Paclobutrazol in the cultivation of *Adenium obesum*

Roberto Jun Takane[1,*], Lydio Luciano de Gois Ribeiro Dantas[1], Antonio Ulisses Medina Gurgel[1], Lamartine Soares Cardoso de Oliveira[1], José César Pontes Moreira[1] and Marcelo de Almeida Guimarães[1]

[1]Departamento de fitotecnia, Centro de Ciências Agrárias, Universidade Federal do Ceará, Av. Mister Hull, 2977, Bloco 805, Campus do Pici, CEP 60356-001, Fortaleza, Ceará, Brazil. *Corresponding author, E-mail: robertotakane@ufc.br.com

ABSTRACT

In floriculture, more specifically in the segment of ornamental packaged plants, *Adenium obesum* has gained popularity. This is due to the important role it plays in the market of ornamental plants. Although its aesthetic standardization as a potted plant has not been defined yet, studies with focus on reducing its size are necessary. Thus, this paper aimed at evaluating the effects of different doses of the vegetal growth retardant paclobutrazol (PBZ) in *Adenium obesum* plant development and quality, in order to enable a differentiated product for the market of flowers and potted plants. The experiment was conducted in a vegetation house in randomized complete blocks with four repetitions. Six treatments were provided, that is, one single application of: 5; 10; 20; 40 and 80 mL of the active ingredient of PBZ, and the control treated with water only. For the biochemical evaluations, we verified the effect of treatments for the anthocyanin variable, with PBZ impairing the concentration of anthocyanin present in leaf tissues. The dose of 80 mL of PBZ reduced the length of aerial part in 37.29% in relation to the control. Despite the decrease observed in the plant final size, the phytotoxic effects caused by PBZ prevented its utilization in the *Adenium obesum* commercial production.

Key words: *Apocynaceae*, floriculture, growth retardant, potted plant.

INTRODUCTION

According to a study on productive chain of ornamental flowers and potted plants done by Lima Júnior *et al.* (2015) in Brazil in 2014, there were 8,248 producers of ornamental flowers and plants, with an average area of 1.82 hectares. They cultivated more than 2,000 species classified in three main product categories: (1) cut flowers and foliage, (2) potted flowers and plants, and (3) ornamental plants intended for landscaping, except grass.

The potted flowers and plants commercialization in Brazil has gained some relative importance over cut flowers and foliage due to a better cost benefit relationship, lower relative costs, higher durability, and higher practicality in decorative use and in domestic or professional environment handling (Junqueira and Peetz 2014). In the segment of packaged plants, *Adenium* sp. has become popular in floriculture due to its resistance to hydric stress, easy to care , flowers with different shapes and hues/colors, abundance and long duration (Oyen 2008; Varella *et al.*, 2015).

The *Adenium obesum* species [(Forssk.), Roem. & Schult.] from the *Apocynaceae* family is relatively new in the segment of packaged flowers in the commercialization of ornamental plants (Mcbride *et al.*, 2014). They swell during root maturation, increasing their size along the stem, thus adding an important sculptural element (Dimmitt *et al.*, 2009).

Al though *Adenium* sp. has a good market acceptance, there are countless factors which interfere with its commercial production. A great emphasis is given to the plant appearance, recipient size, substrate, hydric and nutritional needs, as well as to the control overgrowth. This makes the study on plant size reduction necessary in order to guarantee a production pattern, which is already done for other potted flowers and plants such as *Dendranthema grandiflora* and *Kalanchoe blossfeldiana*. A harmonious relationship between plant and pot size is important, combined with blooming, for the consumer´s aesthetic demands are assured. (Barbosa *et al.*, 2011).

However, an aesthetical standardization as a potted plant is not culturally defined, and that would enable the integration of features such as a more compact size, blooming closer to the commercialization time, and easier logistics to the final consumer. In the production of some pot ornamental plants, the kind of handling that is highly used is the exogenous application of growth retardant, used with the objective of

producing more compact plants and with higher commercialization acceptance (Bonacin *et al.*, 2006).

In light of all of the above, this work aims at evaluating the effects of different doses of the vegetal growth retardant paclobutrazol (PBZ) in the development and quality of *Adenium obesum* plants, in order to enable a differentiated product for the potted flower market.

