

**Quality of *Myrceugenia myrtoides*  
O. Berg seedlings as a function of nitrogen fertilization**

**Calidad de plantas de *Myrceugenia myrtoides*  
O. Berg en función de la fertilización con nitrógeno**

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## Abstract

*Myrceugenia myrtoides* is a naturally occurring species in Rio Grande do Sul and Uruguay, with potential for being used as ornamental plant and for the recovery of degraded environments. The use of native species for these purposes has increasing, but information on their cultivation is still scarce. Thus, the objective of this study was to verify the influence of nitrogen fertilization on the quality of *M. myrtoides* seedlings. The seedlings were placed in 0.4 L capacity containers filled with composted pine bark as substrate. They were fertirrigated fortnightly with nutrient solutions containing different nitrogen concentrations (0.00; 6.90; 11.2; 15.5 and 19.8 mg plant<sup>-1</sup>). A completely randomized design with five doses of nitrogen fertilization and four replications of seven plants was used. The results showed the influence of nitrogen fertilization on aerial part dry mass, ratio between height and aerial part dry mass, and aerial part dry mass and root system ratio, which fit a quadratic model with increasing nitrogen doses. Thus, nitrogen concentrations between 15.5 and 19.8 mg per plant provide superior morphological quality of *Myrceugenia myrtoides* seedlings produced in pots filled with composted pine bark substrate.

**Key words:** *guamirim, Myrtaceae, nitrogen, substrate.*

## Resumen

*Myrceugenia myrtoides* es una especie que se encuentra naturalmente en Rio Grande do Sul y Uruguay, con potencial ornamental y para la recuperación de ambientes degradados. El uso de especies nativas para estos fines ha ido en aumento, pero la información sobre su cultivo aún es escasa. Así, el objetivo de este estudio fue verificar la influencia de la fertilización nitrogenada en la calidad de las plantas de *M. myrtoides*. Las plántulas se establecieron en contenedores con una capacidad de 0.4 L que contenían como sustrato corteza de pino compostada. Estos fueron fertilizados quincenalmente con soluciones nutritivas que contenían diferentes concentraciones de nitrógeno (0.00; 6.90; 11.2; 15.5 y 19.8 mg planta<sup>-1</sup>). Se utilizó un diseño experimental completamente al azar, con cinco dosis de fertilización nitrogenada y cuatro repeticiones de siete plantas. Los resultados mostraron la influencia de la fertilización con nitrógeno en la masa seca de la parte aérea, en la relación altura y masa seca de la parte aérea y en la relación entre la masa seca de la parte aérea y el sistema radicular, que muestra un ajuste a un modelo cuadrático con dosis crecientes de nitrógeno. Así, concentraciones de nitrógeno entre 15.5 y 19.8 mg por planta proporcionan una calidad superior de plantas de *Myrceugenia myrtoides*, producidas en contenedores con sustrato de corteza de pino compostada.

**Palabras clave:** *guamirim, Myrtaceae, nitrógeno, sustrato.*

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## 1. Introducción

The Myrtaceae is one of the most important families in the Brazilian flora, comprising 23 genera and 1030 species (BFG, 2015; Flora do Brasil 2020, under construction). In Rio Grande do Sul, it is the family with the largest number of species in the tree flora, accounting for approximately one fifth of the total richness (Sobral, 2003). Among the genera, *Myrceugenia* has about 15 species, out of which *Myrceugenia myrtoides* O. Berg is restricted to the state of Rio Grande do Sul and Uruguay, occurring mainly in areas of the Pampa biome and in its ecotone with the Atlantic Forest (Landrum, 1981; Flora do Brasil 2020, under construction).

Popularly known as “guamirim”, *M. myrtoides* is characterized as a tree or shrub of up to 4 m high, easily recognized by its grayish trichomes covering the branches, petioles and abaxial leaf surface, which are discolors, lanceolates and with acuminate apex (Landrum, 1981). Similar to other species of the Myrtaceae family, such as *Myrcia cuprea* (Ferreira *et al.*, 2013), *Acca sellowiana* (Sazima and Sazima, 2007) and *Myrciaria cauliflora* (Gobato *et al.*, 2018), *M. myrtoides* has ornamental potential, because of its size and glauca shade, for use in urban afforestation and in the recovery of degraded environments, since the consumption of fruits by bird fauna facilitates the settlement of areas (Paim *et al.*, 2018). However, although it has multiple uses, few phytotechnical studies related to the species have been conducted, thus limiting its use.

