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Impact of Nitrate and Ammonium ratio on Nutrition and Growth of two Epiphytic Orchids

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ABSTRACT

Phalaenopsis and *Dendrobium* do not grow and flower well with 100% ammonium (NH_4-N); and there are detailed studies on the effects of nitrate (NO_3-N) and ammonium ratios on the flowering, but no information about accumulation of other nutrients and the effects of ammonium toxicity on orchids. For this reason, two experiments were carried out with orchids: *Phalaenopsis* 'Golden Peoker' and *Dendrobium* 'Valentine'. Six months after acclimatization the plants were transplanted to individual plastic vessels and the treatments consisted of five ratios (%) of nitrate / ammonium (0/100, 25/75, 50/50, 75/25, 100/0). The sources of NO_3-N and NH_4-N were calcium nitrate and ammonium sulfate, respectively. After 12 months treatment, when the plants were beginning to issuance of flower stem, the accumulation of: N, P, K, Ca and Mg in the shoot and biometric variables were evaluated for both species. The NH_4-N ratio of 40% and 50% of the total nitrogen benefited the growth of *Phalaenopsis* and *Dendrobium*, respectively. The application of higher proportions of ammonium resulted in decreased N, K, Ca and Mg absorption, index of green color and increased leakage of electrolytes in *Phalaenopsis* and *Dendrobium*. NH_4-N proportions greater than 75% for 12 months caused toxicity in *Phalaenopsis* and *Dendrobium*.

Key words: nutritional status, fertigation, Orchidaceae, ammonium toxicity.

INTRODUCTION

Nitrogen fertilization is an essential practice for the cultivation of orchids. Among the various forms, such as NO_3-N, NH_4-N and urea, the NO_3-N is the best form of N absorbed by orchids (Wang and Chang 2017).

The beneficial effect of NH_4-N is due to the lower energy consumption, compared with NO_3-N, due to ammonium direct incorporation into the carbon chain in the assimilation of N, without the need for reduction phases by enzymatic action (Bittsánszky et al. 2015), and may increase N utilization efficiency (Sarasketa et al. 2014).

However, when the ammonium application is the only source of N it may result in physiological and morphological disturbances leading to decrease plant growth and N toxicity (Esteban et al. 2016).

Additionally, high application amounts of ammonium inhibits cation uptake (K^+, Ca^+ and Mg^+), increases oxidative stress and requires high energy costs to maintain low levels of NH_4^+ in cytosolic content (Britto and Kronzucker 2002, Bittsánszky et al. 2015), causes changes in root architecture and causes chlorosis in the leaves

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resulting in decrease in plant growth. There are no studies reporting the effects of ammonium toxicity on orchids.

There are differences between varieties with respect to their response to ammonium (Cruz et al. 2011) and nitrate (Huang et al. 2013) even through they are of the same species.

The balance between the ratios NO_3-N/ NH_4-N in the nutrient solution for growth of the *Phalaenopsis* orchid was determined by Wang (2008). When 0.221 g L^{-1} of N via fertigation was applied to *Phalaenopsis* Blume x Taisuco Kochdian growth was reduced with excessive supply of ammoniacal N and the best growth occurred with the application of 75% of the N in the form of NO_3-N and 25% in the form of NH_4-N. However, this study did not evaluate nutrient accumulation and ammonium toxicity, and the plants were cultivated for eight months without the supply of micronutrients.

The pattern of uptake of ammonium and nitrate varies according to the carbon source supplied, pH of the nutrient medium and its concentration. *Dendrobium* Multico White tissues grown in liquid culture media showed a preferential uptake of ammonium ions over nitrate (Hew and Yong 2004).

Ruamrungsri et al. (2014) cultivated *Dendrobium* Sonia 'Ear Sakul' plants with different NH_4-N: NO_3-N ratios (0:0, 0:200, 50:150 and 100:100 mg L^{-1} of N) once a week and the results showed that presence of NO_3-N in the solution increased the absorption of NH_4-N; they recommended to supply N-fertilizer in the combination of NH_4-N: NO_3-N via leaves to stimulate growth and the uptake of ions into plant organs for assimilation. However, the nutritional status of the plants in relation to the other nutrients and the ammonium toxicity were not considered.

