








EFFECT OF DAMINOZIDE ON THE GROWTH AND FLOWERING OF *Eustoma grandiflorum* PROPAGATED IN POTTED

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ABSTRACT

Daminozide (B-Nine) it is a plant growth regulator, is translocated from the leaves to the stem to avoid elongation and produce more compact plants and uniform growth and vigor. For the cultivation of Lisianthus the information of the damonizide application is scarce despite of great marketing potential, so the objective of this study was to evaluate the effect of daminozide on the vegetative growth and flowering of Lisianthus grown in pot. Granulated Lisianthus seeds were germinated and transplanted into pots under nursery conditions. Three foliar applications of daminozide were realized; two applications in vegetative stage and one in flowering, adding three concentrations in each of them (0.5, 1.0 and 1.5 ml L⁻¹) were used and water was applied as a control during the vegetative and flowering phases. The applications of daminozide at 1.0 and 1.5 ml L⁻¹ were optimal for the vegetative development in Lisianthus (plant height – 15 cm, size of the internodes – 2.5 cm, number of branches – 3, width and length of the leaves – 3 cm and 6.5 cm), while the dose of 0.5 ml L⁻¹ was favorable for all flowering stages (beginning of flowering – 182 buttons, sepals closed and larger than the petals – 53), visible petals larger than the sepals – 25, floral opening and full flowering – 3 flowers per plant). Based on these results, the application of B-Nine at low concentrations (0.5 and 1 ml L⁻¹) is recommended for the development of Lisianthus in pots.

Key words: Lisianthus, B-Nine, greenhouse production, vegetative phase, flowering phase, application rate

INTRODUCTION

Eustoma grandiflorum (known as Lisianthus in Mexico) is an ornamental plant of increasing demand. This species is native to the arid areas of the southern USA and northern Mexico [Gómez-Pérez et al. 2014]. It is an herbaceous plant of the Gentianaceae family that forms a rosette of leaves on a rigid stem that reaches heights ranging between 0.50 and 0.90 m. The native plants have blue to purple flowers, but the hybrids have different shades [Camargo et al. 2004, Velasco-Ramírez et al. 2020].

Lisianthus is grown to produce cut flowers or potted plants and has commercial potential for its variety of colors; its growth depends largely on adequate mineral nutrition [Hernández-Pérez et al. 2015]. In greenhouse production, stems that are too long or that have too much foliage are often a problem in handling and transportation; however, container production guarantees better handling and transportation and therefore lower costs. In addition to Lisianthus being a perennial species, its useful life can be from

three to five years achieving perennality in a container.

Growth regulators, such as daminozide (B-Nine), it's a product containing daminozide and maleic hydrazide can be used to manage height, branching, improve the flower freshness and the better quality of Lisianthus since it inhibits the biosynthesis of gibberellins reducing the length of the internodes, also used to obtain higher quality yield and control of morphological characteristics in various plant species [Karlović et al. 2004, Kazaz et al. 2010, Lodeta et al. 2010, Hayashi et al. 2019] and to counteract excess stem elongation [Lipaei et al. 2014].

Retardants also improve stress tolerance, green color in foliage, and postharvest [Sharma et al. 2011]; however, the type of species, crop, application method, and environmental conditions must be considered since they may affect this type of growth regulator [Khangoli 2001].

Zizzo and Fascella [2000] pointed out that the use of B-Nine in *Tulbaghia violaceae* was favorable for stem growth, but not for optimal flower development. Kelly et al. [2004] argued that the use of B-Nine in *Euphorbia pulcherrima* and *Viola wittrockiana* at low concentrations was optimal for good quality flower development. Pobudkiewicz and Treder [2006] demonstrated that the application of B-Nine twice during the phenological process of *Aeschynanthus radicans* at concentrations of 2500 to 4500 mg L⁻¹ was effective in retarding the growth of stems without adverse side effects, and flowering was not affected. Kahar [2008] recommended a concentration increase of 5000 mg L⁻¹ B-Nine in *Crhysanthemum morifolium* to guarantee a long vase life. Chen et al. [2018] reported that applications of daminozide in *Gerbera jamesonii* produced in containers increased the diameter of the flowers, number of buttons, and good quality in the bearing of the plant.