The initial formation of seedlings is an essential step to ensure the survival and development of individuals after planting. According to Tucci *et al.* (2009), among the main management practices that affect the quality of seedlings, fertilization is one of the most relevant, reflecting on productivity, tolerance to abiotic stress and resistance to pests and diseases (Dias *et al.*, 2012). Nitrogen is one of the main elements most required by plants and contributes the most to biomass production, as it constitutes several compounds of the plant cell, such as chlorophyll, amino acids and nucleic acids. Thus, when nitrogen becomes limiting, plant growth is readily inhibited (Taiz *et al.*, 2017).

Each species has particularities about its nutritional requirements, although the adoption of uniform fertilization for all individuals in nurseries is a common practice, in order to simplify operations (Davide and Silva, 2008; Dias *et al.*, 2012). Because the levels of each compound are usually adjusted to the most demanding species, high fertilizer rates may be detrimental to certain groups, causing loss in the quality and reduction in the growth (Cruz *et al.*, 2006; Fernandes *et al.*, 2019). In contrast, the shortage of essential elements may cause abnormalities in plant development, growth and reproduction, preventing individuals from completing the life cycle (Taiz *et al.*, 2017).

Thus, considering the importance of the species and the lack of information on fertilization for seedling production, the objective of this study was to verify the influence of nitrogen fertilization on the quality of *Myrceugenia myrtoides* seedlings grown in pots with the composted pine bark substrate.

## 2. Material and methods

The study began in April 2017, with the collection of ripe fruits in two adult

plants of *M. myrtoides*, located in Pântano Grande, Rio Grande do Sul state (RS) (30°17'25" S latitude and 52°27'56" W longitude). The collection region has a climate characterized as moderately humid and rainfall around 1500 to 1700 mm per year distributed over 90 to 110 days of rain. The average annual temperature varies from 17 to 20 °C, with the average temperature of the coldest month ranging between 11 and 14 °C and the warmest month between 23 and 26 °C (Maluf, 2000; Peel *et al.*, 2007).

After collection, the fruits were taken to the Biotechnology Laboratory of the Department of Horticulture and Forestry of the Federal University of Rio Grande do Sul (UFRGS) in Porto Alegre, RS. Therefore, the fruits were placed on a bench, where they remained for three days at room temperature and relative humidity. Seed processing consisted of the manual removal of the fruit pulp, which was then washed in running water. Afterwards, the seeds were placed on paper towels to remove excess of water for 24 hours. The seeds were homogenized in only one batch and kept in a refrigerator at 5 to 8 °C for 90 days.

The seeds were placed in multicellular polyethylene trays containing the commercial substrate Carolina Soil®. When the seedlings reached an average height of 10 cm, they were transplanted to 0.4 L containers containing composted pine bark as a substrate, which was subjected to the characterization of the physical and chemical properties in the Substrate Laboratory of the Faculty of Agronomy of UFRGS (*Tab. 1*).

**Table 1.** Chemical and physical properties of composted pine bark substrate.

Chemical properties	
EC <sup>a</sup> (mS cm <sup>-1</sup> )	0.51
pH <sup>b</sup> (H <sub>2</sub> O)	6.69
Physical properties	
Dry density (kg m <sup>-3</sup> )	438.90
Bulk density (kg m <sup>-3</sup> )	510.14
Current humidity (%)	013.97
Total porosity (%)	064.70
Air space (%)	020.23
Easily available water (%)	010.31
Buffering water (%)	001.66
Remaining water (%)	032.50
Available water (%)	011.97

<sup>a</sup>EC = electrical conductivity obtained in 1:5 substrate:water (v/v); <sup>b</sup>pH = hydrogen potential.