Therefore the aim of this work was to determine the effects of ratios of NO_3-N and NH_4-N on the accumulation of N, K, Ca and Mg in the shoot and the effects of ammonium toxicity on growth of *Phalaenopsis* Golden Peoker and *Dendrobium* Valentine.

MATERIALS AND METHODS

The experiment was carried out in an orchid greenhouse located in Itapolis, São Paulo, Brazil. The orchid seedlings used, *Phalaenopsis* 'Golden Peoker' and *Dendrobium* 'Valentine', were obtained via *in vitro* propagation, acclimatized in plastic trays with dry *Sphagnum* moss substrate, and received the complete nutrient solution of Sarruge (1975) via fertigation every two weeks.

The plants were kept in a greenhouse with average PPF of 300 mmol m^{-2} s^{-1} at noon, and maximum and minimum temperatures of 34 and 15°C, respectively. After six months, the seedlings were transplanted individually into black polyethylene vessels (upper diameter: 13 cm, lower diameter: 8.4 cm, height: 10.6 cm) with 0.9 L volume.

The pots were filled with a layer of expanded clay at the bottom (25% of the total volume) and with a 2:1 (v/v) mixture of pinus bark and charcoal medium, and placed on hanging tables at a height of 0.65 m. The position of the pots was randomly changed after each treatment application, every 15 d.

The plants were irrigated twice and three times a week in the winter and summer, respectively, with 100 mL of distilled water (pH = 6.8) per pot.

The concentration of nitrogen used in the nutrient solution was 136 mg L^{-1} of N, which promotes adequate growth of *Phalaenopsis* plants in Brazilian climatic conditions (Mantovani et al. 2015). The other nutrient concentrations, in mg L^{-1}, in the nutrient solution used were: 31 P, 234 K, 200 Ca, 48 Mg, 64 S, 0.5 B, 0.5 Mn, 0.05 Zn, 0.02 Cu, 0.01 Mo, 5 Fe (Sarruge 1975).

The calcium nitrate (MW = 164 g mol^{-1}) was used as a source of NO_3-N, and ammonium sulfate

(MW = 132 g mol^{-1}) was used as the source of NH_4-N.

The treatments consisted of five proportions of NH_4^+ / NO_3^- (%): 0/100, 25/75; 50/50, 75/25, 100/0. The experimental unit consisted of one plant per pot, eight plants were grown for each treatment, arranged in a completely randomized design.

The application of the nutrient solution of Sarruge (1975) with the source of nitrogen modified, according to the treatments was carried out via fertigation, once a week. The volume of solution applied per plant varied with the growth of the seedlings, approximately 50 ml in the first six months and 90 ml in the last six months of the experiment.

At each treatment, the pH of the solution was adjusted to 5.7 to 5.9 and it is noted that calcium concentrations were balanced with calcium chloride to maintain 0.29 g L^{-1} of Ca in all treatments.

After 12 months, when the plants were beginning to issuance of flower stem, evaluations were made: plant stem diameter (mm) measured at 2 cm from the base of the stem using a digital caliper (Starrett®727-2001 manufactured in Itu, São Paulo, BR); index of green color (with chlorophyll portable unit CCM-200 model OptiScience®) and electrolyte leakage (Dionisio-Sese and Tobita 1998) in the central part of the adaxial surface of the last fully developed leaf of each plant).

The number of pseudobulbs and plant height (cm) were determined only for *Dendrobium* 'Valentine' while the number of leaves and plant width (corresponding to the distance between the apex of the last two fully expanded leaves, cm) were measured only for *Phalaenopsis* 'Golden Peoker'.

