg L^{-1} had no effect on the number of black lily leaves (AL-Khassawneh et al., 2006). The size of physalis (*Physalis angulate* L.) was reduced by using of growth retardants (Bosch et al. 2016). Treatment with 25 mg L^{-1} of UN reduced the number of leaves in Rako and Gold Strike kalanchoe cultivars (Hwang et al., 2008).

In the present study, the lowest amount of gibberellin was detected in plants treated with 10 mg L^{-1} UN. The reduction of the internal gibberellin level is a prerequisite for the induction of flowering that is obtained by spraying growth retardants (Olsen et al., 1995; Bosch et al., 2016). Delay in flowering with application of growth retardants is related to stimulation of cytokine synthesis (Opik and Rolfe, 2005).

Reduced fresh and dry weight of roots after application of growth retardants may be related to their synergistic effect with the surface auxin, which limits root growth, root system and ultimately limited production of carbohydrates (Blanchard et al., 2008). In the current experiment, growth retardants did not have a significant effect on fresh and dry weight of roots, but all treatments increased root length compared to control treatment. It was reported that root length increases by UN application on the roots of *Hibiscus rosa sinensis* (Nazarudin, 2012).

Conclusion

All growth retardants had an effective role on improving the quantitative and qualitative parameters of zinnia when compared to the control treatment. Among the treatments, 10 mg L^{-1} UN was the most effective in controlling plant height. In addition, this treatment increased flower diameter and chlorophyll content. On the other hand, soil application of UN was more effective in controlling plant height than foliar application. The shortest time for flowering was observed in 1000 and 1500 mg L^{-1} CCC in soil application method. In conclusion, the use of growth retardants for the improvement of vegetative and reproductive parameters of zinnia is recommendable.

Acknowledgements

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Conflict of interest

The authors declare that there is no conflict of interest for this study

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