MATERIAL AND METHODOS

The experiment was conducted in a greenhouse with transparent plastic film cover, and shading screen on the roof and on the sides, which allowed the reading of an average value of photosynthetically active radiation (luminosity) of 525.81 $\mu mol \cdot m^{-2} \cdot s^{-1}$, obtained in ºlux and converted to $\mu mol \cdot m^{-2} \cdot s^{-1}$, using a portable digital luximeter LD-200, once a week at noon until the final evaluation. The daily average temperature in the experimental area was 31.19 °C. A digital thermal hygrometer was used, and the average relative air humidity was 67.26%.

The substrate used in all the treatments was coconut powder (Table 1). We used black polyethylene vases, with 10 cm of height, 14.5 cm of superior diameter, 11 cm of inferior diameter, and capacity of 1.4 L.

Table 1. Chemical analysis of the coconut powder substrate used in the cultivation of the *Adenium obesum* plants.

pH (H_2O)	C.E. dSm^{-1}	P	K	Na^+	Cu $mg \cdot dm^{-3}$	Fe	Mn	Zn
5.90	1.94	33.1	1619.1	420.1	0.00	7.4	0.8	1.22

Ca^{2+}	Mg^{2+}	Al^{3+}	(H + Al)	SB	T	CEC	V	m	ESP
			$cmol_c \cdot dm^{-3}$					%	
1.00	1.20	0.05	1.65	8.17	8.22	9.82	83	1	19

P, K and Na^+: extractor Mehlich^{-1}; Ca^{2+}, Mg^{2+} and Al^{3+}: extractor 1 mol L^{-1} KCl; H+Al: potential acidity, extractor 0.5 mol L^{-1} calcium acetate; SB: sum of bases; t: effective cation exchange capacity; CEC: cation exchange capacity at pH7; m: aluminum saturation; V: base saturation; ESP: exchangeable sodium percentage.

We used seedlings of *Adenium obesum* [(Forssk.), Roem. & Schult.]) propagated by seeds in a commercial nursery, and acquired after 227 days of cultivation. These seedlings were transplanted into vases containing coconut powder substrate, acclimated in the experimental area environment and fertilized weekly with 100 mL of solution at 10% of N-P-K (20-20-20) soluble fertilizer for four months.

For the solution preparation, we used a commercial product composed of 25% active ingredient (a. i.) of PBZ and 83.5% of inert ingredients. The PBZ concentrations were formulated by diluting the quantities of 0; 0.16; 0.32; 0.64; 1.28 and 2.56 mL of commercial product (pipetted) in 8 liters of water (pH corrected at 5.0), in order to obtain the proportions referring to the respective doses of 0; 5; 10; 20; 40 and 80 mL of a. i. of PBZ.

We performed one single application of growth regulator doses at 4 pm, with the help of a Becker and a plastic container to hold the exceeding solution volume. The solutions were manipulated in 20 L plastic buckets, with one bucket for each concentration, which were used for watering up to reach the substrate full retention capacity. During applications, the plants were temporarily removed from the green house to an outdoor environment for treatment application.

For the experiment, we selected the plants that were more uniform in size, with an average height of 15 cm total. The product dose applications began after 117 days of transplanting (DAT). Two days after the applications, we performed the fertilization of each plant, with 6 g of controlled release fertilizer, Basacote® Plus 6M of formulation 16-8-12 (N - P_2O_5 - K_2O). During the experiment, the plants were watered every other day with 100 mL of water.

The experiment was conducted in a randomized complete block, made out of six concentrations of a. i. of PBZ (0; 5; 10; 20; 40 and 80 mL). As a treatment control, the plants were watered with water. Each treatment was composed of 4 plants and 4 repetitions, with one plant per vase.

Three hundred and thirty-five days after transplanting (DAT), we analyzed the following characteristics: aerial part length (cm), defined as the distance from the substrate level up to the tip of the highest stem; root length (LR; cm), the distance from the substrate level down to the bottom of the root, by using a metric ruler; stem diameter (SD; mm), measured above the plant stem by using a digital caliper ruler; the

number of leaves (NL; unit), which were manually removed and counted. With the help of pruning shears and a knife, the plants were cut off at ground level, having the stem and the root separated.

