Nitrogen doses in the growth of *Calceolaria* x *herbeohybrida* in pot[1]

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ABSTRACT

Calceolaria x *herbeohybrida* Voss (pocketbook plant, calceolaria) is an herbaceous perennial and floriferous species that can be grown in pots or beds. Nitrogen is usually the most required nutrient, being necessary to conduct studies in order to provide useful information for agronomic practices. Thus, this study aimed to evaluate nitrogen doses in the growth of *Calceolaria* x *herbeohybrida* in pots. Initially, assorted and pelleted calceolaria seeds were sow in multicellular trays containing a commercial substrate based on peat and kept in a floating irrigation system in a protected environment. At the greenhouse, seedlings with four true leaves were transplanted into 1 L pots containing the same substrate and a drip irrigation system. The treatments consisted of different nitrogen doses (0.0, 0.5, 1.0, 2.0 or 4.0 g of N L^{-1}) applied weekly using ammonium nitrate as source. Fertilization with P and K was also done, and all treatments received de same dose. At the end of the experimental period, shoot length, leaf area, shoot and root dry matter, number of buds and flowers, chlorophyll *a*, *b* and total chlorophyll were evaluated. The experimental design was completely randomized with four replications and five plants per plot. The data were treated with ANOVA and the means were treated with regression analysis using the SigmaPlot 11.0 software. There was no survival of plants at the highest N dose (4.0 g L^{-1}). Shoot length showed a quadratic behavior, with a maximum point at 1.25 g L^{-1}, where plants reached 13.3 cm. There was a similar behavior for the variable leaf area, with the highest point at 1.24 g L^{-1}. Shoot dry mass presented the maximum point at a dose of 1.20 g L^{-1}, resulting in 4.21 g of dry matter. Regarding root dry matter, no equation could be adjusted. For the variable number of buds and flowers, the highest point was at a dose of 1.20 g L^{-1}, with 35.86 buds and flowers per plant. For chlorophyll *a*, *b* and total chlorophyll, the maximum point was at 1.5 g L^{-1}. The ideal nitrogen dose is between 1.2 and 1.5 g L^{-1} for most variables.

Keywords: floriculture, ornamental, propagation, mineral nutrition.

RESUMO

Doses de nitrogênio no crescimento de *Calceolaria* x *herbeohybrida* em vaso

Calceolária (*Calceolaria* x *herbeohybrida* Voss) é uma espécie herbácea, perene e florífera, que pode ser cultivada em vasos ou canteiros. O nitrogênio (N) geralmente é o nutriente requerido em maior quantidade, sendo necessário realizar pesquisas, a fim de fornecer informações úteis para práticas agronômicas. Diante disso, o trabalho teve como objetivo avaliar doses de nitrogênio no crescimento de *Calceolaria* x *herbeohybrida* em vaso. Inicialmente sementes sortidas e peletizadas de calceolária foram semeadas em bandejas multicelulares contendo substrato comercial à base de turfa e mantidas em sistema de irrigação por *floating*, em ambiente protegido. Na casa de vegetação, mudas com quatro folhas verdadeiras foram transplantadas para vasos de 1L contendo o mesmo substrato e sistema de irrigação por gotejamento. Os tratamentos consistiram de doses de N (0,0; 0,5; 1,0; 2,0 ou 4,0 g L^{-1} de N), aplicados semanalmente, utilizando nitrato de amônio como fonte. Também foi feita adubação com P e K, sendo que todos os tratamentos receberam a mesma dose. Ao final do período experimental foram avaliados: comprimento da parte aérea, área foliar, massa de matéria seca da parte aérea e do sistema radicular, número de botões e flores, clorofila *a*, *b* e total. O delineamento experimental foi inteiramente casualizado, com quatro repetições e cinco plantas por parcela. Os dados foram submetidos à ANOVA e após as médias foram submetidas à regressão pelo SigmaPlot 11.0. Não houve sobrevivência das plantas na maior dose (4,0 g L^{-1} de N). O comprimento da parte aérea apresentou comportamento quadrático com ponto de máxima em 1,25 g L^{-1} onde as plantas atingiram 13,3 cm de altura, apresentando comportamento similar para a variável área foliar, com o ponto de máxima em 1,24 g L^{-1}. A massa de matéria seca da parte aérea teve o ponto de máxima na dose de 1,20 g L^{-1}, com 4,21g de massa de matéria seca, já para a massa de matéria seca do sistema radicular não foi possível ajustar nenhuma equação. Para a variável número de botões e flores, o ponto de máxima foi na dose de 1,20 g L^{-1} com 35,86 botões e flores por planta e para a clorofila *a*, *b* e total o ponto de máxima foi 1,5 g L^{-1}. A dose ideal está entre 1,2 e 1,5 g L^{-1} de N para a maioria das variáveis analisadas.

