

Initial growth of *Calendula officinalis* L. plants treated with paclobutrazol

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Abstract

Calendula officinalis L. is widely used in flowerbeds composition and cultivated as cut flowers. However, this species' plant height (30 - 60 cm) makes a stable disposition over tables a challenge, consequently impairing its sales as a potted plant. Paclobutrazol (PBZ) is a vegetal regulator that reduces the growth of plants. This study aimed to evaluate the initial growth of *C. officinalis* L. treated with PBZ. The experiment was carried out in a greenhouse environment in the Floriculture Sector, of the Department of Agronomy, of the Federal University of Viçosa from October to November 2020. A completely randomized experimental design was used composed of the treatments control, 2500, 5000, 7500, and 10000 ppm of PBZ doses with five replications. Two applications of PBZ doses were performed, with the first at 15 days and the second 30 days after transplantation. Plant height, leaf area, stem length and diameter, root length, leaf number, and photosynthetic pigments were evaluated. Application of PBZ, regardless of concentration, reduced plant height, leaf area, stem length, and diameter. No statistical difference in root length, leaf number, and photosynthetic pigments were found among the doses tested. The initial growth of *C. officinalis* L. was efficiently reduced with PBZ doses.

Keywords: flowering, marigold, potted plants, vegetal growth

Introduction

Calendula officinalis L. is used mainly as an ornamental plant (Zarrinabadi et al., 2019) in gardens, flowerbeds, borders, and, frequently, as a potted plant. Nevertheless, its plant height (30 – 60 cm) makes its disposition over tables difficult (França et al., 2018). Even under pot and substrate limitations, the potted plants reach a size that surpasses the esthetic pattern determined for gardens centers. However, plants within the esthetic pattern may be obtained using growth regulators (Téllez et al., 2020).

Growth regulators exert an important function in the growth of ornamental plants (Soumya et al., 2017). Paclobutrazol ([2RS-3RS)-1-(4-chlorophenyl)4,4-dimethyl-2-(1,2,4-triazole-1-yl)-pentan-3-ol]) (PBZ) is one member of the triazole family, and acts in plant growth reduction (França et al., 2018), accumulation of photosynthetic pigments (Soumya et al., 2017), stomata opening and

osmolytes accumulation (Waqas et al., 2017). PBZ can be applied via irrigation, foliar spray, or directly into the soil (drench), where the roots will absorb it and, through the acropetal movement, reach the leaves and apical meristems (Brito et al., 2016). However, PBZ efficiency varies with species and plant physiology, applied dose, and environmental conditions (Mog et al., 2019).

Studies with PBZ in potted *C. officinalis* L. plants are scarce, and studies on this subject are necessary. Therefore, this study aimed to evaluate the initial growth of *C. officinalis* L. plants treated with PBZ.

Material and Methods

Experiment Location

The experiment was carried out in a greenhouse in the Floriculture Sector at the Department of Agronomy, of the Federal University of Viçosa (20° 45'S, 42° 52' N, altitude 690 m) from October to November 2020 (average

temperature 27 °C and 85% RH).

Plant Material

C. officinalis L. seeds were obtained from Isla Sementes® company and were cultivated on recipients with commercial substrate Tropstrato® in a greenhouse.

The seedlings containing two pairs of true leaves were transplanted into 900 mL pots filled with substrate. Irrigation, fertilization and cultural treatments were carried out as needed. Fertilization was performed weekly with 2 g liter⁻¹ of NPK 20-20-20 (Peters).

Two PBZ doses applications were performed at 15 days and the second 30 days after transplantation. Thirty days after the first application, the following analyzes were performed:

Plant height

Plant height was measured with a ruler. Results were expressed in centimeters (cm).

Leaf Area

The leaf area index was obtained by a non-destructive method with a leaf area meter model AM350. Results were expressed in square centimeters (cm²).

Stem Length and Diameter

Stem length was determined with a ruler. Results were expressed in centimeters (cm). The stem diameter was determined with a digital caliper. Results were expressed in millimeters (mm).

Root Length

The seedlings were removed from the pots, and the substrate adhered to the roots was removed with weak water jets. After washing, the roots were dried and measured with a ruler, starting from the plant collar. Results were expressed in centimeters (cm).

Leaf Number

The leaf number was assessed manually by counting the total number of fully expanded leaves at the end of the experiment.

Photosynthetic Pigments

The leaf chlorophyll a and b and carotenoids content were determined by collecting two leaf discs of 0.6 mm in diameter in fully expanded leaves and placed in glasses containing dimethyl sulfoxide (DMSO). Subsequently, the extraction was performed in a water bath at 65 °C for three hours. Aliquots were removed to perform spectrophotometric readings at 480, 646 and 665 nm wavelengths. The chlorophyll a and b and total

carotenoids were calculated according to the equation proposed by Wellburn (1994).

Statistical Analysis

The experiment was conducted in a completely randomized design with five treatments (control, 2500, 5000, 7500 and 10000 ppm of PBZ) and five replications.

