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Research Article

Dwarfing Responses of *Leonotis leonurus* (L.) R.Br. Lamiaceae Using Foliar Growth Regulator Applications to Induce Compactness in Flowering Potted Plants

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Abstract

The insatiable demand for novel floral forms in the ornamental horticulture industry continues to drive the search for such plant species, particularly in biodiversity-rich regions such as South Africa. The aim of the present study was to evaluate the effectiveness of Cycocel®, a plant growth retardant, in manipulating growth and compactness in *Leonotis leonurus*, a plant with potential high ornamental value in the potted flower industry. Application of Cycocel®, especially at a concentration of 4 mg/L significantly reduced both height and plant width of *L. leonurus* plants growing in a soilless hydro culture system. In addition, application of the growth retardant had a significant influence on increasing the shoot proliferation in *L. leonurus* plants. The number of new shoots produced in week 6 of the 8-week growing period was about threefold higher compared to the control. The reduction in height and width observed in the present study may be due to the interference with key enzymes involved in the gibberellin biosynthesis pathway. Overall, application of Cycocel® had the desired effect in controlling growth parameters in *L. leonurus* plants, thereby improving compactness and enhancing its commercial value in the flowering potted plant market.

Keywords: Axillary Shoots; Cycocel®; Hydroponics; Ornamental Horticulture; Plant Growth Retardant

Introduction

Leonotis leonurus (L.) R.Br. (Lamiacaeae) is a shrub, which is commonly known for its essential oil production through glandular trichomes [1]. The plant species is indigenous to South Africa and other tropical regions of America (Watt and Breyer-Brandwigk, 1962). Its aerial parts are widely used in oriental traditional medicine [2]. In South Africa, it is used by the Xhosa and Zulu as a repellent, laxative, numbing agent against snake and scorpion bites, bee stings and in the treatment of skin diseases (Watt and Breyer-Brandwigk, 1962). The plant has been reported to have anticonvulsant (Bienvenu., *et al*. 2002), anti-inflammatory and hypoglycemic activities (Ojewole, 2005) as well as mood-altering properties (Richard., *et al*. 2001). Besides its medicinal properties, *L. leonurus* is more renowned for its landscape applications namely, autumn colour, perennial borders, cottage gardens, xeriscapes, sunny exposed locations and patio containers (Clausen and Ekstrom, 1989). In the ever-expanding local and international market of Landscape Horticulture, there is a growing demand for new and interesting potted flowering plants [3]. In its natural habitat *L. leonurus* is a vigorous shrub which grows to 2 - 3m tall and 1.5m wide. It produces attractive bright orange flowers during the autumn season, which attract a host of pollinators, namely butterflies, bees, and hummingbirds [4].

The successful control of plant height and form through the use of plant growth retardants such as ((2-chloroethyl) trimethylammonium chloride (Cycocel®) remains an important factor in ornamental horticulture, especially for potted plants [5]. In essence, height control enhances visual appeal and market value of potted plants [6]. The concentration and method application of

plant growth retardants affect their effectiveness [7]. Excessive and adverse reductions in growth may be caused by inappropriate application rates of plant growth retardants (Cox and Keever, 1988). Some of the ornamental crops that are effectively controlled by Cycocel® include herbaceous crops, such as *Pointsettias* and *Geraniums*, as well as woody flowering crops such as *Hibiscus* and *Azaleas* (Olympic Horticultural Product, 2005). Although Cycocel® is versatile in its methods of application; it is usually applied as a foliar spray [7]. One of the effect of the growth retardant, 2-chloroethyl) trimethylammonium chloride, relates to its plant growth regulating properties [8]. Thus, Cycocel, a well know plant growth retardant, can shorten plant form, thereby enabling plants to withstand high doses of fertilization without succumbing to lodging [9].

The current study was done using a hydroponic system, in which the plants were grown in a medium other than soil, using mixtures of the essential plant nutrients dissolved in water [10]. The Nutrient Film Technique (NFT), a closed system that requires large amount of water per plant, was used to grow *L. leonurus* plants in the present study (Carruthers, 1998).

Aim of the Study

The aim of the study was to evaluate the growth responses of *L. leonurus* to different dosages of Cycocel® for manipulation of compactness for suitability in ornamental pot plant production.

Materials and Methods

Plant selection

Mature plants of *L. leonurus* were obtained from the Cape Peninsula University of Technology Nursery, Bellville Campus, South Africa. All cuttings were taken from the same mother stock.

