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Scientific Article

Growth of fertigated desert rose in different nitrate/ammonium proportion

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Abstract

This study aimed to determine NO_3^-/NH_4^+ proportion delivered by fertigation that allows greater desert rose plants growth in pots. Plants were cultivated in greenhouse in polypropylene pots, using a mixture of sand and composted Pinus powder (1:1, v v^{-1}) as substrate. Experimental design was completely randomized, with six treatments (control, 0/100 25/75, 50/50, 75/25 and 100/0 of NO_3^- and NH_4^+ respectively), and ten replications. Fertigation was performed weekly in the amount of 100 mL per pot for 180 days. Variables evaluated were: shoot height, basal diameter of the caudice, number of branches, roots, leaves and caudice dry mass and macronutrient contents determination and accumulation in leaves, caudice and root. NO_3^- and NH_4^+ proportion promoted a greater change in nutrient content in leaves compared to caudice and roots. The proportion 25/75 NO_3^-/NH_4^+ is recommended for desert rose cultivation, as it provides the best results for most of the characteristics studied.

Keywords: *Adenium obesum*, nitrogen, nutrition, pot flower.

Resumo

Crescimento de rosa do deserto fertirrigada com diferentes proporções de nitrato/amônio

Este estudo teve como objetivo determinar a proporção de NO_3^-/NH_4^+, fornecidos por fertirrigação que permita o melhor crescimento de plantas de rosa do deserto cultivadas em vaso. As plantas foram cultivadas em casa de vegetação, em vasos de polipropileno, tendo como substrato uma mistura de areia e pó de pinus compostado (1:1, v v^{-1}). Utilizou-se o delineamento experimental inteiramente casualizado, com seis tratamentos (controle, 0/100 25/75, 50/50, 75/25 e 100/0, de NO_3^- e NH_4^+ respectivamente), e dez repetições. As fertirrigações foram realizadas semanalmente na quantidade de 100 mL por vaso durante um período de 180 dias. As variáveis avaliadas foram: altura da parte aérea, diâmetro basal do cáudice, número dos ramos, massa seca de raízes, folhas e cáudice e determinação dos teores e acúmulo de macronutrientes de folha, cáudice e raiz. As proporções de NO_3^- e NH_4^+ promoveram maior alteração no teor de nutrientes nas folhas comparado ao cáudice e raízes. Recomenda-se para o cultivo de rosa do deserto a proporção 25/75 de NO_3^-/NH_4^+, por proporcionar melhor acúmulo de massa seca pelas plantas, bem como aumento na altura e diâmetro do cáudice das plantas.

Palavras-chave: *Adenium obesum*, nitrogênio, nutrição, flor de vaso.

Introduction

Floriculture is one of the segments in Brazilian agribusiness that present greater profitability per cultivated area, as it includes cut flowers, leaves and potted plants cultivation (Junqueira and Peetz, 2016). Allied to the potential of market expansion and to the search for differentiated products, *Adenium obesum*, popularly known as desert rose, is on a leading place in national scenario. It is a plant cultivated in pots and appreciated for developing swollen roots and/or shoots that serve as primary organs for water storage, and for presenting a variety of flowers shades and forms (Dimmitt et al., 2009; Brown, 2012; Colombo et al., 2015; Colombo et al., 2017).

Although there has been an evolution in ornamental plants commercialization, there are still innumerous factors that interfere in production. For better performance in plant final quality it is fundamental to know about the mineral nutrition of cultivated species (Barbosa et al., 2011). Among the few scientific data available about desert rose mineral nutrition, it is worth mentioning McBride et al. (2014) study, which reports nitrogen (N) as the nutrient with highest total level present in *A. obesum* plants; however, Colombo et al. (2016) reported in their study that N is the second nutrient

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most absorbed in the referred species. Therefore, to better understand fertilization and its usage in desert rose plant nutrition, more scientific studies are needed.

Nitrogen is required in higher quantities for most of plants. In the soil or in the substrate there are two mineral N predominant forms available for plants: nitrate (NO_3^-) and ammonium (NH_4^+) (Schloerring et al., 2002; Lane and Bassirirad, 2002; Silva et al., 2010).