The orchids were divided into aerial part/shoot and root and dried in forced circulation oven at 65 to 70ºC temperature, until constant weight. The dry matter was determined and the plant material was ground to determine contents of N, K, Ca and Mg following the methodology described by Bataglia et al. (1983). Nutrient content and dry matter were used to calculate accumulation of N, K, Ca and Mg in the aerial shoot.

From these data, use efficiency of N = (total dry matter produced)^2 / (total content of N in the plant) (Siddiqi and Glass 1981) and absorption efficiency of N = (total content of N in plant) / (dry matter of roots) (Swiader et al. 1994) were calculated.

The results were analyzed by the F-test at 1% and 5% probability; and when significant for proportions of NH_4^+, polynomial regression studies and the means comparison test (Tukey at 5% probability) were performed. The calculations were performed with the statistical program AgroEstat (Barbosa and Maldonado 2014).

RESULTS

The average accumulation of macronutrients in the aerial part of *Phalaenopsis* 'Golden Peoker' and in *Dendrobium* 'Valentine' followed the following increasing order of accumulation: K> Ca> N> Mg (Figure 1). There was an increase in quadratic form and then a decrease in the accumulation of these nutrients with the increase of ammonium proportions.

The increase of the ammonium concentration in the nutrient solution increased and then decreased the accumulation of total N reaching the maximum point in the ammonium proportion equal to 28% and 36% for *Phalaenopsis* and *Dendrobium*, respectively (Figure 1a).

It was observed that the balance of the ammonium proportion equal to 33% and 45%, favored the absorption of K by *Phalaenopsis* and *Dendrobium* orchids, respectively (Figure 1b).

The increase of the ammonium concentration in the nutrient solution increased and then decreased the accumulation of Ca reaching the maximum point in the ratio of ammonium equal to 31% and 42% for *Phalaenopsis* and *Dendrobium*, respectively (Figure 1c).

