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ARTICLE

Growth inhibition of potted begonia via ethanol treatment

Redução do crescimento de begônia envasada via tratamento com etanol

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Abstract: The application of plant growth regulators is a viable tool to produce potted plants, but its use is limited due to the risk of toxicity. Alternative treatment with ethanol has shown potential in restricting growth, but its morphological effects on ornamental plants are not yet well-explored. This study evaluated the application of different concentrations of ethanol (0, 2%, 4% and 6%) on the growth of potted Dragon Wing Begonia, a hybrid of great ornamental potential that presents vigorous growth, seeking to select the best concentration to reduce plant height. The experiment was conducted in a 2x4 factorial scheme, with two varieties, 'Red' and 'Pink', and four concentration levels of ethanol treatment. Plant growth and development aspects were evaluated. Ethanol showed great potential to reduce plant size, as it linearly restricted all growth variables (height, diameter, number of leaves, leaf area, shoot, and root dry mass) and flowering variables (dry mass and number of flowers and buds). The highest concentration (6%) caused phytotoxic symptoms and senescence in plants. On the other hand, treatments with concentrations of 2% and 4% resulted in compact plants with great market quality. In conclusion, ethanol treatment can be a viable alternative to reduce the growth of potted ornamental plants.
Keywords: *Begonia* x *hybrida* 'Dragon Wing', ornamental plant, plant growth regulator, potted plant.

Resumo: A aplicação de reguladores de crescimento de plantas é uma ferramenta viável para a produzir plantas envasadas, mas seu uso tem sido limitado devido ao risco de toxicidade. O tratamento alternativo com etanol tem mostrado potencial em restringir o crescimento, mas seus efeitos morfológicos nas plantas ornamentais ainda não foram bem explorados. Este estudo avaliou a aplicação de diferentes concentrações de etanol (0, 2%, 4% e 6%) no crescimento de Begônia 'Dragon Wing' em vaso, um híbrido de grande potencial ornamental que apresenta crescimento vigoroso, buscando selecionar a melhor concentração para reduzir a altura da planta. O experimento foi conduzido em esquema fatorial 2x4, sendo duas variedades, 'Red' e 'Pink', e quatro níveis de concentração de tratamento com etanol. Foram avaliados aspectos de crescimento e desenvolvimento das plantas. O etanol mostrou grande potencial para reduzir o tamanho das plantas, pois restringiu linearmente todas as variáveis de crescimento (altura, diâmetro, número de folhas, área foliar, massa de matéria seca da parte aérea e radicular) e de florescimento (massa de matéria seca e número de flores e botões). A concentração mais alta (6%) causou sintomas fitotóxicos e senescência das plantas. Por outro lado, os tratamentos com concentrações de 2% e 4% resultaram em plantas compactas com ótima qualidade de mercado. Em conclusão, o tratamento com etanol pode ser uma alternativa viável para reduzir o crescimento de plantas ornamentais envasadas.
Palavras-chave: *Begonia* x *hybrida* 'Dragon Wing', planta envasada, planta ornamental, regulador de crescimento vegetal.

Introduction

Compact ornamental plants, with reduced plant height and diameter, are desirable by consumers and producers due to their beauty and practicality, and by occupying smaller spaces coupled to low maintenance. Plant growth regulators have been considered the most viable option for controlling plant height due to their efficiency; however, the use of these products has been restricted because of their toxicological potential and residual effect on soil and water, which turn imminent the search for practical alternatives with low risk of toxicity (Bergstrand, 2017; Al-Nasir et al., 2020; Nishijima, 2023).

Ethanol treatment has shown great potential in controlling the vegetative growth of potted plants. To date, only a few studies have demonstrated the effect of ethanol treatment in ornamental plants. In the first work of Miller and Finan (2006), with *Narcissus papyraceus* 'Ziva' grown in pots, the authors observed that the application of diluted ethanol in the root zone showed to be efficient in controlling plant growth and reducing height. Mibus et al. (2014) and Gargul et al. (2015) found the same effect in *Kalanchoe* spp. and Gehl et al. (2022) in *Campanula portenschlagiana*, in which the ethanol treatment showed efficiency in compacting the plant.

Exogenous ethanol application causes several metabolic effects since it can be mobilized by tissues, metabolized, and transformed into other compounds (Ackermann and Stanislas, 2020). In contact with the membrane, it can increase permeability and denature proteins due to changes caused in the lipid system. Although, the reasons that lead to the reduction of plant growth are not yet known. In general, the use of ethanol as an alternative treatment in potted ornamental plants has several advantages related to the fact that this substance is a biodegradable molecule, low cost, easy and safe to apply, and non-toxic in the necessary concentrations (Janeczko, 2011; Gehl et al., 2022).