Some species absorb N preferably in ammoniacal form (Malagoli et al., 2000), while others absorb it in nitric form. This may occur according to the species and environmental factors (Mengel and Kirkby, 1978). To be absorbed by plants, nitrate must be reduced in an energy dependent process and mediated by nitrate reductase enzyme (NR) that reduces $NO_3^–$ to $NO_2^–$, while nitrite reductase (NiR) reduces nitrite to NH_4^+ whereas ammonium does not require this step to be assimilated (Taiz and Zeiger, 2004; Hachiya et al., 2012).

Nitrogen addition under different ionic forms causes effects on plant growth, vigor, production and reproduction (Guo et al., 2012), and may result in positive or negative physiologic responses (Bartelheimer and Poschlod, 2013). NH_4^+ high concentration can be cytotoxic, causing chlorosis and reducing size, when compared to NO_3^- in equal concentration (Walch-Liu et al., 2001; Britto and Kronzucker, 2002; Miller and Cramer, 2005). NH_4^+ negative effects are often associated with rhizosphere acidification, lower cations absorption, hormonal imbalance and organic acids depletion (Roosta and Schjoerring, 2007; Hachiya et al., 2012).

Some studies report that combining $NO_3^–$ and NH_4^+ may reduce this last ion toxicity in some species such as wheat, tomato and corn (Britto and Kronzucker, 2002; Garnica et al., 2009). Muniz et al. (2009) studying production and quality of potted chrysanthemum fertigated with different nitrate/ammonium relations concluded that the best plant growth was achieved when ammonium proportion was 50% of total N.

This contradiction about the best nitrogen form to use and the lack of information in Brazilian literature about how NO_3^-/NH_4^+ relations affect ornamental plants growth and development, show the importance of this study. Therefore, the aim of this work was to determine NO_3^-/NH_4^+ proportion, delivered through fertigation that allows a better growth in potted desert rose.

Material and Methods

This experiment was conducted under a Van der Hoeven® greenhouse, with 50% light retention and temperature varying from 28 ± 3 ºC, from August 2015 to March 2016.

Adenium obesum seedlings were produced from germinated seeds in polystyrene trays with composted Pinus powder substrate. These seeds were donated from Sandro Takemura (from Flora Takemura), a desert rose grower (Londrina, Paraná). During transplanting, 60 seedlings with 60 days mean age were selected, with the following characteristics: shoot height (4,0 ± 0,6 cm), caudice diameter (11,5 ± 2 mm) and dry mass (0,12 ± 0,02 g), which was determined using a sample of ten plants. Plants were transplanted to polypropylene pots with diameter of 13 cm, height of 9.8 cm and volume of 1000 mL, filled with a sand and composted Pinus powder mix, in 1:1 (v v^{-1}) proportion. Irrigation was performed daily, manually, except in fertigation days, applying a six-millimeter water line per pot.

Experimental design was completely randomized with ten replications, being considered one plant per experimental plot. Six proportions of nitrate/ammonium ($NO_3^–/NH_4^+$) were used as treatments (control, 0/100, 25/75, 50/50, 75/25 and 100/0) (Table 1). Fertigation was performed weekly through manually watering in the nutritive solution amount of 100 mL per pot.

Table 1. Nutritive solution composition with NO_3^-/NH_4^+ different proportion, used in desert rose fertigation.

Macronutrients (mmol L^{-1})	NO_3^-/NH_4^+ proportion				
	0/100	25/75	50/50	75/25	100/0
KH_2PO_4	0	0	0	0	0.07
KNO_3	0	0	0	0.65	1.73
KCl	1.80	1.80	1.80	1.15	0
$Ca(NO_3)_2$	0	1.95	3.90	5.20	5.20
$CaCl_2$	2.60	1.62	0.65	0	0
$MgSO_4$	0	0	0	0	0.50
$MgCl_2$	0.60	0.60	0.60	0.60	0.10
NH_4Cl	6.73	4.78	2.83	0.88	0
$NH_4H_2PO_4$	0.07	0.07	0.07	0.07	0
$(NH_4)_2SO_4$	1.00	1.00	1.00	1.00	0
$NaNO_3$	0	0	0	0	0.87
NaCl	0.87	0.87	0.87	0.87	0