Palavras-chave: floricultura, ornamental, propagação, nutrição mineral.

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1. INTRODUCTION

Plants of the genus *Calceolaria* L. occur in South America, Central America, Mexico, in temperate and tropical regions (DI FABIO et al., 1995), and in New Zealand (WOLDEMICHAEL et al., 2003). They previously belonged to the family Scrophulariaceae, however, they are currently called Calceolariaceae, being separated from the former through differences identified by molecular analyses (MAYR and WEBER, 2006). The genus comprises approximately 250 species, which occur mainly in high altitude regions along the Andes, but are also recorded at sea level, mainly on the coasts of the Atlantic and Pacific oceans, in the Patagonia region (COSACOV et al., 2009).

Calceolaria x *herbeohybrida* Voss is an herbaceous, perennial and floriferous species, developed from the following species: *C. crenatifolia*, *C. corymbostum* and *C. cana* (DOLE and WILKINS, 2005). The leaves are pubescent, rough, fleshy with jagged edges; the inflorescences are erect and branched, with numerous flowers that have an inflated lower lip resembling a bag, and yellow or red coloration with orange and brown dots and stripes (LORENZI, 2008). The flowers are formed in the winter and spring periods and cultivation is usually carried out in pots (LORENZI, 2008), but can also be done in flower beds (NAU, 2011). The propagation of the species is through seeds (LORENZI, 2008; NAU, 2011), which are small, and plants may show 17,000 to 40,000 seeds per gram. Germination occurs 8 to 10 days after sowing, with preferential temperature of 18 to 20 °C (LARSON, 1980).

The use of agricultural practices such as fertilization requires knowledge from producers, so that they can use fertilizers rationally and economically (ARAÚJO et al., 2009), and this knowledge often comes from research. It is important to establish an adequate fertilization, identifying the main problems related to the mineral nutrition of the species by determining which nutrients are limiting, in which quantity, also considering the best season and form of application (LUZ et al., 2014). Mineral nutrients play essential and specific roles in plant metabolism (ROSA et al., 2012). The most commonly required nutrient is nitrogen (N), which must be supplied in a balanced way, especially in relation to phosphorus and potassium, since it may cause delay in flowering, as well as predispose plants to pest attack (BELLÉ, 2008).

Nutritional deficiencies of N can significantly affect plant growth and influence the quality and number of flowers. Notwithstanding, excess application of this nutrient has implications for the cost of production and constitutes one of the causes of water contamination, both by surface runoff and by leaching (WANG et al., 2012). Research related to the proper balance of N is necessary to provide useful information for agronomic practices, with the purpose of preventing or avoiding nutrient loss by leaching, and consequently reducing the environmental impact (NARVÁEZ et al. 2013).

Lack and excess of nutrients are detrimental to the quality of ornamental plants, since floriculture is a relatively recent activity when compared to other commercial crops, so the available literature on the mineral nutrition of ornamental plants is still scarce (FURTINI NETO et al., 2015). Therefore, the present work had as objective to evaluate nitrogen doses on the growth of potted *Calceolaria* x *herbeohybrida* Voss.