The inhibitory effect of ethanol on the growth of potted plants has been investigated for some species; despite that, there are no reports in the literature of its effects on Begonia plants. The genus *Begonia* attracted attention due to the new hybrids on the market with beautiful ornamental characteristics, such as Dragon Wing Begonia (*Begonia* x *hybrida* 'Dragon Wing') with shiny dark green, wing-shaped leaves, and with inflorescences of red or pink colors, which are produced throughout the year and contrast with the foliage. The species is easy to grow, and adapts to environments from full sun to half shade. However, it is necessary to fit its size to pot market standards, where the height and shape of the plant are the main criteria observed (Hvoslef-Eide and Munster, 2007; Paiva et al., 2020).

Therefore, this study evaluated the application of different ethanol concentrations (0, 2%, 4%, and 6%) on the growth of potted Dragon Wing Begonia to verify its potential in reducing the height and diameter of the plant without depreciating ornamental aspects.

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Material and Methods

Dragon Wing Begonias seedlings (*Begonia* x *hybrida* 'Dragon Wing') of two varieties 'Red' and 'Pink' were used with an average of four leaves and 10 cm in length (Ball Horticultural ®). The seedlings were repotted into 21 cm-diameter plastic pots with a substrate made of pine bark and Sphagnum peat with a pH of 5.8 and a density of 252.1 kg cm^{-3} from Multiplant Grow Mix. The pots were placed in a greenhouse with 50% shading under a temperature average of 35°C/20°C (day/night) and 60% relative humidity (RH) from December 2020 to March 2021. Plants were fertigated twice a day for one minute (at 8 a.m. and 4 p.m.), with the application of nutrients according to Kämpf (2000).

Solutions containing 0, 2, 4, or 6% (v v^{-1}) ethanol (Absolute ethyl alcohol P.A ACS from Êxodo Científica®) diluted in distilled water were applied to the substrate of three-week-old plants in a volume of 50 ml per plant once a week for eight weeks. Treatments were applied in the morning with irrigation suspended.

The design used was completely randomized blocks, each block being a bench, with four replicates, in the factorial scheme 2x4, two varieties of Dragon Wing Begonia ('Red' and 'Pink'), and four ethanol concentrations (0, 2%, 4%, and 6%). Each plot was conducted with two plants, using eight plant replications per treatment. Weekly, we determined the plant's height (from the substrate surface to the highest point of the plant) and diameter (average between two perpendicular measurements of the diameter in the top view). We evaluated the number of flowers at the bud point and opening point, and possible visible physiological disorders (chlorosis or necrosis in leaves and flowers, deformations in leaves, and senescence).

At 90 days of cultivation, we selected four plants per treatment to evaluate the number of leaves, leaf area, and shoot and flower dry mass. In addition, the roots were separated and cleaned for the measurement of the size of the largest root and root dry mass. For the dry mass, the plant materials were kept in a forced air circulation oven regulated at 70°C until they reached constant mass. Leaf area was estimated using ImageJ® software, where all the leaves were detached at the base of the petiole and arranged open on a white background with scale. We took images to use in the program.

Data were statistically evaluated by performing the F-test of the analysis of variance, and a regression adjustment when the F-test was significant for the different concentrations, using PROC MIXED of SAS® 9.4 software (SAS Institute, 2013).

Results

Ethanol treatment reduced the growth of the two varieties of Dragon Wing Begonia, showing great susceptibility of the species, as plant height and diameter decreased significantly with increasing concentration. For all parameters investigated, the linear regression model fitted the data better, revealing a continuous decrease in values with increasing concentration. The lowest concentration (2%) caused a decrease of 18% in height and 7% in diameter, and the intermediate concentration (4%) provided plants almost 50% smaller in height, with a decrease of 18% in diameter. However, the highest concentration (6%) caused an exaggerated growth suppression, reducing height by almost 75% and diameter by 47% (Figs. 1a, 1b).

We observed the same pattern for the other parameters, with a reduction directly proportional to the increase in ethanol concentration. The lowest concentration of ethanol showed less than 5% restriction when compared to the control for the variables of shoot dry mass, number of leaves, and leaf area, while the concentration of 4% resulted in a reduction of 32% shoot dry mass, 23% of the number of leaves and 10% leaf area and the concentration of 6% restricted more than 50% of these variables (Figs. 2a, 2b, 2c). There was inhibition of inflorescence development. The intermediate concentration decreased the number of flowers by 58%, the number of buds by 28%, and the flower dry mass by 39%. The highest concentration caused stronger adverse reactions on flower development, decreasing more than 80% in these parameters (Figs. 2d,2e, 2f).