After 180 days, plants were removed from pots and roots were washed in running water, to remove substrate. Lately, these plants were separated in roots and shoot, from which leaves were separated. Different tissues were washed in distilled water for a posterior evaluation of the following characteristics: shoot height (cm), caudice basal diameter (mm), number of branches, dry mass of leaves (g), caudice (g) and roots (g), as well as measurement of macronutrients levels and accumulation in leaves, caudice and root. Plant height was obtained measuring from the bottom of the plant to its apex, with the help of a graduated ruler, caudice basal diameter was obtained from the mean of two diameter measures, with the help of a digital caliper. Number of branches per plant was obtained through counting. Leaves, caudice and roots dry mass were obtained after drying tissues under a forced ventilation oven at 55 °C until reaching constant mass and subsequent weighting in an analytical balance with an 0.001 g accuracy.

After this process, tissues were milled in analytical mill model A11IKA® for macronutrients level determination and its accumulation. Nitrogen, phosphor, potassium, calcium and magnesium levels were quantified following methodologies described by Malavolta et al. (1997). In nitro-perchloric digestion extract was quantified phosphor by colorimetric, calcium and magnesium by atomic absorption spectrophotometry and potassium by flame photometry. Nitrogen was obtained through sulfuric digestion and quantified by Kjeldahl method (Instituto Adolfo Lutz, 2008). Results were expressed in g kg^{-1}.

In order to obtain plant nutrient accumulation, dry mass values were multiplied by nutrient levels. Concerning substrate, pH and electrical conductivity (EC) were evaluated. Their determination followed methodology described by Abreu et al. (2007), through extraction method 1:2 (v/v) of substrate and deionized water, and readings with the help of portable pH meter and conductometer.

For normality and homogeneity assumption, variance analysis and Tukey test were used, using the software R for these analyses. When the tests did not meet the expectation, data were transformed or was applied Kruskal Wallis non-parametric test (Pimentel-Gomes, 2009; Barbin, 2013).

Results and Discussion

Results showed that nitrate/ammonium proportion do not interfere in characteristics as shoot height, caudice basal diameter and number of branches; however, they showed significance difference comparing to control (no fertigation) (Figure 1).

Figure 1. Desert rose (*Adenium obesum*) vegetative growth in function of NO_3^-/NH_4^+ proportion in nutritive solution, after 180 days. C: (Control) without nutritive solution application.

For leaves, caudice and roots dry mass, plants submitted to nitrate/ammonium proportion (25/75) showed greater means, differing significantly from other treatments by Tukey test (5%) (Table 2).

Table 2. Means for characteristics: shoot height (HGT), caudice basal diameter (CBD), number of branches (NB), leaves dry mass (LDM), caudice dry mass (CDM) and roots dry mass (RDM), in function of NO_3^-/NH_4^+ proportion applied via fertigation in *Adenium obesum* plants.

NO_3^-/NH_4^+	HGT (cm) **	CBD (mm) **	NB**	LDM (g) **	CDM (g)	RDM (g) **
0/100	13.12 a*	25.95 a	4.60 a	1.28 b	2.03 b	0.89 d
25/75	13.08 a	26.06 a	4.50 a	1.60 a	2.72 a	1.25 a
50/50	12.86 a	26.58 a	4.50 a	1.03 c	1.99 b	1.11 b
75/25	12.90 a	26.06 a	4.40 a	0.99 cd	1.97 b	0.96 c
100/0	12.96 a	26.10 a	4.70 a	0.93 d	1.93 b	0.91 d
Control	5.84 b	18.92 b	1.50 b	0.17 e	0.79 c	0.19 e
C.V. (%)	9.60	8.82	12.80	11.73	5.12	9.94

*Means followed by the same letter in columns do not differ by Tukey test 5%. ** Kruskal Wallis non-parametric test (HGT, CBD, NB, LDM, RDM).