In order to obtain the leaf dry matter (LDM; g), the materials were packed in paper sacks and taken to dry in a drying oven with circulation and air exchange at 60 °C for 72 hours until they reached a constant weight. Since the aerial part dry matter (APDM; g) and the root system dry matter (RSDM; g) are juicy and thick, it was necessary to cut the plant into smaller parts for further drying. These materials were placed in disposable aluminum packaging, which were properly identified and taken to dry in a drying oven with circulation and air exchange at 70 °C for 72 hours until they reached the constant weight. After this time, the samples were weighed down on an analytical scale with three decimals of accuracy.

The leaf area (LA; cm^2) was determined by the use of a leaf area integrator by LI-COR® 3100. The detached plant leaves were processed one by one, and the LA values were shown and noted down. The LA measurements were adjusted by the leaf area integrator to a linear regression.

Variables such as chlorophyll a (Chl *a*), chlorophyll b (Chl *b*), total chlorophylls (Chl *t*) and carotenoids presented estimated concentrations following the methodology proposed by Wellburn (1994). This method consisted of selecting the first pair of leaves that were completely expanded, removing the three disks measuring 1.0 cm diameter, and placing them in test tubes (previously coated with aluminum foil) containing 2 mL of dimethyl sulfoxide solution (DMSO) saturated with $CaCO_3$. After that, they were kept covered through the entire analysis.

Subsequently, the samples were incubated and back-burned at 65 °C for 30 minutes. Then, after this time and after the samples had reached room temperature, the leaf disks were separated (for further weighing) and the pigment extract was used to determine the absorbance at 665, 649 and 480 nm in a spectrophotometer, model Ultrospec 6300 pro. The separated disks were washed down to remove the excess of solution and dried out in a drying oven with circulation and air exchange at 60 °C for 48 hours until the dry matter value was reached.

The concentration estimates of Chl *a*, Chl *b*, Chl *t* and carotenoids were obtained according to the following equations:

$$\text{Chl } a = (12.47 \times A_{665}) - (3.62 \times A_{649});$$
$$\text{Chl } b = (25.06 \times A_{649}) - (6.5 \times A_{665});$$
$$\text{Chl } t = (7.15 \times A_{665}) + (18.71 \times A_{649});$$
$$\text{Carotenoids} = (1000 \times A_{480} - 1.29 \times \text{Chl } a - 53.78 \times \text{Chl } b) / 220,$$

where *A* represents the absorbance in a corresponding wave length expressed in $mg \cdot g^{-1}$ DM (Dry matter).

The anthocyanin concentrations in the *Adenium obesum* plant leaves were calculated according to the equation proposed by Murray and Hackett (1991). Five disks measuring 1.0 cm diameter and containing about 160 mg of leaf matter were removed from the youngest pair of leaves that were completely expanded. These disks were placed in test tubes containing 5 mL of a mixture composed of a solution of HCl at 3 M, deionized water and absolute methanol at the proportion of 1:3:16 (v:v:v). After, the tubes were placed under constant and smooth agitation at 4 °C in the dark for 48 hours and the samples were removed from the agitation and taken to be centrifuged at 3,000 × *g* for 10 minutes. The supernatant obtained was used to determine the absorbance at 530 and 653 nm in a spectrophotometer Ultrospec 6300 pro. The anthocyanin contents were obtained through the following equation:

$$\text{Anthocyanin contents} = A_{530} - 0.24 \times A_{653},$$

in which *A* represents the absorbance in a corresponding wave length, expressed in $mmol \cdot g^{-1}$ FM (Fresh matter).

RESULTS AND DISCUSSION

The variance analysis turned out successful for the PBZ concentrations tested for the Length of aerial part, TL, NL, APDM, RSDM, LDM and TDM variables, whereas for the LR, SD and LA variables they were not successful for the F test, with average values of 15.78 cm, 37.69 mm, 1714.88 cm^2, respectively (Table 2).