Plant growth retardants have a special advantage, mainly in situations where crops require greater control overgrowth under a greenhouse. Foliar applications of daminozide are and have been a common and effective application method as a growth retardant in ornamental plants, but there is no information on the effectiveness of B-Nine on the elongation rate of Lisianthus stems or on the flowering stage. Therefore, the objective of this work was to evaluate the effect of

B-Nine on the vegetative growth and flowering stage of *E. grandiflorum* grown in pots.

MATERIALS AND METHODS

Establishment of the experiment inside the nursery garden

The experiment was carried out in 2020, in the nursery garden of the University Center of Biological and Agricultural Sciences of the University of Guadalajara in Jalisco, Mexico (20°45'N, 103°31'W; 1650 m a.s.l.).

Pelleted seeds of Lisianthus (*E. grandiflorum*) variety 'Mariachi mix' (100) from the SAKATA® commercial house were sown in a germination tray in a mixture of peat moss, Agrolite, and agricultural lime (50 : 40 : 10) and irrigated daily at field capacity. They were kept at a temperature of 20°C to 24°C and allowed to grow for 15 weeks. When they reached a diameter of 6 cm and had a well-developed root system, they were transplanted into 6" plastic pots. One plant was placed per pot, which contained a substrate mixture of 40% leaf soil, 15% compost, 25% agricultural land, and 20% rock of volcanic origin. The plants were maintained in the greenhouse at 21.7°C under natural light conditions with irrigation intervals every 24 h with an application of 300 mL per pot directly to the substrate. Relative humidity and luxes were monitored every day in the morning throughout the experiment.

The fertilization program began 15 days after transplanting with an application of 1.5 g L⁻¹ of ammonium sulfate + 1.5 g L⁻¹ of monoammonium phosphate (MAP), (12–52–00), replacing the irrigation of that day with the application of 300 ml of solution.

Subsequently, the nutritive solution was dosed in three water-soluble MAP fertilizations (12–52–00) at a dose of 3 g L⁻¹ and a dosage of 250 ml per plant with an interval of 72 h between applications, after which an application of 2.5 ml L⁻¹ liquid urea (45–00–0) and 2 g L⁻¹ magnesium sulfate (00–00–00 + 16 + 13) was applied a dosage of 350 ml per plant with an interval of 72 h between applications. Fifteen days later, the first granulated fertilization was applied by adding 2 g Nitrofoska® (12–12–17 + 2 + 20) per plant to the substrate, repeating the same procedure two more times.

Additionally, a TRADECORP®Az microelement fertilizer was added at a dose of 0.5 g L⁻¹ with a dos-

age of 200 ml per plant as a nutritional supplement in the vegetative stage.

At 41 days after transplanting, an apical bud was manually cut from each plant, ensuring that they all reached an average height of 10 cm and four pairs of true leaves.

The nutrient solution was modified at the time of the flowering stage, which consisted of six water-soluble fertilizations of potassium nitrate (12–00–46) and magnesium sulfate (00–00–00 + 16 + 13) and two applications of calcium nitrate (12–00–00 + 23 Ca) at a dose of 3 g L⁻¹ and a dosage of 450 mL per plant with an interval of 72 h between applications.

The cultural tasks were carried out alongside the nutritional applications throughout the experiment. The control of pests and diseases consisted of scheduled explorations every 48 h in random plants, observing possible alterations in the morphology and normal functioning of the physiological activities, roots, stems, leaves, and flower buds, as well as the programming of activities, such as defoliation or removal of apical meristems for the development of an optimal canopy.

For phytosanitary control, two preventive applications of propamocarb were made at a dose of 1 ml L⁻¹ and a dosage of 150 ml per plant with a fortnightly interval between applications after transplantation to avoid the fungal complex that causes damping off. For the epigeal part of the plant, 1 g L⁻¹ mancozeb and 2 ml L⁻¹ cypermethrin were sprayed with a motor backpack and hollow cone nozzle until the objective was saturated, under favorable environmental conditions, low light intensity, relative humidity greater than 50%, and temperature less than 25°C.