The plants treated with 2 and 4% ethanol showed a compacted appearance, and they were within the market quality standard (with reduced height and diameter covering the entire pot surface) compared to the control (0%) (Fig. 3). However, the concentration of 6% completely inhibited the growth of seedlings from the third application, when plants showed a wilted appearance, with curled leaves and weakened stems (Fig. 4).

Ethanol treatment showed effects on root formation, decreasing length and dry mass when compared to the control. At the lowest concentration (2%), root length reduced 11% and dry mass 31%. At intermediate concentration (4%) the root dry mass was reduced by 57% and the highest concentration (6%) reduced by 90% dry mass and by 50% root length (Fig. 5).

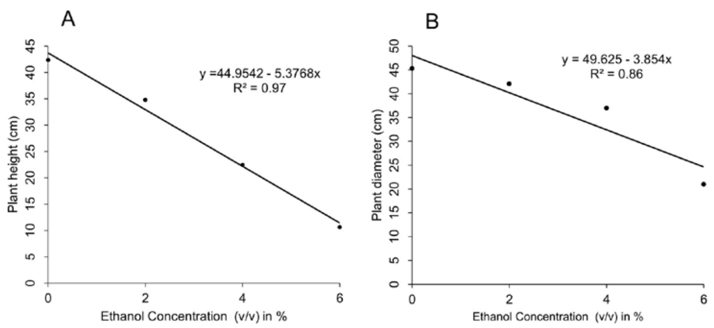

Fig. 1. Plant height (A) and plant diameter (B) of 56-week-old Dragon Wing Begonia plants treated with different concentrations of ethanol.

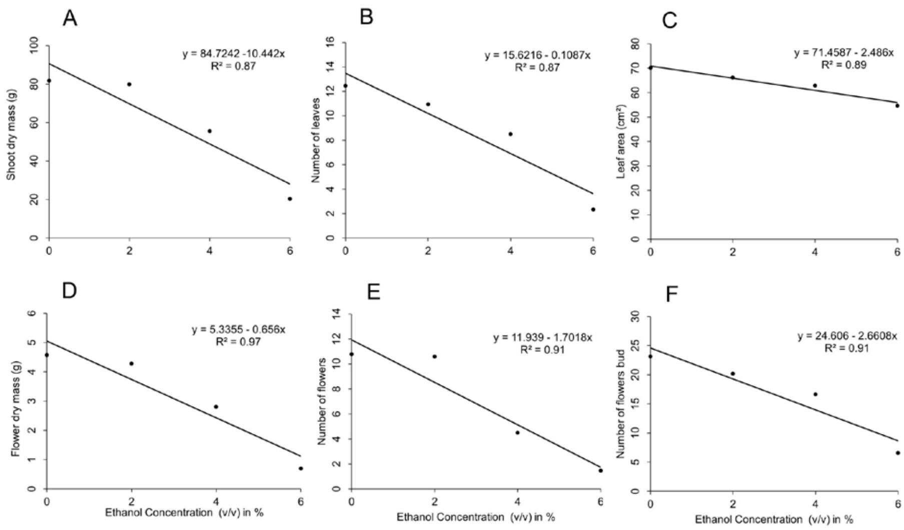

Fig. 2. Shoot dry mass (A), leaf number (B), leaf area (C), flower dry mass (D), number of flowers (E) and number of bud (F) of 56-week-old Dragon Wing Begonia plants treated with different concentrations of ethanol (0, 2, 4 and 6%).

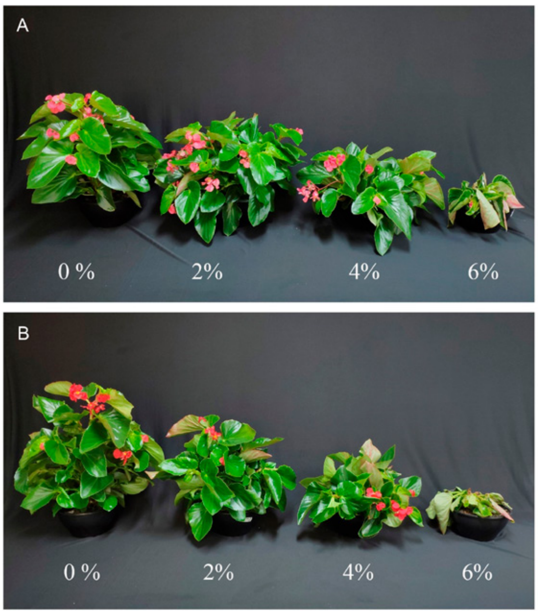

Fig. 3. Effect of ethanol treatment on 56-week-old Dragon Wing Begonia 'Pink' (A) and 'Red' (B) plants after application for eight times.