The results were submitted to the variance analysis at the levels of $p < 0.01$ and $p > 0.05$ of significance, and the quantitative data averages were employed through regression analysis by using the ASSISTAT software (Silva and Azevedo 2016). The response curve fitting procedure for the treatment factors was done through the Table Curve 2D software (Scientific 1991).

The absence of PBZ (control) fostered the highest averages (47.30 cm) for the length of aerial part (Figure 1a), whereas the maximum dose (80 mL) enabled the acquisition of plants with a smaller value (29.38 cm), a reduction of 37.29% when compared to its witness plant. Similar results were also observed for the TL (Figure 1 b), where the absence of PBZ provided the highest values (63.32 cm), higher than the minimum in 29.23%.

Table 2. A summary of agronomical variables of *Adenium obesum* variance analysis according to pacrobutrazol doses.

		Mean square									
VS	DF	LAP	LR	TL	NL	SD	LA	APDM	RSDM	LDM	TDM
PBZ	5	201.36*	12.84^{nm}	208.96**	419.34*	8.86^{nm}	273.250,62^{nm}	27.41*	63.81*	4.58**	213.02**
Block	3	167.16*	41.48*	253.19**	189.25^{nm}	93.00*	902.143,45**	2.74^{nm}	6.82^{nm}	3.12^{nm}	22.57^{nm}
Residue	15	38.45	11.16	44.92	103.52	21.02	119.105,74	1.05	2.23	1.23	8.74
VC (%)	-	14.30	23.88	11.69	18.85	11.70	20.32	12.85	16.88	23.48	13.74

VS – Variation source; DF – Degree of freedom; VC = Variation coefficient; * and ** Significant at p <0.01 and p> 0.05 of probability, respectively; nm – not significant by the F test.

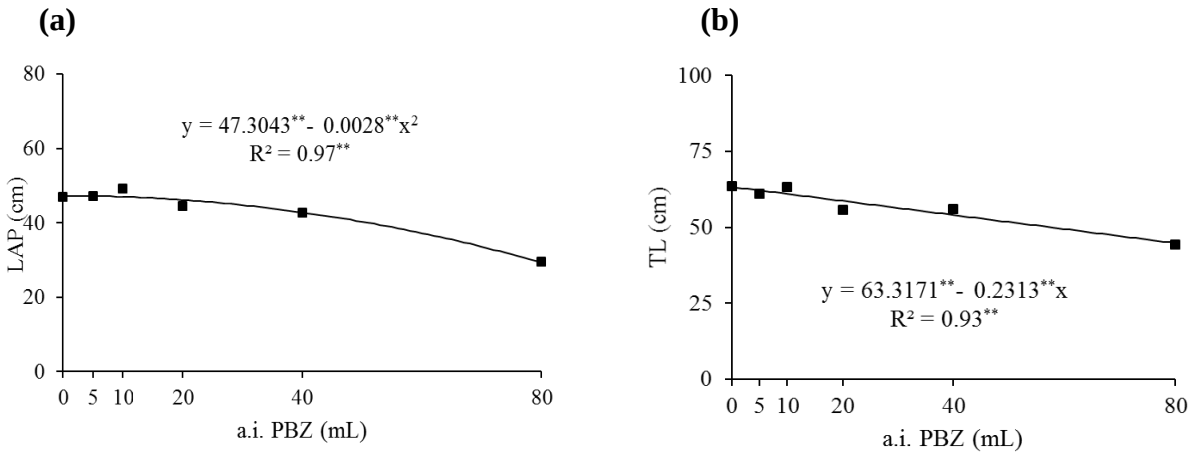

Figure 1. Length of aerial part (a) and total length (TL; b) of *Adenium obesum* plants according to different doses of PBZ.

This result, which is in accordance with the increasing doses of PBZ, is associated both with the APDM (Figure 2b) and the RSDM (Figure 2c) decrease, supporting the effect which is inhibitory to growth fostered by the gibberellin synthesis inhibition. These inhibitors have been useful for the cultures in which a reduction in plant height is wanted (Taiz and Zeiger 2013).