Four different treatments were compared: 1 water application control, 2 foliar application of daminozide (B-Nine) at 0.5 ml L⁻¹, 3 foliar application of B-Nine at 1.0 ml L⁻¹ and 4 foliar application of B-Nine at 1.5 ml L⁻¹. The applications of B-Nine were made according to the label that marks the product, based on doses for *Chrysanthemum morifolium* (B-NINE® WG) since there is no information for application in *Lisianthus* and *C. morifolium* is a plant of similar size and phenology to *Lisianthus*. The applications B-Nine was made 1, 9, and 85 days after cutting the apical bud (first application after the first apical cut, second application nine days after the first application and the third application at the beginning of the first flower

buds, adding the three concentrations of the product according to each treatment per plant), The B-Nine was sprayed on the entire plant canopy consistently in the morning.

Evaluated variables

A randomized complete block experimental design was used with three replications for each treatment.

Variable measurements were made on 16 randomly selected plants per experimental unit (20 plants per treatment). The measurements were: plant height measured with a tape measure (basal area of the plant until the presence of the first flower bud); distance between nodes measured with a vernier digital caliper (from the base of the stem to the formation of the floral calyx); number of branches per plant; width and length of the leaves measured with a digital vernier caliper; leaf area using the ImageJ program; and number of flower buds per plant. Measurements were taken in plants from each of the B-Nine applications. The variable number of flower buds was recorded during the five flowering stages of the *Lisianthus* crop: 1 – beginning of flowering (the time elapsed from the development of the seedling to the first flower bud was considered), 2 – sepals closed and larger than the petals, 3 – visible petals larger than the sepals, 4 – floral opening (recorded from the development of the seedling until when the flower bud started to open) and 5 – full flowering (from the development of the seedling to when the flower presented its largest opening diameter).

Statistical analyses

To identify significant differences between treatments and controls in plants, data were first checked to confirm normality; then, appropriate two-tailed t-tests (normal data) or Mann-Whitney U-tests (non-normal data) were performed. To identify significant differences between treatments, Kruskal-Wallis and one-way analysis of variance (ANOVA) on ranks were used, followed by an LSD post-hoc test. Analyses were performed using Statgraphics Centurion XVIII software.

RESULTS AND DISCUSSION

The results of the study showed that for the variable plant height, the growth regulator B-Nine fa-

vored the development of the plants with respect to the control; however, no significant differences were observed between the doses used ($P = 0.00047$) since the plant height had an approximate growth of 15 cm in all doses tested (0.5, 1.0, and 1.5 ml L⁻¹; Fig. 1A). This shows that the use of B-Nine has a direct effect on the growth of the plant, reducing its size and allowing the plant to be manipulated in adverse light and temperature conditions.

The retarding effect was observed since an average plant of this variety of *Lisianthus* varies from 51 to 91 cm, in addition to showing healthy, thick and vigorous stems in these applications. Daminozide as a growth inhibitor plays a role in inhibiting cell elongation, and the addition of stem segments by reducing biosynthesis of gibberellin.

Similar results were reported by Zizzo and Fascella [2000], who evaluated the use of daminozide