The cuttings were rooted in a mixture of 50% sifted bark and 50% river sand. Cycocel® [(2Chloroethyl) trimethylammonium chloride] was purchased from Nesco Engineering, 7 Strand Rd, Labiance, Bellville, South Africa.

Hydroponics experiment

A recirculation soilless medium setup was used to supply the treatments to the plants. Plastic pots (12.5 cm diameter) were filled with Light Expanded Clay Aggregate (LECA, SA Horticultural Supplies, 94 Frere Rd, Judith's Paarl, Johannesburg, South Africa). The medium was chosen because of its good drainage. After being thoroughly washed in deionized water the rooted cuttings were transplanted into the pots in an environmentally controlled greenhouse fitted with a silver Alunet screen. The greenhouse midday temperatures ranged between 16 - 20ºC and relative humidity between 40 and 88%. The pots were lined inside experimental gutters and placed in five rows on galvanized steel tables measuring 2m × 1m. Each experimental gutter contained 10 pots and was covered with a thick waterproof black plastic sheet to prevent penetration of light that will cause algae growth on the water. A nutrient film technique system was used, in which water was supplied to the pots via connections of 20 mm fittings and 20 mm pipes pumped onto the experimental gutters and drained back to the reservoir.

Preparation of Cycocel® treatments

The reservoir, a 50-litre water tank, contained a nutrient solution of water and a commercial hydroponic fertilizer, Nutrifeed (Starke Ayres, 10 - 14 Evans Ave, Epping Industria 1, Cape Town, South Africa). Cycocel® treatments (1.5 mg/L, 2 mg/L, 3.5 mg/L and 4 mg/L) were mixed in 1-litre spray bottles and applied as foliar feed on a weekly basis, starting at two weeks after transplanting. The control received no treatment. All treatments were applied as a single spray with a hand held spray bottle. The dose of Cycocel®, the plant growth retardant, was applied according to the instructions from the supplier.

Data collection

Prior to planting in the hydroponic system the plants were thoroughly washed in deionized water to remove any foreign matter from their roots. After an 8-week growing period the following plant growth parameters were measured: fresh weight prior planting, plant height, root length, visual compactness (in a 1 - 5 Likert scale), plant width, number of lateral branches, number of new shoot buds and dry root and shoot post-harvest weight. During harvesting plants were removed from medium and the roots were thoroughly washed. The plants were oven-dried overnight at 50ºC and dry weight was measured.

Statistical analysis

Mean values of the plant heights, widths, number of new shoots, number of lateral branches, visual compactness using a 5 - Likert scale and plant dry weights were analyzed using one-way analysis of variance (ANOVA). Statistical analysis was done using STATISTICA (Statsoft, Dell Statistica, 2300 East 14th Street, Tulsa, OK 73104, USA). Where significant differences were observed, the Fisher least significance difference (L.S.D.) was used to separate mean values at the 95% level of significance ($P \leq 0.05$) [11].

Results

Biomass accumulation and compactness in *Leonotis leonurus*

Biomass accumulation in *L. leonurus* was measured as dry mass after a growing period of 8 weeks (Figure 1). The highest shoot dry mass was obtained at a Cycocel® concentration of 1.5 mg/L. The 1.5 mg/L Cycocel®-treated plants accumulated significantly high shoot dry mass compared to the control and other Cycocel® treatments ($P \leq 0.001$). Similarly, root dry mass was also significantly higher for 1.5 mg/L Cycocel® compared to control plants and the other Cycocel®-treated plants ($P \leq 0.01$). After a growing period of 8 weeks, a significant ($P \leq 0.001$) reduction in plant height was observed between the control and Cycocel®-treated plants. The highest reduction in plant height was obtained at a Cycocel® concentration of 4 mg/L (Figure 2A). There was significant reduction in plant width in all Cycocel®-treated plants when compared to the control ($P \leq 0.001$, Figure 2B). The highest reduction in plant width was observed for plants treated with 4 mg/L Cycocel®. When compared to the control, significant width reduction was observed from week 3 to week 8 where the greatest width reduction was observed in the treatment with 4 mg/L. No evidence of compactness was observed before the application of Cycocel® (weeks 1 and 2). However, from week 3 through to week 8 there was a significant increase in compactness ($P \leq 0.001$) in all Cycocel®-treated plants compared to control plants (Table 1). After the 8 weeks growing period, there was no significant difference in the degree of Cycocel®-induced compactness (5 Likert-scale) among the treatments themselves.