Concerning dry mass production, considering the ammonium proportion increase in nutritive solution, this behavior can be related to the lower energy request to assimilate NH_4^+ in comparison to NO_3^- (Sandoval et al., 1995; Bijlsma et al., 2000). Klett and Gartner (1975) found greater dry mass accumulation in chrysanthemum ‘Brigth Golden Anne’, cultivated in pine bark substrate, when plants were fertigated with both nitrogen forms, NO_3^- and NH_4^+, than when only one font was used. Similar results are described by Muniz et al. (2009) in which the better chrysanthemum varieties growth was obtained when ammonium proportion was present in 50% proportion of total N. According to Pan et al. (1997), N is absorbed in *Cymbidium sinense* preferentially as NO_3^-N, although NH_4-N and NO_3-N combination, when supplied in properly amounts, is more indicated for leaves and root growth.

Lower dry mass values obtained in treatment 0/100, comparing to 25/75, may be a result to excessive NH_4^+, which can be toxic to plants in high concentration (Zhou et al., 2017). Regarding substrates pH (Table 3), values varied between 6.58 (75/25) and 7.09 (25/75), with all values tending to neutrality.

Table 3. Observed means for substrate pH and electrical conductivity (CE) in function of NO_3^-/NH_4^+ proportion applied via fertigation in desert rose plants (*Adenium obesum*), after 180 days.

NO_3^-/NH_4^+	pH (H_2O) **	EC (µS cm^{-1})
0/100	6.86 bc*	166.76 b
25/75	7.09 a	146.10 c
50/50	6.87 bc	257.60 a
75/25	6.80 c	252.30 a
100/0	6.92 b	255.50 a
Control	6.93 b	123.60 d
C.V. (%)	2.59	6.96

*Means followed by the same letter in columns do not differ by Tukey test 5%. ** Kruskal Wallis non-parametric test (pH).

Electrical conductivity values (Table 3) varied from 123.60 µS.cm^{-1} (control) to 257.60 µS cm^{-1} (50/50). Takane et al. (2006) characterize as substrate salinization values above 500 µS cm^{-1}, therefore treatments remained below this maximum salinization level recommended by these authors, not affecting desert rose seedlings growth. The lower EC verified in 25/75 substrate was due to the higher nutrient absorption, which can be clearly indicated by the macronutrients accumulation in plants (Table 4).

Table 4. Macronutrients accumulation: nitrogen (N), phosphor (P), potassium (K), calcium (Ca) and magnesium (Mg) in *Adenium obesum* plants, in function of NO_3^-/NH_4^+ proportion applied via fertigation, after 180 days.

NO_3^-/NH_4^+	N	P	K	Ca	Mg
	------------------------------------mg plant^{-1}----------------------------------				
0/100	72.39 c*	16.88 c	114.46 b	51.87 c	13.50 c
25/75	99.11 a	25.51 a	157.62 a	76.95 a	17.88 a
50/50	87.41 b	20.86 b	119.87 b	59.79 b	16.47 ab
75/25	63.66 d	20.64 b	90.95 c	53.13 c	16.09 b
100/0	54.22 e	14.87 d	96.64 c	50.17 c	14.32 c
Control	10.46 f	3.85 e	31.67 d	10.36 d	2.59 d
C.V. (%)	6.87	7.03	6.42	7.58	7.33

*Means followed by the same letter in columns do not differ by Tukey test 5%.

Concerning macronutrients accumulation by *Adenium obesum*, plants submitted to different NO_3^- and NH_4^+ proportion differed significantly by Tukey test. Greater values for N, P, K, Ca and Mg were observed when ammonium was present in nutritive solution around 75% of total N, mainly due to higher plant growth (Table 2). Generally, quantity of absorbed nutrients followed the sequence: K > N > Ca > P > Mg (Table 4), supporting results found by Alves (2016) and Colombo et al. (2016) in studies with the same species. On the other hand, McBride et al. (2014) reported that N is the most absorbed nutrient for this species, followed by K. Regarding different NO_3^- and NH_4^+ proportion and their nutritional effects, these elements significantly affect nutrients level in leaves, caudice and roots of *Adenium obesum* (Table 5). For N level, values found in leaves were from deficient (control) to sufficient (other treatments), considering values used as reference (Table 5) obtained from McBride (2012) study, which states that appropriate N concentration in *Adenium obesum* leaves is among 19 and 24 g kg^{-1}.