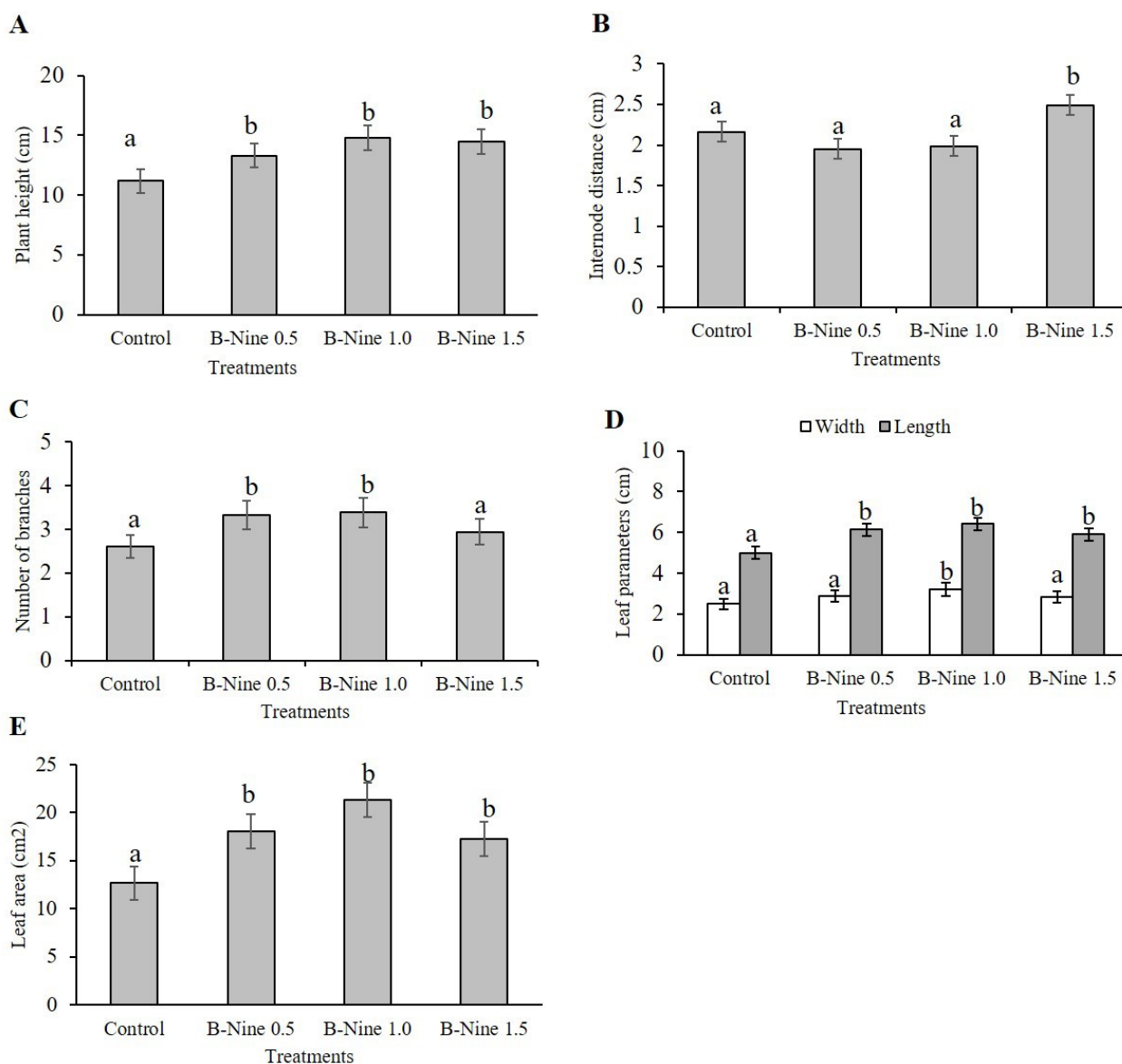


Fig. 1. Effect of daminozide (B-Nine) concentration on the development of *Eustoma grandiflorum* (*Lisianthus* var. ‘Mariachi mix’) plants during the vegetative stage: A) plant height, B) internode distance, C) number of branches, D) leaf parameters (width and length), E) leaf area. Significant differences between treatment and control $P \leq 0.05$, Kruskal-Wallis test

applied in a foliar application to *Tulbaghia violacea*, concluding favorable growth in plant height compared to paclobutrazol. Kahar [2008] recommended the use of daminozide as a growth regulator in the cultivation of *Crysanthemum morifolium*, obtaining good results with foliar applications at the height of the stem at a dose of 1250 mg L⁻¹. Lipaei et al. [2014] emphasized that the use of daminozide in *Calendula officinalis* gave significant results in plant height with doses lower than 500 mg L⁻¹.

In contrast to the other doses tested (Fig. 1B), the effect of B-Nine on the development of internodes of *Lisianthus* plants was significantly greater in the treatment at 1.5 ml L⁻¹ (P = 0.56), obtaining distances of 2.5 cm, considering it optimal according to the height obtained from the plants. The inhibition of ent-Kaurene, whose function is an early intermediary in endogenous gibberellin biosynthesis, is the mechanism of action of daminozide. Because daminozide inhibits this action on GAs, cell elongation is decreased, leading to the reduction of internodes in plants [Sitawati and Ni'mah 2021]. Kahar [2008] reported the effect of daminozide on the vegetative characteristics of development between nodes in *C. morifolium*, obtaining significant results with doses lower than 1000 ml L⁻¹.