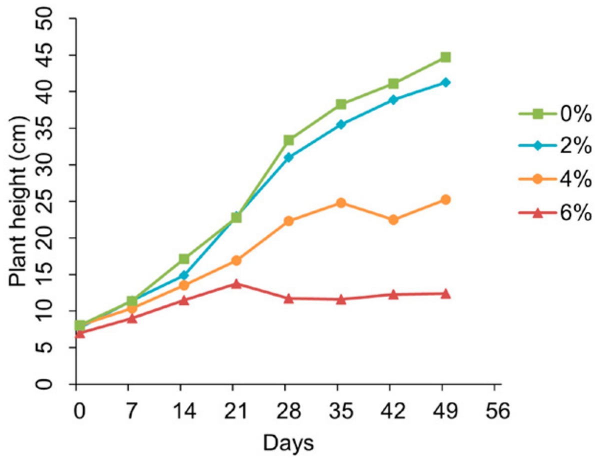

Fig. 4. Plant height of Dragon Wing Begonia plants treated with different concentrations of ethanol during the experiment period.

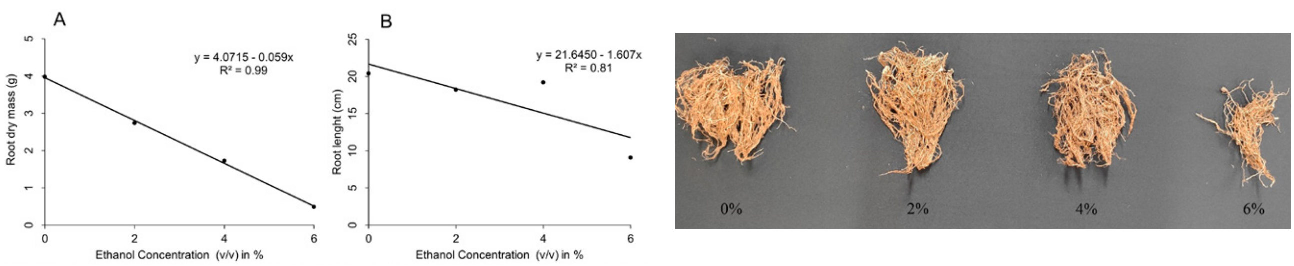

Fig. 5. Root dry mass (A), root length (B) and image below showing the change of roots of Dragon Wing Begonia plants treated with different concentrations of ethanol (0, 2%, 4% and 6%).

Discussion

The use of plant growth regulators is frequent in ornamental plants; however, there are no reports that aim to inhibit the growth of potted Dragon Wing Begonia (Ribeiro et al., 2019; Demir and Çelikel, 2019; Shin et al., 2020; Shi et al., 2021). In the present study, ethanol was used as an alternative plant growth regulator and showed great potential, with all tested concentrations exhibiting reduced plant size. This substance has advantages over other quimical products due to its biodegradable molecule, non-toxic in the necessary concentrations.

The ethanol treatment did not cause a different effect on the varieties, indicating a homogeneity between the responses of the two genotypes to the increase in the concentration of doses. However, Mibus et al. (2014) reported the opposite in *Kalanchoe* species. In their study with ethanol sprays at different concentrations, some genotypes were more resistant to the application of ethanol and showed a significant decrease in height only at high concentrations (over 8%) that were phytotoxic for the most intolerant materials.

There was a directly proportional relationship between plant growth and ethanol concentration, as the decrease in plant height was progressive with increasing concentration. The size reduction was significant in all treatments, with the concentration of 4% reducing plant height by 50% and the diameter by almost 20%, as a result, there was a proportional reduction in the shoot dry mass, the number of leaves, and the leaf area. Other authors showed that moderate concentrations are capable of compacting plants without generating phytotoxic aspects and that very low doses (<1%) do not cause significant effects on growth (Gargul et al., 2015; Mibus et al., 2014; Miller and Finan, 2006). This pattern was reported by Gehl et al. (2022) in *C. portenschlagiana*, who observed a 50% reduction in growth with 2% ethanol treatment and found no significant effects at concentrations lower than this.