Similar results were found by Albornoz *et al.* (2014), when studying different doses of PBZ over the growth of *Nerium oleander* L. (*Apocynaceae)* plants, confirming that the application of this growth regulator was higher than in the control, with the higher dose of growth regulator (40 mg·L^{-1}) presenting a reduction in height of 20 cm, compared to the witness plant. Ochoa *et al.* (2009) presented similar results in a study on *Nerium oleander* L., applying the dose of 20 mg·L^{-1} of PBZ and noticed a reduction of 37% in height when compared to the treatment control, validating the effect of using PBZ for size reduction.

The vegetative growth inhibition associated with PBZ has been linked to the application method (Mauk *et al.*, 1990), which is the application via soil up to reaching field capacity. It proved to be more efficient than the application via aerial part spraying, when it comes to plant size reduction (Pardos *et al.,* 2005; Al-khassawneh *et al.*, 2006). Thus, this application method is more efficient for growth control, since the regulator is rapidly absorbed by the root system and immediately translocated to the aerial part. However, only a small fraction of a. i. reaches the apical meristems, where the inhibitory effects take place (Barbosa *et al.*, 2008).

The high NL of 73.74 leaves·plant^{-1} was obtained with the maximum dose of PBZ (80 mL), surpassing the minimum value of 33.10% in relation to control, which presented a value of 49.33 leaves·plant^{-1} (Figure 3).