Regarding the number of branches, it was significant for B-Nine treatments at 0.5 ml L⁻¹ and 1.0 ml L⁻¹ (P = 0.019), obtaining three branches per plant, on average (Fig. 1C). The daminozide regulator was applied using a foliar spray and was favorable in *Lisianthus* plants for the generation of a number of branches, the inhibitory effect of the gibberellins of B-nine brings with it an increase in the number of lateral stems, stronger plants and a better size. This was consistent with Pobudkiewicz and Treder [2006], who compared the effects of flurprimidol and daminozide in the oriental lily 'Mona Lisa' and found that in these plants, growth was inhibited only by flurprimidol.

Regarding the leaf variable, the width was favorable at 1.0 ml L⁻¹ B-Nine treatment (P = 0.020), obtaining an average of 3 cm, in contrast to the other treatments tested, including the control treatment, which measured less than 2 cm. The length of the leaf was favorable for all tested doses (0.5, 1.0, and 1.5 ml L⁻¹; P = 0.003), being 6.5 cm, on average (Fig. 1D).

Similarly, in the foliar area, it was evident that the foliar application of daminozide (B-Nine at 0.5, 1.0,

and 1.5 ml L⁻¹) was significant (P = 0.003) with an average area of 23 cm² regarding control (Fig. 1E). B-Nine has the ability to redirect carbohydrates towards the root, stimulating root development and the production of cytokinins with a consequent increase in the development of chloroplasts, better growth and coloration of foliage (intense green), it also allows the accumulation of abscisic acid, which increases tolerance to stress conditions due to water deficit.

The number of flower buds registered after the flowering stages of *Lisianthus* was favorable in four of the five phases for the B-Nine treatment at 0.5 ml L⁻¹ (Fig. 2). During the first stage, which was the beginning of flowering (Fig. 3A), an average of 182 flower buds were obtained with 0.5 ml L⁻¹ B-Nine treatment, followed by the 1.5 ml L⁻¹ treatment with an average of 175 buds. The least flower buds (75) were obtained in the control treatment. In the second stage (Fig. 3B), greater evidence of closed sepals and larger petals were obtained with the 0.5 ml L⁻¹ B-Nine treatment with an average of 53 flower buds, closely followed by the 1.0 ml L⁻¹ treatment with an average of 52 flower buds. In the third stage, where the visible petals were larger than the sepals (Fig. 3C), plants treated with 0.5 ml L⁻¹ B-Nine had an average of 25 flower buds. Eleven flower buds were observed in plants treated with 1.0 ml L⁻¹ and 1.5 ml L⁻¹ B-Nine and the control. In the fourth stage, which involved floral opening (Fig. 3D), 1.0 ml L⁻¹ B-Nine treatment was favorable, with an average of four openings, followed by the treatment at 0.5 ml L⁻¹ with an average of 2; No openings were observed in the control treatment. In the fifth stage, which was full flowering (Fig. 3E), only B-Nine treatment at 0.5 ml L⁻¹ had good results, with an average of 3 flowers per plant. The effect of B-Nine on the number of flowers could be due to the fact that the plants with this product had better morphology and, at the same time, a greater number of stomata, which led to an increase in the flow of carbon dioxide in the mesophyll tissue, which can reduce the photosynthates. This process, finally, distributes the photosynth in plant tissue [Lakshminathi et al. 2017] so that the plants that were applied with daminozide had a greater number of flowers.

In this experiment, it was evident that at lower doses the B-Nine product makes its collateral effects more efficient, such as flower development and increased leaf area in *E. grandiflorum* (*Lisianthus*).