2. MATERIAL AND METHODS

Pellet seeds of *Calceolaria* (Sakata®) were sown in multicellular trays containing peat-based commercial substrate. One seed was placed per cell, and then covered by a thin layer of expanded vermiculite. The trays were maintained in a floating irrigation system, in a protected environment. When the seedlings reached four true leaves, they were transplanted to 1.0 L pots containing the same substrate used for sowing. This work was carried out in a greenhouse at the Department of Horticulture and Forestry (DHS) of the Faculty of Agronomy of the Federal University of Rio Grande do Sul (UFRGS), in Porto Alegre, RS, from August 19 to November 4, 2015. During that period, the plants were drip irrigated in an open irrigation system with drainage of excess water.

The treatments consisted of the following N doses: Control – 0.0; T1 - 0.5 g L^{-1}; T2 - 1.0 g L^{-1}; T3 - 2.0 g L^{-1} or T4 - 4.0 g L^{-1} N, applied weekly via fertigation. The source used was ammonium nitrate (NH_4NO_3) with 30% N, being 50% in the ammoniacal form and 50% in the nitric form.

All treatments received phosphorus and potassium fertilization; using as source Krista MKP® (KH_2PO_4) with 52% P_2O_5 and 34% K_2O; and potassium chloride (KCl) with 60% K_2O. Phosphorus and potassium fertilization was performed according to the recommendation of Nau (2011), using P:K ratio of 1:2 from the first to the fourth week of cultivation, corresponding to 0.19 g L^{-1} MKP and 0.23 g L^{-1} KCl. From the fifth to the tenth week, 0:1.5 ratio was used, i.e., 0.25 g L^{-1} KCl. 50 mL per plant of the nutrient solution containing all the nutrients mentioned above were applied weekly via fertigation. No supplementation with micronutrients was performed in this experiment.

The determination of the hydrogenionic potential (pH) and electrical conductivity (EC) was carried out at the Biotechnology Laboratory - Analysis of Horticultural Substrates, belonging to the School of Agronomy of UFRGS. Substrate analysis was carried out before the transplanting of seedlings and at the end of the experiment, i.e., 76 days after transplanting (76 D.A.T), using the 1:5 (v:v) dilution method in water, according to IN No. 17, of May 21, 2007 (BRASIL, 2007).

Shoot length was obtained by means of a millimeter ruler and leaf area (LA) was determined by a Li-3100 scanner, brand Licor. The root system was washed in tap water for removal of the substrate and then, as performed with the shoots, it was oven dried at 65±5 °C until constant weight. Subsequently, the count of shoots and flowers was performed.

Leaf chlorophyll content was evaluated using an electronic chlorophyll meter (clorofiLOG), model CFL1030, brand Falker®, which uses the Falker Chlorophyll Index (FCI) as a measurement unit.

FCI is a dimensionless index, which presents a high correlation with laboratory measurements obtained by the traditional method of acetone extraction. Four leaves per plant were used, which were chosen at random to determine the values of chlorophyll *a*, chlorophyll *b* and total chlorophyll.

The experimental design was completely randomized, with four replications and five plants per plot. Data were submitted to analysis of variance (ANOVA), followed by polynomial regression by SigmaPlot 11.0. The data regarding shoot length and dry mass of shoots did not meet the assumptions of the analysis of variance and therefore both were transformed to log x^{-10}. As for EC, it did not meet the assumptions of the ANOVA even after data transformation, thus the non-parametric Kruskal-Wallis test was performed.

3. RESULTS AND DISCUSSION

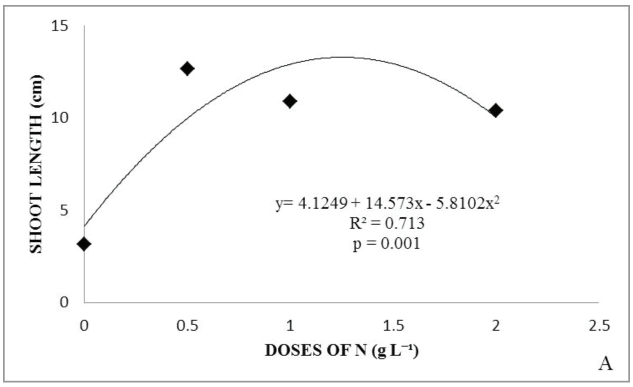
There was no plant survival at the highest dose tested (4.0 G l^{-1} n), therefore, it was decided to disregard this treatment. The analysis of variance showed that for most of the analyzed variables there was a significant difference (Table 1), and it was possible to make a quadratic adjustment, except for the dry mass of roots, where it was not possible to adjust any curve.