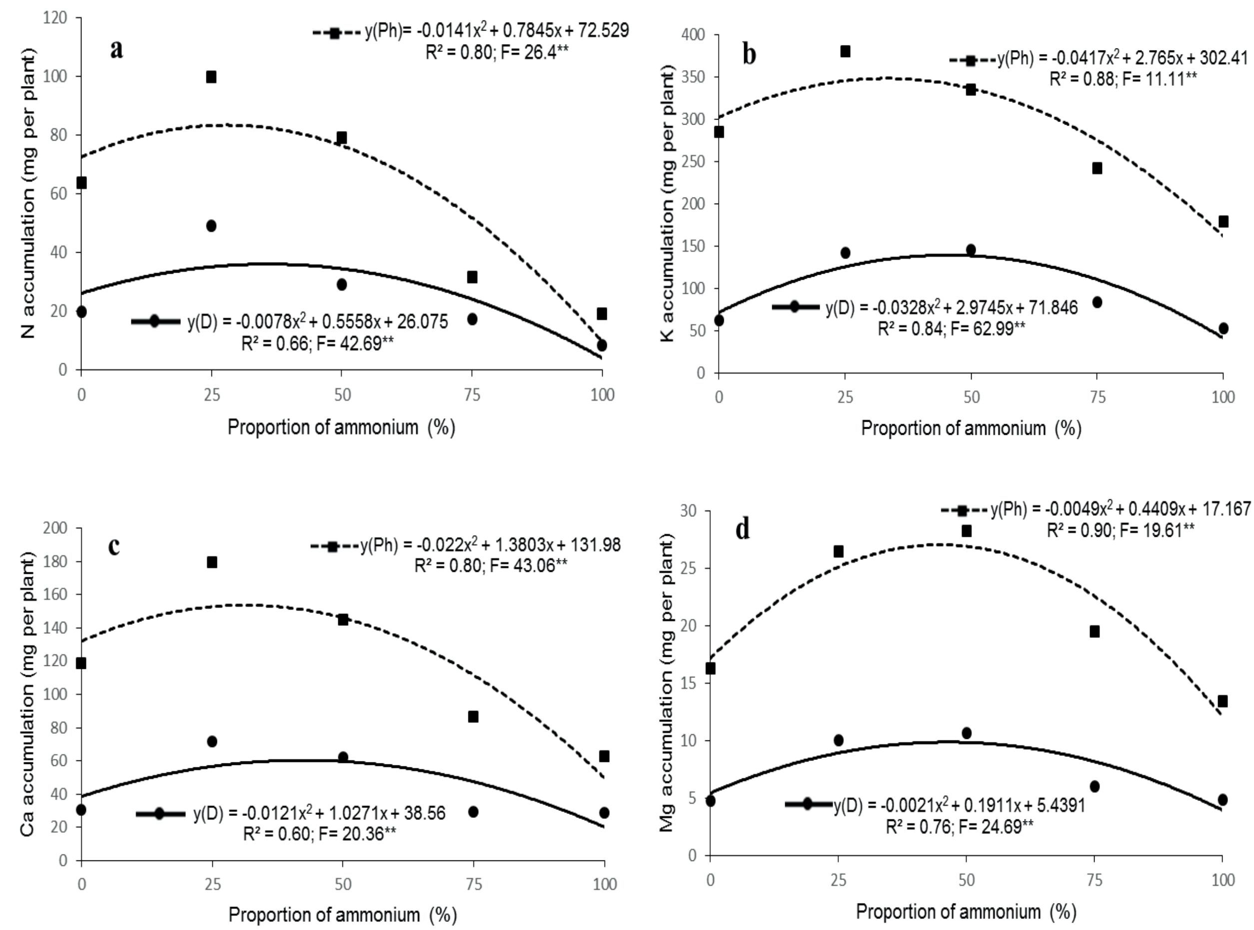

Figure 1 - Accumulation of N (**a**), K (**b**), Ca (**c**) and Mg (**d**) in aerial part of *Phalaenopsis* 'Golden Peoker' (Ph) and *Dendrobium* 'Valentine' (D) as a function of increasing proportions of ammonium in relation to nitrate after 12 months of the beginning of the application of the treatments. ** $p<0.01$ by the F test.

Likewise, the maximum accumulation of Mg was reached with the proportions of ammonium equal to 45% and 46% for *Phalaenopsis* and *Dendrobium*, respectively (Figure 1d).

The absorption efficiency of N decreased linearly and the efficiency of N utilization presented a quadratic increment in *Phalaenopsis* reaching maximum point with the proportion of 38% ammonium. In *Dendrobium*, the N absorption and utilization efficiency increased in a quadratic form with the increase of nitrate ratios, peaking at 38% and 65% ammonium respectively (Figure 2a and 2b).

In *Phalaenopsis* the highest index of green color was with the proportion of 31% of ammonium; and in *Dendrobium* the highest index of green color were obtained for the proportion of 46% ammonium (Figure 2c).

The leakage of eletrolytes increased linearly with the increase of ammonium proportions in the two orchids studied (Figure 2d).

The increase of the ammonium concentration in the nutrient solution in *Phalaenopsis* (Figure 3) and in *Dendrobium* (Figure 4) promoted a quadratic increase in the vegetative variables studied until reaching the maximum point.

In *Phalaenopsis* the largest plant width (31 cm) was calculated to be when the proportion was 41% ammonium; the largest stem diameter (15 mm) with the proportion of 33% ammonium, the