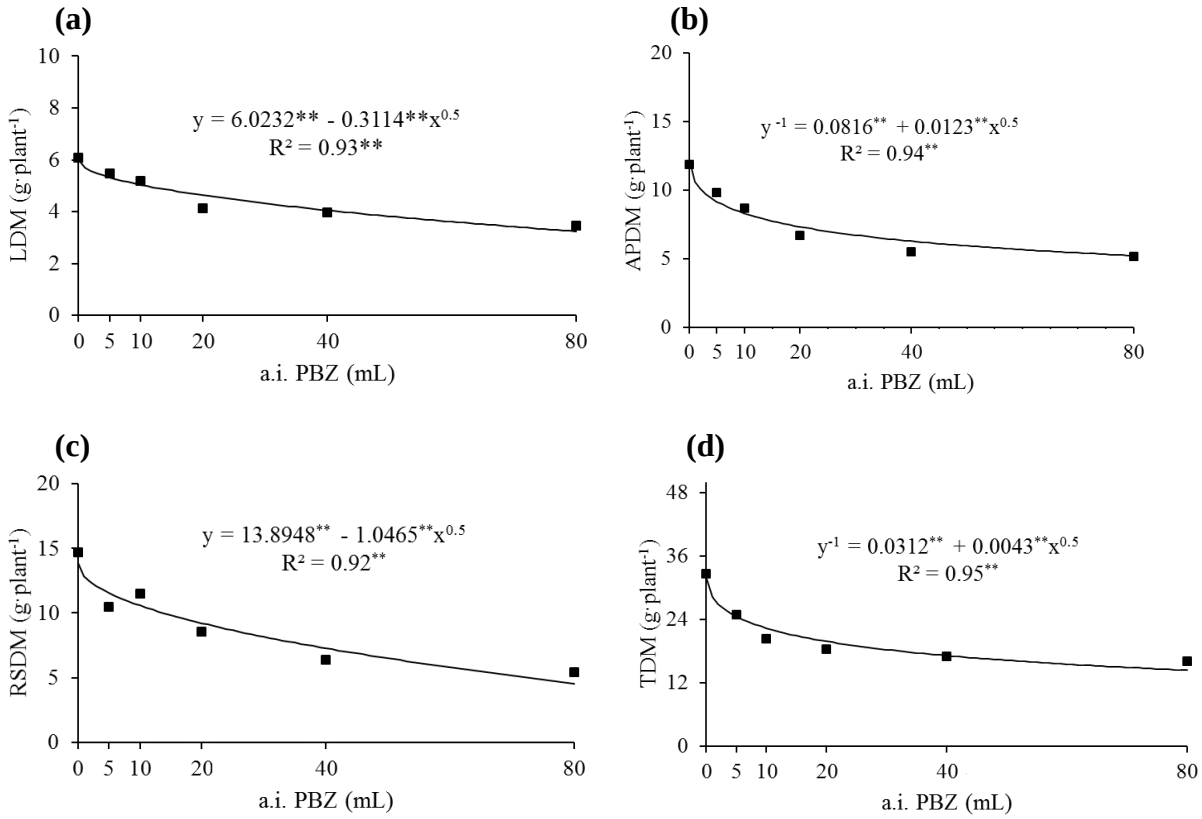

Figure 2. Leaf dry matter (LDM; a), aerial part dry mass (APDM; b), root system dry matter (RSDM; c) and total dry matter (TDM; d) of the *Adenium obesum* plants according to different PBZ doses.

This result was different from that obtained by Albornoz *et al.* (2014), who found smaller values of NL according to the increase in PBZ doses (0; 20 and 40 mg·L^{-1}) in the size reduction of *Nerium oleander* L., being statistically inferior to the control. The result was probably due to the emission of a high quantity of small leaves, commonly found at the base of some *Adenium obesum* plants. This increase in numbers can be justified, but not in mass, since the LDM result (Figure 2a) decreased according to the increase in PBZ doses, confirming a reduction.

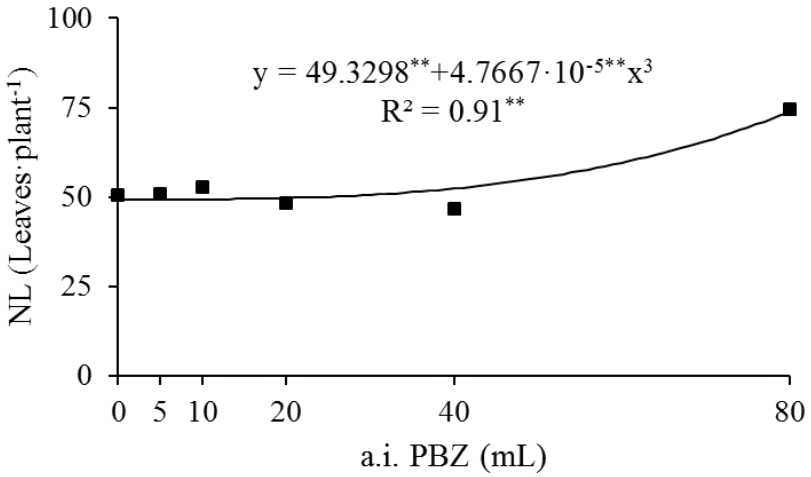

Figure 3. Number of leaves (NL) of *Adenium obesum* plants according to different PBZ doses.

For the *Adenium obesum*, we noticed that increasing doses of PBZ significantly reduced the quantity of APDM, which decreased from 12.25 g·plant^{-1} (control) to 5.23 g·plant^{-1} with 80 m in a proportion of 57.33% (Figure 2b). The same event also happened for the RSDM, LDM and TDM according to the increase in PBZ

doses. The highest dose applied (80 mL) reduced LDM in 46.24% (figure 2a), RSDM in 67.36% (Figure 2 c) and TDM in 55.21% (Figure 2d). In general, this behavior in dry matter reduction was expected, since PBZ is a growth reducer that influences the inhibition of cell formation and the internode stretching below the meristem. This is an advantage for the potted plant production system, since it improves the aesthetical conformity of the plant/vase set.

With the application of PBZ, the effect on dry bio matter reduction was more evident on the RSDM variable. This is an advantageous factor for the species cultivated in vases, since they can be used in a smaller container enabling easy handling in their productive chain.

For the biochemical evaluations, we noticed an effect on the PBZ concentrations studied only for the anthocyanin variable, whereas for the remaining variables, Chl *a*, Chl *b* and Chl *t*, as well as for the carotenoids, no effects could be observed (Table 3).

Table 3. A summary of the variance analysis for chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoids and anthocyanin in *Adenium obesum* plants according to paclobutrazol doses.