Auxiliary shoots and lateral branching in in *Leonotis leonurus*

Generally, a significant increase ($P \leq 0.001$) in the number of new shoots was observed in all Cycocel®-treated plants compared to the control (Table 2). The significant effect on axillary shoot proliferation was observed from four weeks after the initiation of the experiment. Across all Cycocel® treatments, the highest induction and proliferation of new shoots occurred at five and six weeks after the start of the experiment. During week 5, significantly high numbers of new shoots (11 shoots/plant and 12 shoots/plant) were produced in plants treated with 3.5 mg/L and 4 mg/L, respectively. This was almost two-fold the number of shoots observed in control plants ($P \leq 0.01$). A similar trend in high soot proliferation was again observed during week 7 of the 8-week growing period, in which in 3.5 mg/L and 4 mg/L Cycocel® produced 9 shoots/plant and 11 shoots/plant, respectively. The level of shoot proliferation during week 7 was approximately two-fold higher than the control plants. Overall, the highest production of axillary shoots (14-16 shoots per plant) occurred during week 6, in which the proliferation rate was almost three-fold higher than the control plants ($P \leq 0.001$). In general, during the entire growing period of 8 weeks, the control had the least number of new shoots per week. Foliar applications of *L. leonurus* plants with Cycocel® at different concentrations did not have any significant effect on the number of lateral branches produced per plant during an 8 week growing period (Table 3).

Cycocel (mg/L)	Week 3	Week 5	Week 6	Week 7	Week 8
1.5	2.70 ± 0.48a	3.50 ± 0.53ab	4.00 ± 0.00a	4.90 ± 0.32a	4.90 ± 0.32a
2.0	2.60 ± 0.52a	3.40 ± 0.52ab	4.00 ± 0.00a	4.70 ± 0.48a	4.90 ± 0.32a
3.5	2.00 ± 0.00b	3.30 ± 0.48a	4.00 ± 0.00a	4.80 ± 0.42a	4.90 ± 0.32a
4.0	2.90 ± 0.32a	3.80 ± 0.42b	4.00 ± 0.00a	4.90 ± 0.32a	4.90 ± 0.32a
Control	2.00 ± 0.00b	3.30 ± 0.48a	3.10 ± 0.32b	4.00 ± 0.00b	4.00 ± 0.00b

Table 1: Effect of different concentrations of Cycocel® on visual compactness using the Likert scale of *L. leonurus* during the 8 week experimental period.

Values presented are means ± standard deviation. Means followed by similar letters in a column are not significantly different from each other at $P \leq 0.05$ according to Fischer least significance difference.

Cycocel (mg/L)	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
1.5	2.60 ± 1.65a	2.60 ± 1.65a	4.90 ± 2.81a	2.50 ± 1.78ab	8.00 ± 3.40ab	15.80 ± 4.16a	5.00 ± 1.94a	1.70 ± 1.49a
2.0	2.00 ± 0.94a	2.00 ± 0.94a	9.50 ± 7.52a	3.10 ± 1.97a	8.40 ± 3.75ab	14.00 ± 3.13a	6.00 ± 3.53ab	2.70 ± 1.77ab
3.5	2.50 ± 2.46a	2.50 ± 2.46a	8.60 ± 5.42a	3.11 ± 2.13a	11.00 ± 4.45bc	14.00 ± 4.1a	9.00 ± 5.52c	2.90 ± 2.13bc
4.0	2.10 ± 0.99a	2.10 ± 0.99a	8.70 ± 7.13a	1.70 ± 1.42ab	12.50 ± 5.15c	16.40 ± 4.7a	10.60 ± 4.9c	4.00 ± 2.31b
Control	3.10 ± 1.91a	2.70 ± 2.11a	5.10 ± 4.25a	1.30 ± 1.42b	6.40 ± 2.95a	5.40 ± 3.44b	4.60 ± 2.50a	1.70 ± 1.57a

Table 2: Effect of different concentrations of Cycocel® on the number of new shoots of *L. leonurus* during the experimental period.

Values presented are means ± standard deviation. Means followed by dissimilar letters in a column are significantly different from each other at $P \leq 0.05$ according to Fischer least significance difference.