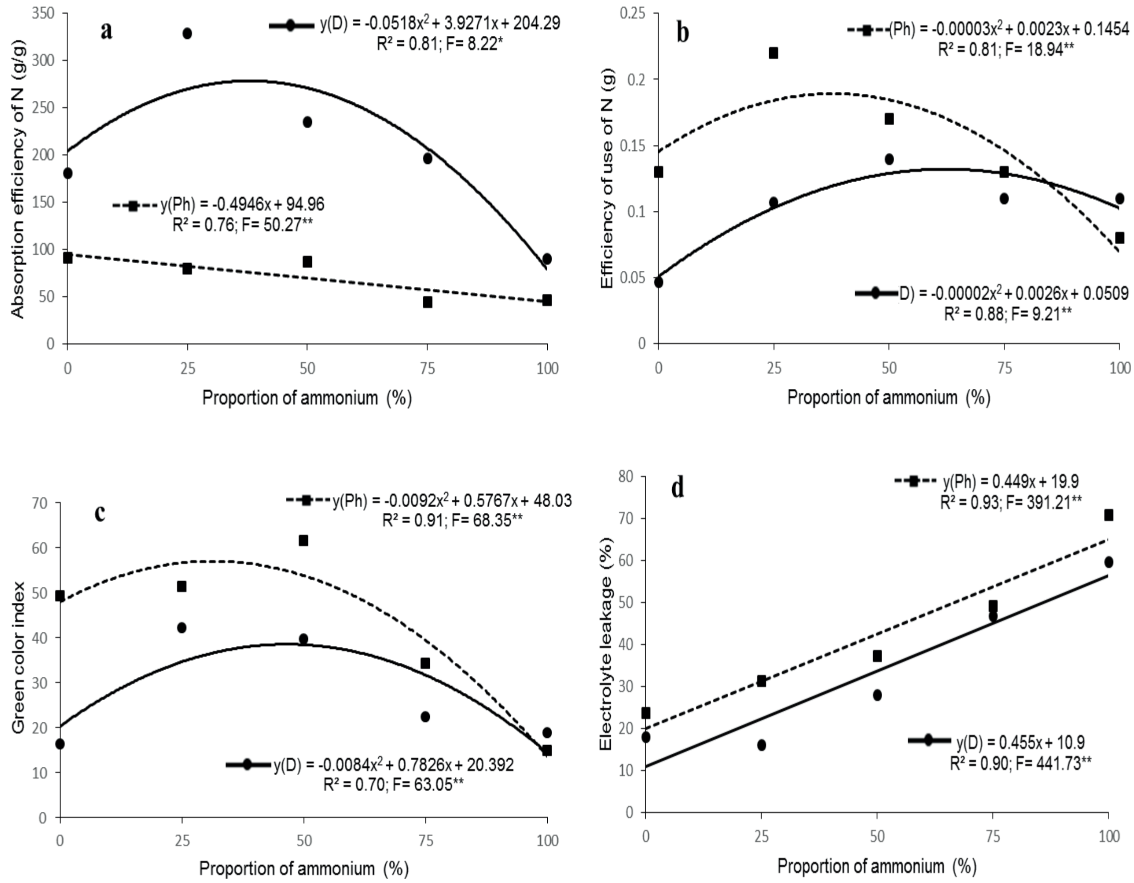

Figure 2 - Absorption efficiency of N (g / g) (**a**), use efficiency (g) (**b**), green color index (**c**) and electrolyte leakage from aerial part (%) of (**d**) *Phalaenopsis* 'Golden Peoker' (Ph) and *Dendrobium* 'Valentine' (D) as a function of increasing proportions of ammonium (%) in relation to nitrate, after 12 months of starting the treatments.* $p<0.05$; ** $p<0.01$ by the F test.

largest number of leaves (6) with the proportion of 36% ammonium and the highest dry matter (4.9 g) with a proportion of 35% of ammonium (Figure 3).

In *Dendrobium* Valentine, bigger plants (22 cm), the largest diameter of the pseudobulb (9 mm), the highest average number of pseudobulbs (3.6) and the largest dry matter (2.3 g) were grown with the proportion of 50% ammonium (Figure 4). Therefore, *Dendrobium* Valentine is more tolerant to ammonium as the source of N than *Phalaenopsis* Golden Peoker.

With the proportion of up to 57% of ammonium there was no toxicity in *Phalaenopsis*, whereas *Dendrobium* grew well with the proportion of ammonium up to 74%, reinforcing the greater tolerance of this orchid genus to ammoniacal N.