The data obtained were submitted to a normality (Shapiro-Wilk) and homogeneity (Bartlett) of variances test, analysis of variance (ANOVA), and the means compared by the Tukey test at 5% probability. All analyses were performed in the statistical R software 3.6.3 (R Core Team, 2020). Graphs were made using Sigma Plot software.

Results and Discussion

Plant Height

The lower height of *C. officinalis* was obtained at the dose of 10000 ppm of PBZ (Figure 1).

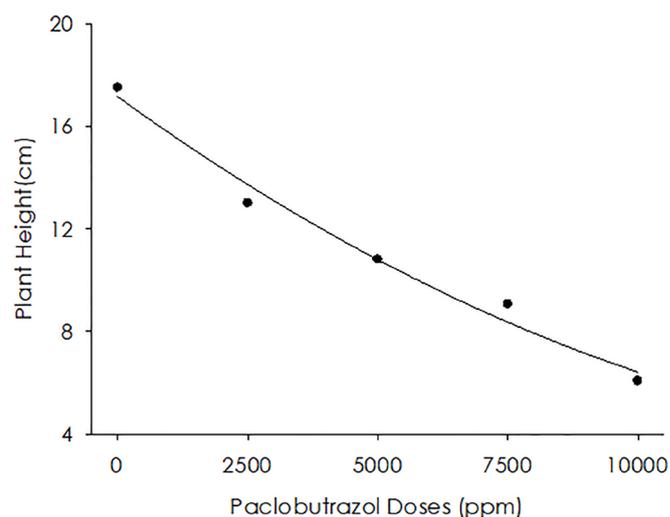


Figure 1. Plant height of *C. officinalis* L. plants treated with PBZ doses.

PBZ reduced the growth and elongation of the internodes and, consequently, the plants' height. These changes are due to the alteration of the isoprenoid pathway, which originates the basic diterpenoid carboxylic acid skeletons of gibberellins. This alteration inhibits the synthesis of gibberellin induced by the action of PBZ, which directly interferes in the process of cell division, suppressing plant elongation (Desta & Amare, 2021; Tesfahun & Menzir, 2018). As a result, several species treated with PBZ tend to be shortened and have darker green leaves (Hamdani et al., 2018), as observed in this study. The reduction in plant height is the most expressive morphological result of PBZ application (Tefahun, 2018), as it allows plants with high heights to be potted and sold as ornamentals.

Leaf Area

Leaf area decreased as the PBZ doses increased, with the lowest values obtained at the dose of 10000 ppm (Figure 2).

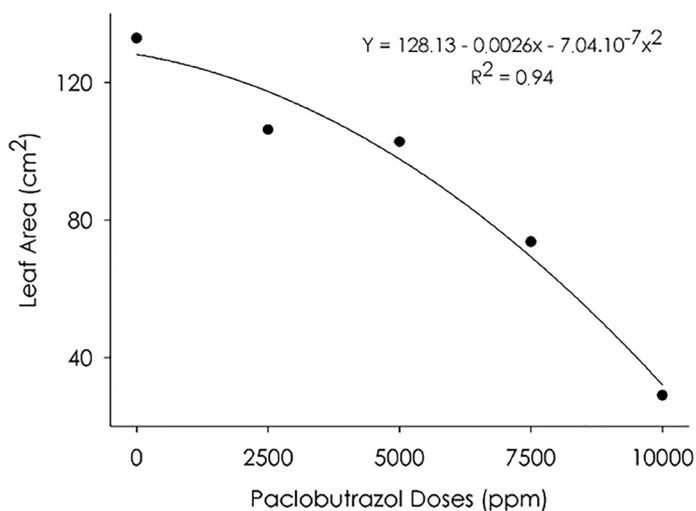


Figure 2. Leaf area of *C. officinalis* L. plants treated with PBZ doses.

The leaf area provides the photosynthetic surface available, working as an indicator of environmental impact factors on plants (Benetti et al., 2014). The reduction in leaf area of *C. officinalis* L. plants was due to the PBZ inhibiting effect on endogenous gibberellins levels, which are responsible for cell elongation (Mog et al., 2019).

Stem Length and Diameter

The PBZ dose at 10000 ppm promoted the lowest values for stem length and diameter (Figures 3 A and B).

Although PBZ concentrations were not sufficient to suppress the effects of GA signaling and change the leaf number, they reduced the stem growth and diameter and branches, which is attributed to the inhibitory interference of cytochrome P450 oxygenase that catalyzes the formation of ent-kaurenoic acid, resulting in reduced cell division and elongation (Dayan et al., 2012; Desta & Amare, 2021).