The highest concentration (6%) decreased almost 75% of the plant height and 90% of the root dry mass; as a result, plants started to exhibit a wilted appearance, with curled leaves, weakened stem, and stunted root system, leading to senescence, this effect is usually observed at high concentrations of ethanol, varying its intensity according to the genotype susceptibility. On one hand, some species showed less tolerance, such as *Kalanchoe* spp. (Mibus et al., 2014; Gargul et al., 2015) and *C. portenschlagiana* (Gehl et al., 2022); for these species, the concentration of 4% caused negative symptoms on growth, with stunted and yellowed leaves and the appearance of necrosis. On the other hand, *Narcissus papyraceus* 'Ziva' plants showed notable resistance since only higher concentrations (over 10%) caused toxicity symptoms (Miller and Finan, 2006). The same authors also observed inhibition of root growth, but as in the present study, only doses higher than 4% resulted in damage to the plant.

Ethanol treatment affected flower development as well. A delay of 10 days in the appearance of the first flower and twice lower flower production during the weeks were observed at 4% and 6% ethanol concentrations (data not shown). After eight applications, the highest concentration provided more than 80% reduction in dry mass and number of flowers and buds. In Kalanchoe, treatments with concentrations higher than 2% also delayed flower induction and decreased flower size (Gargul et al., 2015; Mibus et al., 2014). The delay in flowering time is usually observed when using plant growth regulators, especially with gibberellin inhibitors, because of the complex interaction between hormones and flowering induction. Ethanol can induce inhibition of gibberellin synthesis and a hormone disbalance in plants, that could lead to the suppression of flowering reported in this study (Chen et al., 2023).

In contrast, in *Narcissus papyraceus* ‹Ziva›, authors observed no changes in flowering and inflorescences up to 5% concentration, and foliar application of ethanol resulted in an increased duration of inflorescences and a higher number of flowers and buds (Miller and Finan, 2006). Other authors demonstrated the same effect on cut flowers. Gebremedhin (2020) reported a longer vase life of Rose cut flowers and Maia et al. (2019) of Gerbera cut flowers with 4% ethanol as a preservative solution. Ethanol proved to decrease ethylene activity in cut flowers, resulting in respiration reduction and extended maintenance of flower quality; however, high concentrations may cause loss of membrane integrity and decrease flower vase life (Maia et al., 2019).

The reasons why ethanol inhibits growth and flowering when applied to the substrate are still uncertain. An osmotic interaction may occur in the root zone that causes a decrease in water supply, reproducing drought stress and leading to a deficiency in growth and flower development. Ethanol can also be toxic to the root system, showing a direct consequence on the cells due to the direct contact of the roots with the solution. There is also the possibility that ethanol is absorbed by the plant and metabolized, causing physiological changes (Janeczko, 2011; Mibus et al., 2014).

Matsui et al. (2022) showed that ethanol treatment can increase heat stress tolerance in lettuce plants, but the increasing concentration resulted in growth inhibition. These authors reported that ethanol has physiological effects on plants, as treated plants exhibited a lower level of ion leakage and an increased level of polyamines regulated by a set of differentially expressed genes.

However, the growth reduction effects observed in the shoot and the inhibition of flowering may be a combination of factors since other authors observed more than one evidence of ethanol action. Gehl et al. (2022) reported that ethanol can induce osmotic stress in the roots by decreasing the osmotic potential in the plant, leading to lower absorption of water and nutrients and contributing to plant compaction. In this report, ethanol proved to be toxic to the root system, as well, due to the significant reduction in root growth even at the lowest concentration and necrotic spots and damage in the vascular cylinder of the plant root observed at high concentrations (6%).

Despite that, moderate levels of ethanol in the root zone do not cause toxic effects on plants and have shown to be effective as a growth regulator. In the present study, plants treated with 2 and 4% showed the size and quality of commercial plants, with reduced height and diameter filling the entire pot. In these concentrations, plants did not show symptoms of toxicity, such as wilting and chlorosis of the leaves.

Conclusions

Ethanol treatment applied to the substrate proved to be efficient in reducing the growth of potted Dragon Wing Begonia, 'Pink' and 'Red', resulting in high quality plants within the commercial standard. However, due to the progressive reduction of vegetative and flowering parameters with the increase of the tested concentration, phytotoxic effects were observed at 6% concentration.

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Author Contribution

CFMM: conceptualization, validation, methodology, resources, writing- reviewing and editing, supervision. **TAS**: conceptualization, methodology, investigation, data curation, writingreviewing and editing

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

Data will be made available on request.

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