DISCUSSION

The two species of orchids fertilized with excess ammonium induced a decrease in the accumulation of cations, a fact also reported for K (Hoopen et al. 2010, Hess et al. 2006) and Ca (Siddiqi et al. 2002) in other cultivated plants.

This ionic imbalance occurs due to the increase of NH_4^+ influx and cation efflux across the plasma membrane, with extrusion of these cations to the cellular vacuole, which was confirmed with the increase of electrolyte leakage, which may cause

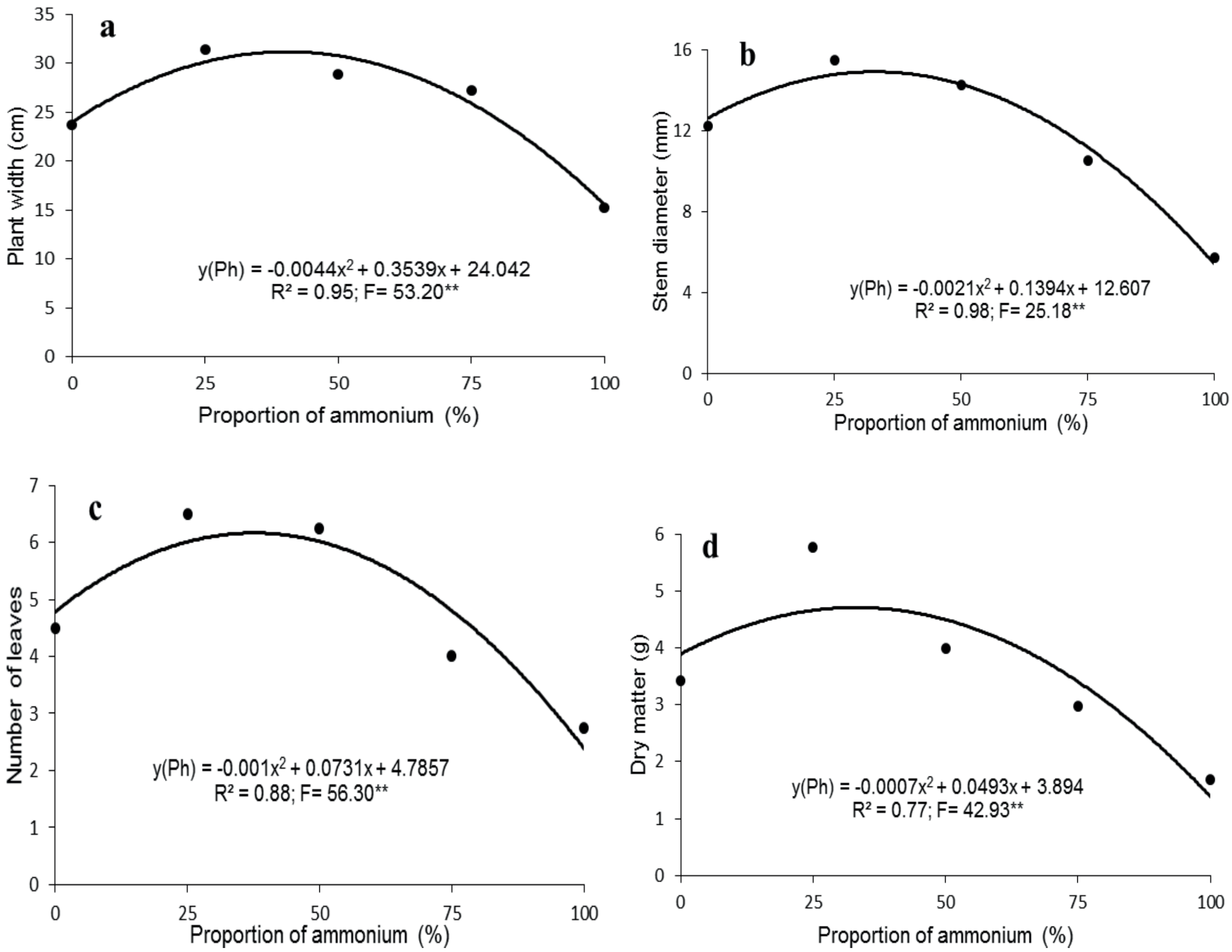

Figure 3 - Plant width (**a**), stem diameter (**b**), number of leaves (**c**), and total dry matter (**d**) of *Phalaenopsis* 'Golden Peoker' as a function of increasing proportions of ammonium (%) in relation to nitrate, after 12 months from the beginning of the application of the treatments. ** $p<0.01$ by the F test.