Cycocel (mg/L)	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
1.5	2.50 ± 0.71a	2.50 ± 0.71a	2.90 ± 1.10a	3.601.58a	3.60 ± 1.58a	3.60 ± 1.58a	5.00 ± 1.63a	5.00 ± 1.63a
2.0	2.60 ± 0.52a	2.60 ± 0.52a	3.40 ± 0.84a	4.10 ± 1.37a	4.10 ± 1.37a	4.10 ± 1.37a	5.20 ± 1.55a	5.20 ± 1.55a
3.5	3.30 ± 0.67a	3.30 ± 0.67a	3.90 ± 1.20a	5.44 ± 1.93a	5.20 ± 1.93a	5.20 ± 1.93a	6.20 ± 1.55a	6.20 ± 1.55a
4.0	2.90 ± 0.57a	2.90 ± 0.57a	3.70 ± 1.70a	4.00 ± 1.76a	4.00 ± 1.76a	4.00 ± 1.76a	4.80 ± 1.93a	4.80 ± 1.93a
Control	2.80 ± 0.63a	2.80 ± 0.63a	3.30 ± 0.95a	4.00 ± 1.25a	4.00 ± 1.25a	4.00 ± 1.25a	5.60 ± 1.35a	5.60 ± 1.35a

Table 3: Effect of different concentrations of Cycocel® on the number of lateral branches of *L. leonurus* during the experimental 8 week period.

Values presented are means ± standard deviation. Means followed by similar letters in a column are not significantly different from each other at P ≤ 0.05 according to Fischer least significance difference.

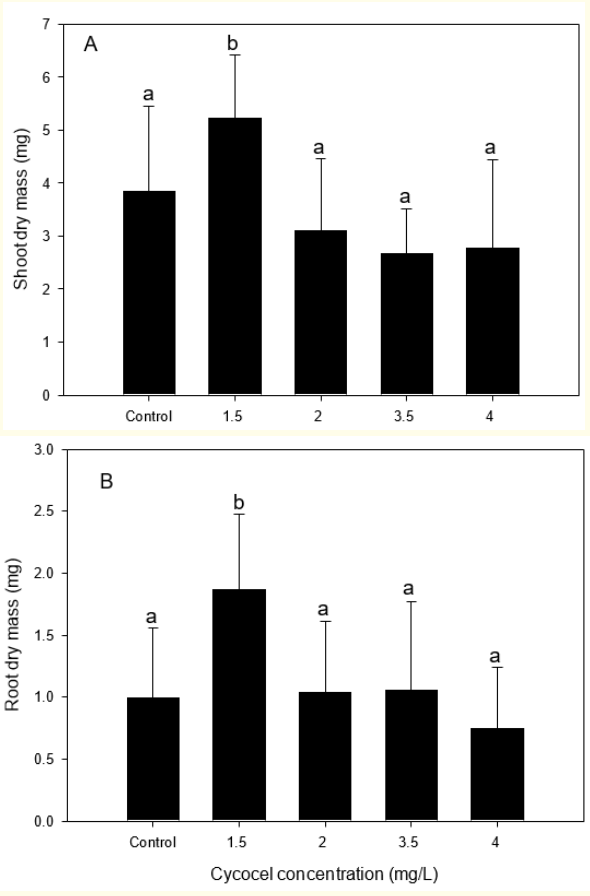

Figure 1: Effect of different concentrations of Cycocel® on plant biomass accumulation in *L. leonurus* after 8 weeks of growth in a hydroponics system. (A) Shoot dry mass. (B) Root dry mass. Values presented are means ± standard deviation. Means followed by dissimilar letters are significantly different from each other at P ≤ 0.05 according to Fischer least significance difference.