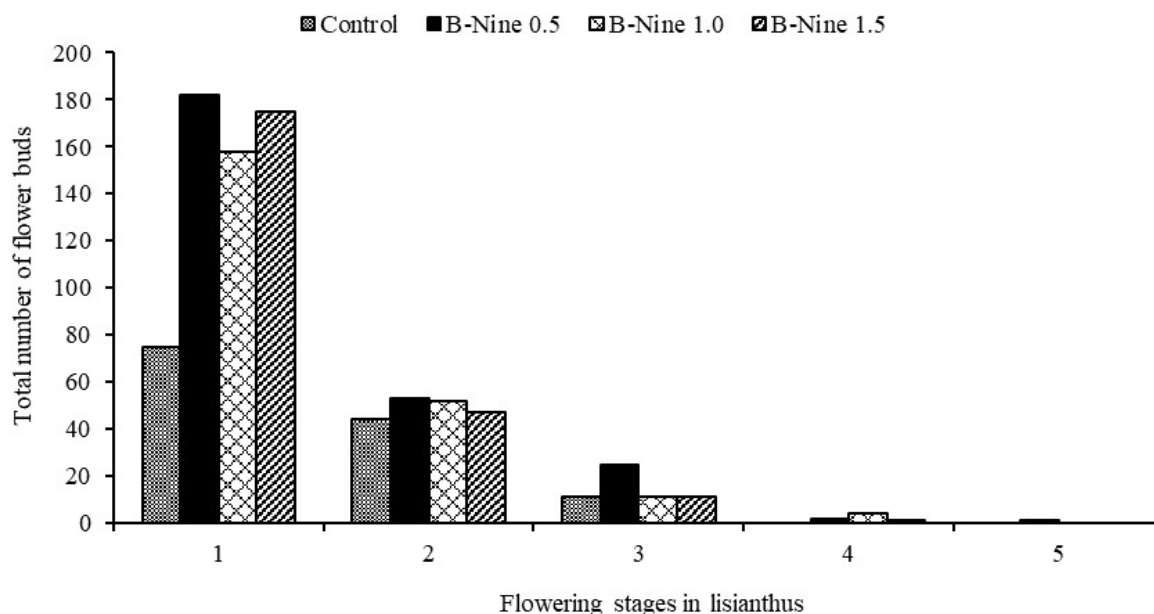


Fig. 2. Effect of daminozide concentration (B-Nine: 0.5, 1.0, and 1.5 ml l⁻¹) on the development of flower buds during the five flowering stages



Fig. 3. Flowering stages in lisianthus. A) stage 1 – beginning of flowering, B) stage 2 – sepals closed and larger than the petals, C) stage 3 – visible petals larger than sepals, D) stage 4 – floral opening, E) stage 5 – full bloom

The number of flower buds showed an increase with the dose of B-Nine (0.5 ml L⁻¹), improving the commercialization expectations of Lisianthus in pots. Increasing the added value of the plants subjected to the treatment with daminozide will increase its acceptance in the market. Plants with larger flowers are preferred over those with small flowers; this coincides with Esquivel-Pool et al. [2005] and Tolotti et al. [2003], who mentioned that with applications of 1000 mg L⁻¹ of daminozide, it was possible to obtain plants

with standard commercial quality and promote floral development.

Daminozide is presented as a growth regulator that impacts plants directly. It has been demonstrated that its use as in ornamental crops generates the inhibition of gibberellin synthesis, influencing quality variables, such as length of the stems and internode shortening [Salachna and Zawadzińska 2017].

Shoot growth reduction of many ornamental plants is achieved through the use of plant growth retardants

[Lipaei et al. 2014]. The risk of lodging in plants with very long stems and their requirement for more space implies greater labor and increased transportation costs [Kazas et al. 2010].

Plant growth retarders decrease the length of the internodes and eliminate apical dominance [Khangoli 2001], and daminozide (B-Nine) is an important plant growth retarder. It has been shown to reduce plant height and shorten the distance between nodes, but it generates a greater number of branches and flower buds, achieving an attractive appearance in the plant [Pobudkiewicz and Treder 2006, Kahar 2008, Lipaei et al. 2014]. Furthermore, in evaluations where the response of daminozide was measured in foliar applications in *Helianthus annuus* plants grown in pots, Whipker and McCall [2000] confirmed that it reduced the size of the plants, but the flowers were large.

CONCLUSIONS

The use of daminozide at lower doses is effective for the cultivation of *Lisianthus*, since it was favorable in all flowering stages and reduce the size of the internodes and therefore the height of the plant, achieving a size of 15 cm, compared to its usual height without affecting vegetative development, showing avigorousness and a significant number of branches.

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