Table 1. Analysis of variance of *Calceolaria* x *herbeohybrida* Voss under different doses of nitrogen fertilization.

ANALIZED VARIABLES	p-VALUE	AVERAGE	CV (%)
Shoot length (cm)	0.001	9.25	45.28
Leaf area (cm^2)	0.003	365.80	53.81
Dry mass of shoots (g)	<0.001	2.61	56.67
Dry mass of roots (g)	0.853	0.26	49.41
Number of buds and flowers	<0.001	20.33	68.56
Chlorophyll *a* (FCI)	<0.002	242.06	12.29
Chlorophyll *b* (FCI)	<0.003	80.03	20.12
Total chlorophyll (FCI)	<0.004	322.09	14.12

FCI = Falker Chlorophyll Index; CV = coefficient of variation.

The application of N influenced positively the variable shoot length, the maximum point being at 1.25 g L^{-1} so that the plants reached 13.3 cm in height (Figure 1A). The height of ornamental potted plants is one of the main quality characteristics (BARBOSA et al., 2011), being observed in the cultivation of *Nidularium fulgens* Lem. an increase in plant height as the N dose increased, demonstrating linear behavior (SANTOS et al., 2015), different from that observed in this study.



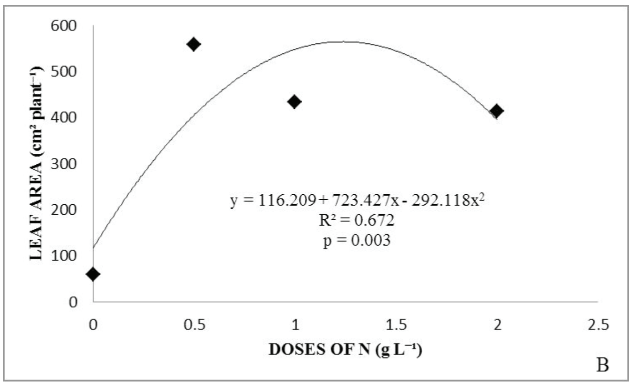


Figure 1. Shoot length (A) and leaf area (B) of *Calceolaria* x *herbeohybrida* as a function of different doses of nitrogen fertilization.

The variable leaf area also showed similar behavior, with a maximum point at 1.24 g L^{-1}, so that the plants had 564.10 cm^2 $plant^{-1}$ (Figure 1B). In the cultivation of *Polianthes tuberosa* L., the application of the maximum dose of N provided an increase of 32.86% in the leaf area when compared to the control treatment, without application of N (RATHORE and SINGH, 2013); the same was verified for *Zinnia elegans* Jacq. (Khan et al., 2004). In the calceolaria cultivation, the leaf area increased approximately 8 times when compared to the control treatment (without N), with treatments T1 and T2 (0.5 and 1.0 g L^{-1} N).

The dry mass of shoots had the maximum point at the dose of 1.20 g L^{-1}, with 4.21 g, yet for the dry mass of roots, it was not possible to adjust any equation (Figure 2A and B). The total dry mass of *Weigela florida* (Bunge) A. DC. 'Red Prince' also had a quadratic behavior with respect to the N dose applied (SANDROCK et al., 2005), as did the dry mass of shoots in the present work.