VS	Mean square					
	DF	Chl *a*	Chl *b*	Chl *t*	*Carotenoids*	*Anthocyanin*
PBZ doses	5	0.03^{nm}	0.09^{nm}	0.16^{nm}	0.003^{nm}	0.38**
Block	3	0.06^{nm}	0.26^{nm}	0.37^{nm}	0.009^{nm}	0.03^{nm}
Residue	15	0.05	0.09	0.24	0.005	0.05
VC (%)	-	14.38	15.69	14.10	20.20	18.06

VS = Variation source; DF = Degree of freedom; VC = Variation coefficient; * and ** Significant at $p < 0.01$ and $p > 0.05$ of probability, respectively; nm –not significant by the F test.

For the anthocyanin variable, the highest values were inversely proportional to the increase in PBZ doses (Figure 4). The treatment control provided the highest value observed of 1.27 mmol·g^{-1} of leaf dry matter, whereas the highest dose (80 mL) presented the smallest value, 1 mmol·g^{-1} of leaf dry matter, being higher than the minimum value of 21.71%. Although this is a small difference, the level of anthocyanin in *Adenium obesum* leaves may be correlated to the increase in LDM, with the highest average among the treatment control.

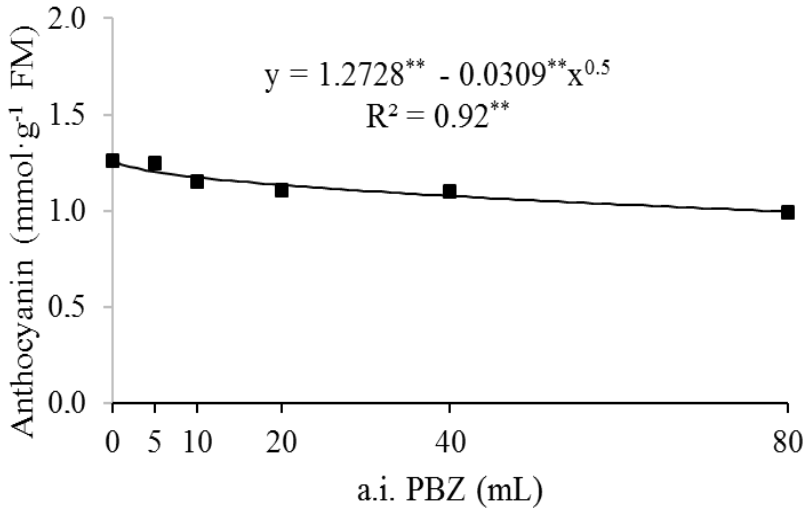

Figure 4. Anthocyanin in *Adenium obesum* plants according to different PBZ doses.

According to Scatena and Nunes (1996), the anthocyanin synthesis in leaves may be associated with several environmental stresses such as nutritional, hydric and fungal attack, as well as related to intense

radiation. According to Araújo and Deminics (2009), ecophysiological functions of anthocyanin are attributed to the osmotic adjustment in stressful conditions of drought and cold, like antioxidant, protection against ultraviolet and visible lights, suggesting the anthocyanins act as filters of visible light and that, during leaf expansion, senescence and responding to abiotic stresses, the anthocyanin synthesis takes place in the leave epidermal layers.

Contradicting this last aspect, the abiotic stress provided by PBZ application likely influenced the anthocyanin synthesis decrease in the plants studied, presenting as a negative effect due to the protective benefits of this flavonoid.

Another negative effect provided by the growth regulator studied was the phytotoxicity emphasized in the treated plant leaves with higher intensity according to the dose increase, where they presented to be deformed, twisted or even with a deformed appearance.

CONCLUSIONS

From the results obtained with this research, we can conclude that the dose of 80 mL of a. i. of PBZ reduced the length of aerial part in 37.29% in relation to the control. However, the phytotoxic effects caused by the growth regulator could hinder the *Adenium obesum* commercial production.

REFERENCES

Albornoz A, Fernández M, Vilchez J, Fernández C and Martínez L (2014) Efecto del paclobutrazol sobre el crecimiento de plantas de berbería (*Nerium oleander* L.) en fase de vivero. Revista de la Facultad de Agronomía de la Universidad del Zulia 31(Suppl 1):301-311.

Al-khassawneh NM, Karam NS and Shibli RA (2006) Growth and flowering of black iris (*Iris nigricans* Dinsm.) following treatment with plant growth regulators. Scientia Horticulturae 107(2):187-193.