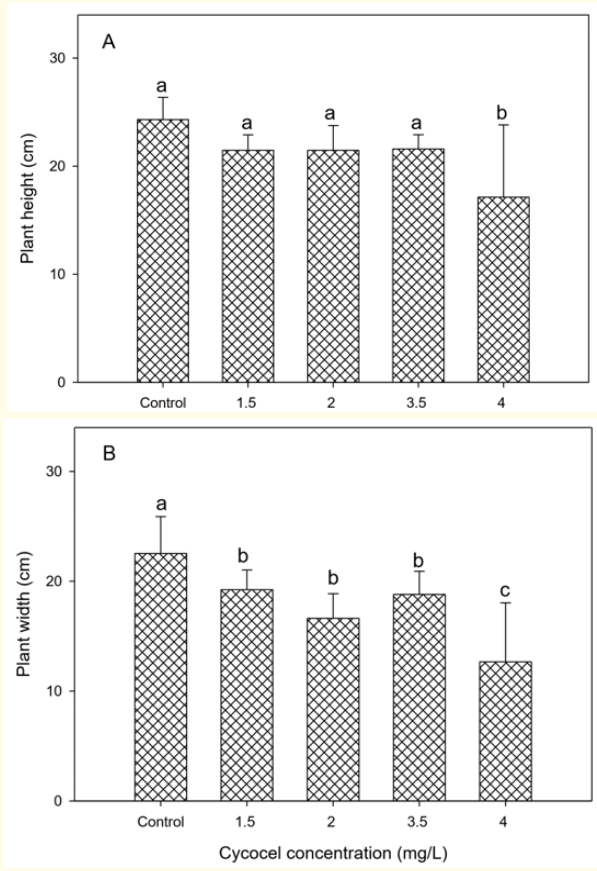

Figure 2: Effect of different concentrations of Cycocel® on *L. leonurus* plant growth parameters as measured after 8 weeks of growth in a hydroponics system. (A) Plant height of *L. leonurus* plants. (B) Plant width of *L. leonurus* plants. Values presented are means ± standard deviation. Means followed by dissimilar letters are significantly different from each other at P ≤ 0.05 according to Fischer least significance difference.

Discussion

The association of flowers with beauty and aesthetic value has over the centuries, led to selection and domestication of many floral plant species, emanating in the emergence of a multi-billion dollar ornamental horticultural industry [12]. The global value of this international floriculture industry is estimated to be US$9 billion per annum [13]. Chief among the ornamental flower industry is plant pot production, in which the use of plant growth regulators remains a cornerstone technology. The majority of plant growth regulators that are used in ornamental plant culture are essentially chemical growth regulators, which control plant size, improve compactness and enhance flowering [14,15]. In the present study, with the exception of 1.5 mg/L Cycocel®, there was no significant difference in the dry mass of *L. leonurus* plants between the treatments and control.

The observed results in our study were contrary to those reported by El-Mokadem and Hadia [16], who established that in *Encelia farinosa* both Cycocel® and B-nine® reduced dry mass of treated plants. The reduction in height and width observed in the present study was possibly due to the dwarfing effect of Cycocel®. Similar results have been reported by Rajala., *et al.* [17], whereby Cycocel® induced a reduction in growth of wheat, oat and barley seedlings.

Teto., *et al.* [18] reported significant reduction in height using paclabutrazol on *L. leonurus.* The dwarfing effect of plant growth retardant such as Cycocel® is due to reduction of stem elongation resulting from inhibition *ent*-kaurene, a key enzyme regulating the giberellin (GA) biosynthesis pathway [19]. Although the term quality is difficult to define with regards to ornamental horticulture, it is commonly used in marketing of these products (Meijón., *et al*. 2009). In this regard, the height/diameter (H/D) ratio has been regularly used as a development index in assessing quality of ornamental plants [20]. The reduction in plant height observed in the present study at a concentration of 4 mg/L Cycocel® may be due to inhibition of GA production [21]. This interference with GA biosynthesis in turn reduces cell division and cell elongation leading to inhibited shoot growth and stem elongation [21].

For compactness the results revealed that if pinching (pruning) is done at the right time then more lateral shoots will be produced, this resulting in well-shaped, bushy and attractive plants. It was also observed that the plants in the control which were not treated with Cycocel® also produced comparatively fewer new axillary shoots throughout the experimental period. Apical dominance and lateral branch production are not only affected by physical removal of bud but also a number of other factors [22]. There was a marked increase in shoot proliferation, notably during week 5 to week 7 in *L. leonurus* plants treated with 3.5 and 4 mg/L Cycocel®. The observed high shoot proliferation may be due to an increase in endogenous cytokinin levels induced by the growth retardant. Cytokinins, a group of naturally occurring adenine derivatives that carry either an isoprene-derived side chain or an aromatic side chain at the N^6-terminus, play multiple crucial roles in plant growth and development including cell division and shoot proliferation [23]. For increasing the number of new shoots, the use of Cycocel® was most effective from week 4 until the end of the experimental period. Thus, for effective induction of new shoots, Cycocel® should be applied from week 4 onwards. On the other hand [24] did not observe an increase in the number of new shoots following the application of plant growth regulators in poinsettia. These contradictory results may suggest a plant species-dependent response to plant growth regulators with regards to shoot proliferation [25-33].

Conclusion

In this study, application of Cycocel® had a significant and desired influence in the control growth in *L. leonurus* plants, which can improve and increase its commercial value in the flowering potted plant market. The application of Cycocel® produced plants with desired compactness characteristics for pot plant floriculture production. Nevertheless, further studies are required to understand the underlying physiological mechanisms involved in the observed growth parameters.

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Author Contributions

KB conducted the experiments, analyzed data and wrote the manuscript. CPL conceptualized the idea, designed the experiments together with KB and supervised the work. MAM participated in analysis and interpretation of data, revising the work critically for important intellectual content and manuscript preparation.

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