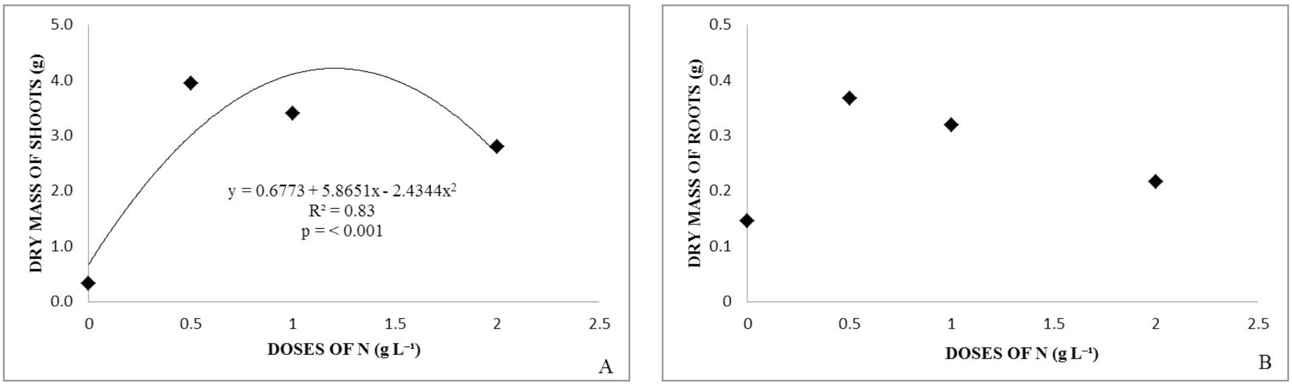


Figure 2. Dry mass of shoots (A) and dry mass of roots (B) of *Calceolaria* x *herbeohybrida* as a function of different doses of nitrogen fertilization.

For the variable number of buds and flowers, the maximum point was at the dose of 1.20 g L^{-1} with 35.86 buds and flowers per plant (Figure 3). The same was observed in the cultivation of potted *Calendula officinalis* L. 'Tokaj', where nitrogen fertilization exerted a significant impact on the number of flowers and extended the flowering period (KRÓL, 2011).

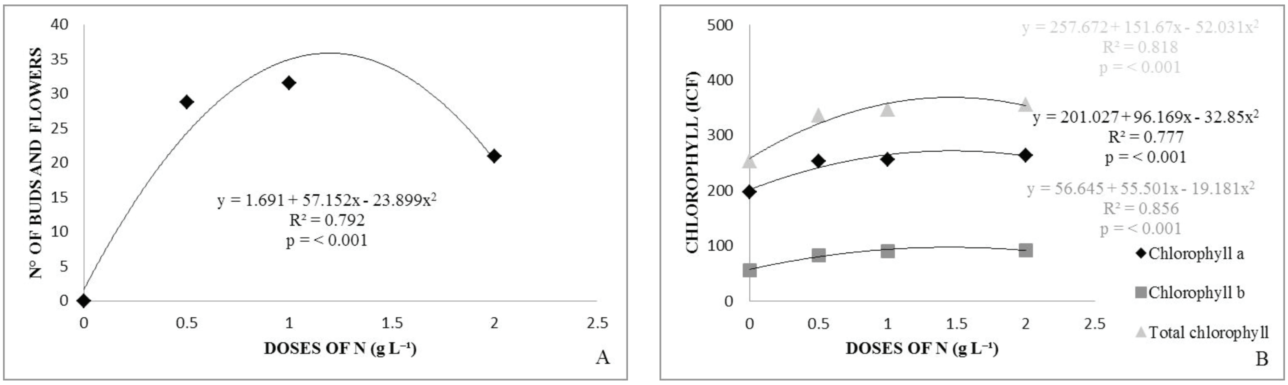


Figure 3. Number of buds and flowers (A) and Falker Chlorophyll Index (FCI) (B) of *Calceolaria* x *herbeohybrida* as a function of different doses of nitrogen fertilization.