symptoms of nutritional deficiencies to the plants (Mendonza-Villarreal et al. 2015).

The excess of NH_4^+ caused a decrease in the efficiency of N uptake by the orchids probably due to the damage caused to the root system, which reflected in the accumulation of nutrients in the plant. Already decreased efficiency of utilization of N in the shoot was due to excess ammonium in the nutrient solution causing high accumulation of this cation in chloroplasts and can block the metabolism enzyme complex GS-GOGAT, responsible for the assimilation of N by plants (Bittsánszky et al. 2015).

It was observed that, for high ammonium proportions, the values of the green color index were lower, indicating damage in *Phalaenopsis* and *Dendrobium* due to excess of this cation. This was due to excess NH_4-N cause changes in several metabolic reactions, inducing an increase in the content of reactive oxygen species, O_2 and H_2O_2, which can cause oxidative peroxidation, reducing the levels of chlorophyll a and b (Jampeetong et al. 2012), and as a result of these reactions, the leaves had lower index of green color (Li et al. 2014, Prado 2008).

The combination of NO_3-N and NH_4-N attenuates the ammonium toxicity in many species (Britto and Kronzucker 2002). The orchids in this study grew better in the treatments with balance

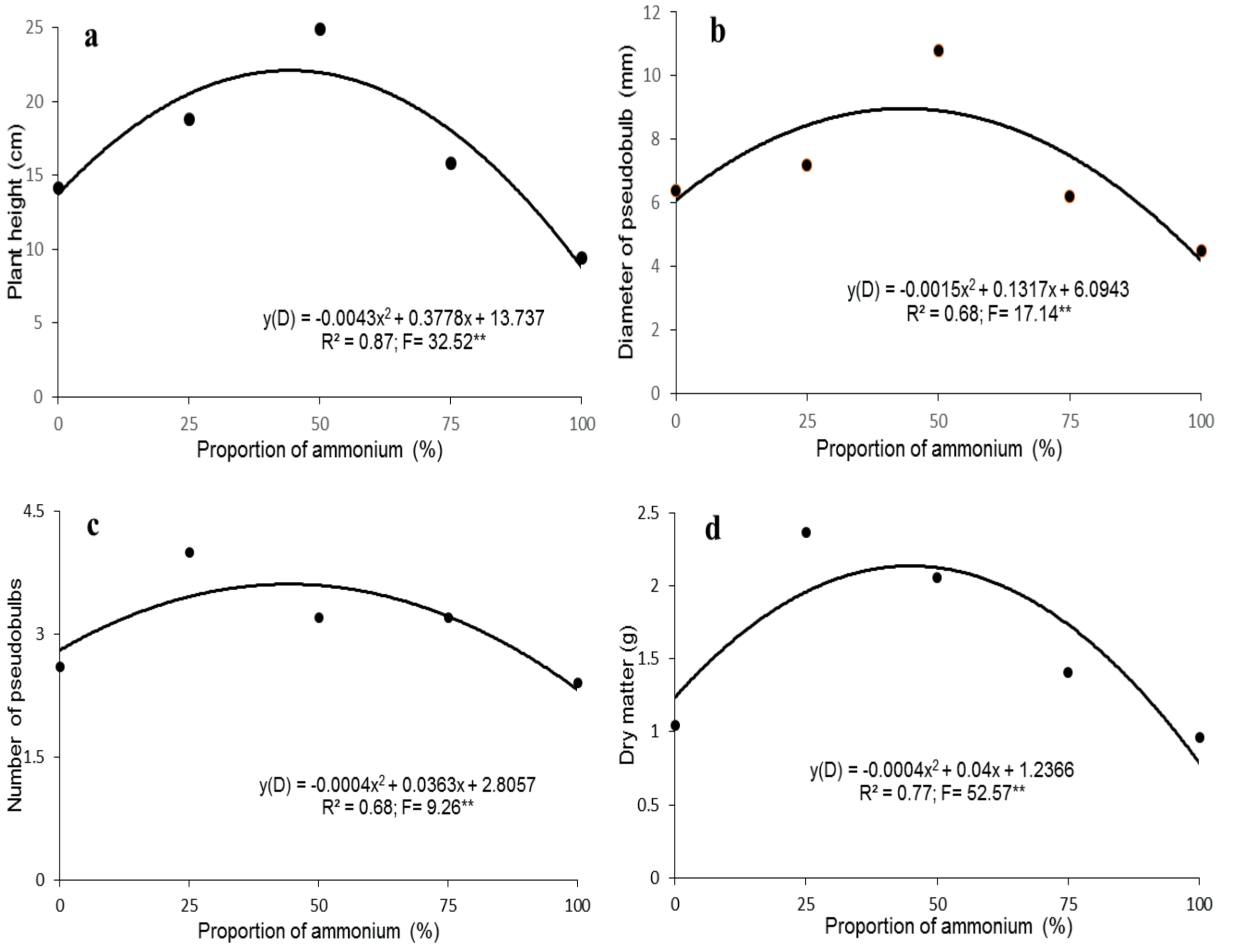

Figure 4 - Plant height (**a**), diameter of pseudobulb (**b**), number of pseudobulbs (**c**), and total dry matter (**d**) of *Dendrobium* 'Valentine' as a function of increasing proportions of ammonium (%) in relation to nitrate, after 12 months from the beginning of the application of the treatments. ** $p<0.01$ by the F test.

between these N sources, corroborating with the results obtained by Wang (2008) in *Phalaenopsis* and by Ruamrungsri et al. (2014) in *Dendrobium*.