The macronutrients P, K and Ca levels were above reference values in all studied organs (Table 5) and showed significant difference among treatments. However, increasing these nutrients levels did not influence on height and caudice diameter values, which did not differ among treatments, except when relating to control. It´s common to find on literature conclusions on K consume for several cultures (Epstein and Bloom, 2005; Castro et al., 2005).

Table 5. Macronutrients levels: nitrogen (N), phosphor (P), potassium (K), calcium (Ca) and magnesium (Mg) in *Adenium obesum* leaves, caudice and roots, in function of NO_3^-/NH_4^+ proportion applied via fertigation, after 180 days.

NO_3^-/NH_4^+	N	P	K	Ca	Mg
Leaves	------------------------------g kg $^{-1}$------------------------------------				
0/100	27.59 b*	4.59 bc	32.72 b	17.44 b	3.75 bc
25/75	29.92 a	4.28 d	36.34 a	24.28 a	3.97 ab
50/50	31.63 a	5.02 a	30.49 c	25.61 a	4.51 a
75/25	24.35 c	4.48 cd	23.39 f	18.57 b	4.49 a
100/0	27.25 b	4.81 ab	28.18 d	17.47 b	4.35 a
Control	17.35 d	3.13 e	26.29 e	16.69 b	3.39 c
C.V. (%)	4.97	3.88	4.18	9.41	10.83
***VR	19-24	0.9-1.2	4.5-5.5	15-16	8.4-8.8
Caudice					
0/100	13.07 bc	3.24 c	22.57 b	11.37 c	2.85 c
25/75	12.09 c	4.30 b	22.68 b	10.86 c	2.41 d
50/50	18.61 a	5.42 a	25.68 a	13.30 b	3.94 ab
75/25	13.67 b	5.67 a	23.06 b	14.55 a	4.14 a
100/0	9.59 d	3.44 c	22.70 b	14.88 a	3.69 b
Control	7.96 e	3.01 c	27.20 a	7.85 d	1.86 e
C.V. (%)	7.36	11.7	7.82	7.3	5.49
***VR	15-21	1.0-1.4	8.9-13.4	8.2-10.6	5.3-7.8
Roots					
0/100	11.85 d	4.97 abc	30.07 a	7.26 a	3.28 b**
25/75	14.68 b	5.57 a	30.23 a	6.85 a	3.98 a
50/50	16.03 a	4.42 bc	33.66 a	6.26 ab	3.59 ab
75/25	13.15 c	5.24 ab	23.30 b	6.34 ab	3.63 ab
100/0	11.39 d	4.13 c	29.25 a	5.72 b	3.47 b
Control	6.45 e	4.95 abc	30.06 a	6.93 a	2.89 c
C.V. (%)	7.77	13.02	11.64	15.32	14.28
***VR	15-25	1.3-2.1	16-28	3.4-4.6	7.0-9.8

*Means followed by the same letter in columns do not differ by Tukey test 5%. ** Kruskal Wallis non-parametric test (Mg in roots). ***VR: reference value for each nutrient based on McBride study (2012).

Mg remained below the references values in all studied organs (Table 5). Although, despite its level improvement comparing to control, treatments used may not have made provided the required amount of Mg to plant growth. Mg acts in chlorophyll molecule tetrapyrrole ring and as an activator of RUBISCO enzyme, being essential to atmospheric C fixation (Strei et al., 2005; Andersson, 2008). It also works as ribosome cofactor during protein translation (Petrov et al., 2012).

Conclusions

It is recommended, for desert rose cultivation, the use of 25/75 NO_3^-/NH_4^+ proportion, which provided better results for most of the phytometrics characteristics and greater accumulation values for all nutrients evaluated, except for Mg, resulting in better plant performance.

Author Contribution

V.S. 0000-0002-1552-2560: installation of the experiment, analysis and collect of data, preparation of the manuscript. **G.A.C.A.** 0000-0002-4472-1833: Creation idea, installation of the experiment, conduction, preparation of the manuscript. **T.M.R.** 0000-0002-2314-6198: Collaboration in the preparation of manuscript in discussion and results. **R.C.** 0000-0001-66492819: collaboration in the preparation of the manuscript under discussion and results. **G.B.** 0000-0002-0447-3521: statistical analysis of the data. **R.T.F.** 0000-0002-7595-1965: Research orientation, suggestions and ideas.

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