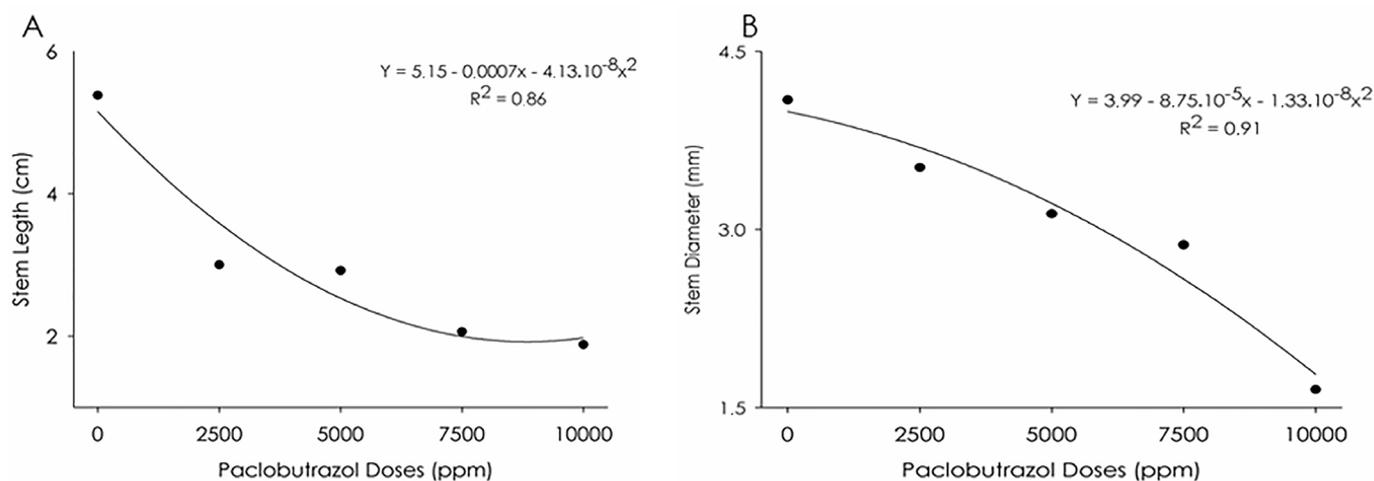


Figure 3. Stem length (A) and diameter (B) of *C. officinalis* L. plants treated with PBZ doses.

Root Length

There was no difference in root length of *C. officinalis* plants treated with different doses of PBZ ($x = 10.2 \pm 3.9$ cm). However, treated plants showed shorter roots compared to the control treatment.

PBZ increases the root diameter, increasing the width of the cortex and facilitating the formation of secondary xylem vessels (Desta & Amare, 2021). This is due to the modification that occurs in the radial expansion of the cells due to the decrease in the endogenous activities of gibberellins in response to the treatment with PBZ (Desta & Amare, 2021).

Nonetheless, the results found in this work may be related to the direct contact of PBZ doses via soil with the root system of *C. officinalis* L. plants, causing

growth reduction. In this case, foliar applications are recommended because PBZ has low mobility in the phloem, not causing changes in the root system growth (Siqueira et al., 2008).

Leaf Number

No difference was observed in the leaf number of *C. officinalis* treated with the different PBZ doses ($x = 3.0 \pm 0.4$ cm).

This result may have occurred because the applied concentration was insufficient to suppress the effects of foliar GA signaling (Carvalho-Zanão et al., 2018). Leaves are potential sources of GA signaling, essential for meristematic differentiation (Dayan et al., 2012) and, consequently, the formation and development of

new leaves. In *Nicotiana tabacum* plants, leaf removal impaired internodes' development, resulting in dwarfism (Dayan et al., 2012) and the formation of new leaves resembling GAs-deficient phenotypes (Dayan et al., 2012). The effect of defoliation exceeded that of PBZ, and even with the exogenous application of GA₃, the growth of internodes and the formation of new leaves was not restored. Therefore, the influence of PBZ on the leaf number and production of new leaves depends on the applied concentration, in which high concentrations reduce the production of new leaves and, consequently, the total leaf number, while low concentrations (< 2500 ppm) do not result in quantitative changes (Carvalho-Zanão et al., 2018).

Photosynthetic Pigments

The leaf concentration of chlorophylls a and b, and carotenoids ($x = 7.05 \pm 0.57 \text{ mL L}^{-1}$; $x = 3.74 \pm 0.95 \text{ mL L}^{-1}$; $x = 1.50 \pm 0.49 \text{ mL L}^{-1}$, respectively) was not influenced by the different PBZ doses.

The imbalance in the concentration of endogenous auxins and cytokinins reduces the number and maturation of chloroplasts, as well as the regulation of pigment synthesis and photosynthetic reactions, directly interfering with the foliar concentration of chlorophylls a and b and carotenoids in *C. officinalis* L. leaves (Carvalho-Zanão et al., 2018; Matos et al., 2017; Hajhashemi, 2018).

Conclusions

The initial growth of *C. officinalis* L. plants was affected by the paclobutrazol doses (PBZ) with a consequent reduction in plant height, leaf area, length, and stem diameter.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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