Araújo SAC and Deminicis BB (2009) Fotoinibição da fotossíntese. Revista Brasileira de Biociências 7(4):463-472.

Barbosa JG, Barbosa MS, Tsuji SS, Muniz MA, Grossi JAS and Rubim M (2008) Cultivo de girassol ornamental (*Helianthus annuus* L.) em vaso sob diferentes doses de paclobutrazol. Ornamental Horticulture 14(2):205-208.

Barbosa JG, Muniz MA, Mesquita DZ, Cota FO, Barbosa JM, Mapeli AM, Pinto CMF and Finger FL (2011) Doses de solução nutritiva para fertirrigação de pimentas ornamentais cultivadas em vasos. Ornamental Horticulture 17(1):29-36.

Bonacin GA, Rodrigues TJD and Mattiuz CFM (2006) Aplicação de retardadores de crescimento em híbridos de girassol ornamental. Ornamental Horticulture 12(1):37-42.

Dimmitt M, Joseph G and Palzkill D (2009) Adenium: Sculptural Elegance, Floral Extravagance. Scathingly Brilliant Idea, Tucson.

Junqueira AH and Peetz MS (2014) O setor produtivo de flores e plantas ornamentais do Brasil, no período de 2008 a 2013: atualizações, balanços e perspectivas. Ornamental Horticulture 20(2):115-120.

Lima Júnior JC, Nakatani JK, Monaco Neto LC, Lima LACV, Kalaki RB and Camargo RB (2015) Mapeamento e Quantificação da Cadeia de Flores e Plantas Ornamentais do Brasil. OCESP, São Paulo, 132p.

Mauk CS, Unrath CR, Blankenship SM and Lehman LJ (1990) Influence of method of application of paclobutrazol on soil residues and growth retardation in a 'Starkrimson-Delicious' apple orchard. Plant growth regulation 9(1):27-35.

Mcbride K, Henny RJ, Chen J and Mellich TA (2014) Effect of light intensity and nutrition level on growth and flowering of *Adenium obesum* 'Red' and 'Ice Pink'. HortScience 49(4):430-433.

Murray JR and Hackett WP (1991) Dihydroflavonol reductase activity in relation to differential anthocyanin accumulation in juvenile and mature phase *Hedera helix* L. Plant Physiology 97:343-351.

Ochoa J, Franco JA, Bañón S and Fernández JA (2009) Distribution in plant, substrate and leachate of paclobutrazol following application to containerized *Nerium oleander* L. seedlings. Spanish Journal of Agricultural Research 7(3):621-628.

Oyen LPA (2008) *Adenium obesum* (Forssk.) Roem. & Schult. Schmelzer GH and Gurib-Fakim A (eds.) Medicinal plants 1. Editor Prota, Wageningen,11:1-7.

Pardos M, Calama R, Montero G and Pardos JA (2005) Growth of container-grown cork oak seedlings as affected by foliar and soil application of paclobutrazol. HortScience 40(6):1773-1776.

Scatena VL and Nunes AC (1996) Anatomia de *Pleurothallis rupestris* lindl. (*orchidaceae*) dos campos rupestres do brasil. Boletim de Botânica da Universidade de São Paulo 15:35-43.

Scientific J (1991) Table Curve: Curve fitting software. Jandel Scientific, Corte Madera, CA.

Silva FAS and Azevedo CAV (2016) The Assistat Software Version 7.7 and its use in the analysis of experimental data. African Journal of Agricultural Research 11(39):3733-3740.

Taiz L and Zeiger E (2013) Fisiologia vegetal. 5. ed. Artmed, Porto Alegre.

Varella TL, Silva GM, Cruz KZCM, Mikovski AI, Nunes JRS, Carvalho IF and Silva ML (2015) In vitro germination of desert rose varieties. Ornamental Horticulture 21(2):227-234.

Wellburn AR (1994) The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrometers of different resolution. Journal of Plant Physiology 144:307-313.

Received: July 10, 2019.
Accepted: August 28, 2019.
Published: November 14, 2019.