Plants need N to develop, since this nutrient is a constituent of proteins, nucleic acids, coenzymes among others (MILLER and CRAMER, 2004). Moreover, N metabolism has a direct relationship with growth, differentiation and morphogenesis, through physiological and biochemical processes (HAYASHI et al., 2002). Symptoms of mineral deficiency can affect different plant organs; in the case of ornamental plants, when these organs are the flowers, inflorescences or foliage, there is a significant impact on the quality of the final product, consequently affecting the final price thereof (FURTINI NETO et al., 2015).

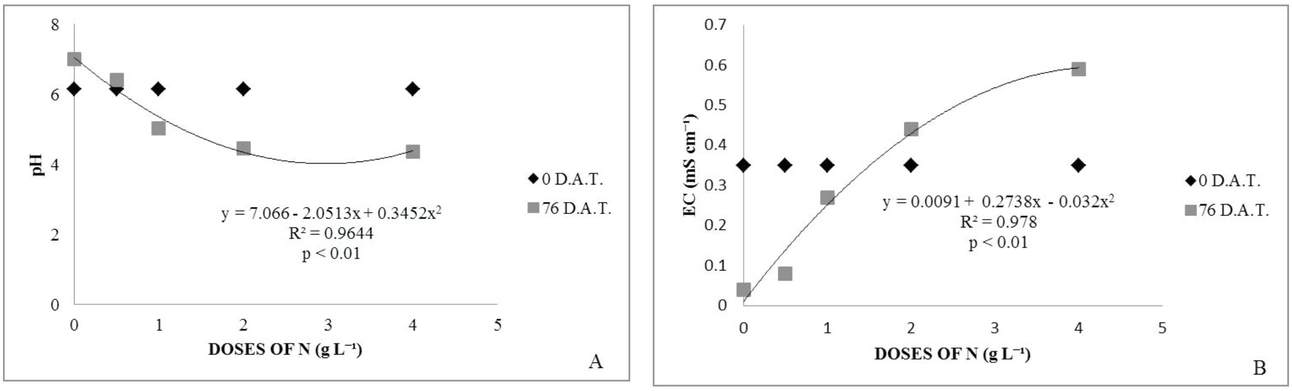


Figure 4. pH value (A) and electrical conductivity (EC) (B) of the substrate by the 1:5 method at 0 and 76 days after transplanting (D.A.T.) of *Calceolaria* x *herbeohybrida* as a function of different doses of nitrogen fertilization.

Figure 5 shows calceolaria plants at 76 D.A.T., where the lack and excess of N significantly affected the growth of this species; at the dose of 0 g L^{-1} there was no plant growth, and growth retardation is a characteristic of N deficiency (EPSTEIN, 1975), whereas at the 4.0 g L^{-1} dose we can observe the non-survival of plants.

Figure 5. General appearance of the growth of *Calceolaria* x *herbeohybrida* as a function of different doses of nitrogen fertilization.

For plants fertilized with 0.5 g L^{-1}, a delay was observed in flowering, since no plant of this treatment presented open flowers, increasing the crop cycle. The flowering period is a critical stage in which fertilizers can be used to promote floral development (ZHANG et al., 2012), since in the cultivation of ornamental potted plants, such as calceolaria, flowering is of fundamental importance for the acceptance and sale of the product to the consumer. Thus, the dose of 1.2 g L^{-1} N is best suited to produce the highest number of buds and flowers.

For chlorophyll *a*, chlorophyll *b* and total chlorophyll, the maximum point was 1.5 g L^{-1}, to reach 271.41, 96.79 and 368.20 FCI, respectively (Figure 3A and B). In the cultivation of *Laelia speciosa* (Kunth) Schltr., the chlorophyll *a* and total chlorophyll content increased as the N dose increased, but for larger doses there was reduction of these pigments; chlorophyll *b*, in turn, showed no dose-dependent difference (DÍAZ-ÁLVAREZ et al., 2015).