The results indicated a tendency of greater accumulation of dry matter in the orchids, associated with a decrease in NH_4^+ concentration in the solution, similar to the results of studies with other cultivated plants (Warren and Adams 2002, Rothstein and Cregg 2005, Hachiya et al. 2007) which showed better plant growth in NO_3-N and NH_4-N mixtures. According to Bijlsma et al. (2000), the simultaneous assimilation of NO_3^- and NH_4^+ by the roots has a better cost-benefit ratio than the assimilation of a single source of N, that is, greater assimilation of N at a lower cost of C per mole of absorbed N.

According to Liu et al. (2009), the higher biomass production capacity serves as an indicator to evaluate the ability of carbohydrate synthesis. The roots of plants fertilized with NH_4^+ alone have high C skeleton demands for ammonium assimilation (Arnozis et al. 1988) and high O_2 consumption (Matsumoto and Tamura 1981), resulting in low sugar concentration in the roots and lower plant growth.

The negative effect of ammonium on plant growth variables, especially plant width and stem diameter of *Phalaenopsis* Golden Peoker, were similar to the results found with *Phalaenopsis* Blume x Taisuco Kochdian (Wang 2008), *Eustoma grandiflorum* (Mendoza-Villarreal et al. 2015) and *Solanum lycopersicum* (Borgognone et al. 2013).

This damage to orchid growth in response to NH_4-N application as the exclusive source of N has been attributed mainly to the toxic effects of free ammonium (Ivanova and Staden 2009) which leads to an excess of cationic charge absorption, which must be balanced by an increase in H^+ efflux (Hachiya et al. 2012; Smith and Raven 1979). Therefore, for maximum growth and adequate nutrition of orchids, the nutrient solution should have a maximum of 40% and 50% ammonium for *Phalaenopsis* Golden Peoker and *Dendrobium* Valentine, respectively.

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