EC and pH are important chemical characteristics of the substrates (KÄMPF, 2005). The pH is the parameter that measures the activity of the hydrogen ion, determining the acidity of a substrate (FERMINO, 2014), and its value influences the availability of nutrients; it also has influence on the physiological processes of the plant (KÄMPF, 2005). In figure 4 it can be observed that before transplanting the seedlings and starting the fertilization (0 D.A.T.), the pH value was in the range of 6.15. Yet at 76 D.A.T. this variable presented a quadratic behavior, with the lowest pH value (4.01) being verified at the dose of 2.97 g L^{-1} N. In the process of ammonia absorption by plant roots, there is H^+ release, which contributes to the acidification of the medium (JOHNSON et al., 2010). The plants fertilized with 2 g L^{-1} were the ones that presented the highest growth, explaining the increased requirement of nutrients and H^+ release, with consequent acidification of the substrate.

When the substrates do not have mineral soil in their composition, the optimum pH value is in the range of 5.0 to 5.8 (KÄMPF, 2005). In the specific case of floriculture, for most species under cultivation, the ideal pH range is between 5 and 6.5 (CAVINS et al., 2000). Since the substrate used in this work did not present mineral soil in its composition, it initially had a pH of 6.15. At 69 D.A.T., the pH of the control treatment was close to 7.0, and for T1 there was practically no change in relation to the initial value, that is, before cultivation. For the other treatments, it was verified that the nitrogen fertilization decreased the pH value, and it should be noted that no pH control was performed on the irrigation water.

The electrical conductivity estimates the concentration of ionized salts present in the solution (SCHAFER et al., 2015), which is an important characteristic to be observed for the efficient management of fertilization according to the plant sensitivity to the concentration of salts in the substrates (KÄMPF, 2000). The plants present different responses with respect to this variable, since this will depend on the cultivar and species in question, as well as the age and the vegetative development, the environmental conditions and the cultivation practices adopted (SCHAFER et al., 2008).

The initial EC of the substrate was, on average, 0.35 mS cm^{-1} for all treatments, and at 76 D.A.T. it was observed a direct and positive relationship with nitrogen fertilization (Figure 4). In T4, 0.59 mS cm^{-1} EC was obtained; when compared to the control treatment (0.04 mS cm^{-1}) in the same period, an increase of 14.75 times was observed.

The use of fertilizers may increase the salinity of the culture medium, causing nutritional imbalances through the alteration of the osmotic potential (SILVA et al., 2013), what may explain the non-survival of all T4 plants, since the amount of salts in this treatment was toxic to this species. Salinity has a negative influence on water uptake by roots and leads to nutritional imbalance, where the excess of certain ions inhibits the absorption of others (SCHOSSLER et al., 2012).

These results are consistent with those found by Cavins et al. (2000), who classified plants according to the sensitivity to salts, being calceolaria a species with low tolerance to saline conditions. Using the 1:5 method, the ideal cultivation range for the species with respect to EC is 0.12 to 0.35 mS cm^{-1} (CAVINS et al., 2000), as the EC in T4 was 0.59 mS cm^{-1}, confirming the sensitivity of the species to salt doses higher than this range.

To produce potted calceolaria, nitrogen fertilization is of fundamental importance for the plants to complete their cycle, since it is directly related to the growth of the species. It is important to observe the adequate dose to be supplied, so that there is no excess or lack of this nutrient. Therefore, the importance of carrying out research in this area is highlighted, which can serve as support for technicians and producers in the sector.

4. CONCLUSION

In the conditions of this work, the ideal dose is between 1.2 and 1.5 g L^{-1} N for the growth of potted *Calceolaria* x *herbeohybrida*.

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AUTHORS CONTRIBUTION

M.C.W.: Responsible for the implementation, evaluation and writing of the article; **G.T.G**.: Assisted in the implantation, conduction and evaluation of the experiment; **P.P**.: Assisted in the implantation, conduction and evaluation of the experiment; **M.T.**: Assisted in the implantation, conduction and evaluation of the experiment; **J.P.**: Assisted in the conduction and evaluation of the experiment; **E.D.A**.: Assisted in tabulating the data, statistical evaluation and revision of the article; **C.S.F.**: Co-adviser, assisted in the statistical evaluation and correction of the article; **G.S.**: Adviser assisted in the preparation of the study and correction of the article;

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