The treatments consisted of five nitrogen doses (*Tab. 2*). The commercial product Kristalon®, calcium nitrate (Ca(NO<sub>3</sub>)<sub>2</sub>) and ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>) were used as nutrient sources. According to manufacturer information, Kristalon® ferti-

lizer (formulation 6-12-36) contains 6% nitrogen (4.5% nitric and 1.5% ammoniacal), 12% phosphorus (P<sub>2</sub>O<sub>5</sub>), 36% potassium (K<sub>2</sub>O), 1.8% magnesium (Mg), 8% sulfur (S), 0.07% iron (Fe), 0.025% boron (B), 0.01% copper (Cu), 0.04% manganese (Mn), 0.004% molybdenum (Mo) and 0.025% zinc (Zn).

**Tabla 2.** Nitrogen fertilization doses used for fortnightly fertirrigation in the production of *Myrceugenia myrtoides* seedlings.

Treatments	Fertilizer dose (g L <sup>-1</sup> )	Nitrogen dose (g L <sup>-1</sup> )	Nitrogen dose (mg plant <sup>-1</sup> )
T1	Control (no fertilization)	0.00	00.00
T2	5 g de Kristalon + 2.5 g de Ca(NO <sub>3</sub> ) <sub>2</sub>	0.69	06.90
T3	5 g de Kristalon + 2.5 g de Ca(NO <sub>3</sub> ) <sub>2</sub> + 1.44 g de NH <sub>4</sub> NO <sub>3</sub>	1.12	11.2
T4	5 g de Kristalon + 2.5 g de Ca(NO <sub>3</sub> ) <sub>2</sub> + 2.88 g de NH <sub>4</sub> NO <sub>3</sub>	1.55	15.5
T5	5 g de Kristalon + 2.5 g de Ca(NO <sub>3</sub> ) <sub>2</sub> + 4.32 g de NH <sub>4</sub> NO <sub>3</sub>	1.98	19.8

Fertilization was performed fortnightly through fertirrigation, where 10 mL of solution was applied per container. The control treatment plants received no fertilization, only the same volume of water (distilled water) supplied to the other treatments. Electric conductivity (EC) and hydrogen potential (pH) of each treatment were monitored interspersed with fertirrigation, biweekly, using the non-destructive Pour Thru method (Cavins *et al.*, 2000).

Over the experiment, the plants were kept under a bench, in a greenhouse with polyethylene cover and anti-aphid walls owned by the Department of Horticulture and Forestry of the Federal University of Rio Grande do Sul, located in Porto Alegre, RS (30°04'26" S latitude and 51°08'07" W longitude). The irrigation system used in the study was a micro-sprinkler with 8 mm water depth day<sup>-1</sup>, distributed in four daily irrigation shift.

After 230 days of conduction of the experiment, the dry mass of the aerial part and root system, and the morphological quality of the seedlings were evaluated through the parameters of the ratio height and aerial part dry mass (H/APDM), ratio between aerial part dry mass and root (APDM/RDM) and Dickson Quality Index (DQI) (Dickson *et al.*, 1960).

The experimental design was completely randomized, with five doses of nitrogen fertilization and four replications of seven plants, totaling 140 experimental units. Data were submitted to one-way analysis of variance (ANOVA) and polynomial regression, seeking to test the effect of nitrogen doses on the quality of *Myrceugenia myrtoides* seedlings, at 5% probability of error, with the aid of Costat 6.4 and SigmaPlot 11.0 software, respectively. It was necessary to transfer the dry mass data of the aerial part to  $\frac{1}{x}$ , for them to pass the normality test.

### 3. Results and discussion

The results showed the influence of nitrogen fertilization on dry mass of the aerial part (APDM), height and aerial part dry mass (H/APDM) and the relationship between aerial part dry mass and root system (APDM/RDM) (*Tab. 3*), which presented quadratic adjustment as doses of nitrogen were increased. However, for root system dry mass and Dickson quality index, no significant difference was found between the evaluated doses.

**Tabla 3.** Significance level, average, coefficient of variation and degree of freedom of *Myrceugenia myrtoides* seedlings as a function of different doses of nitrogen fertilization.

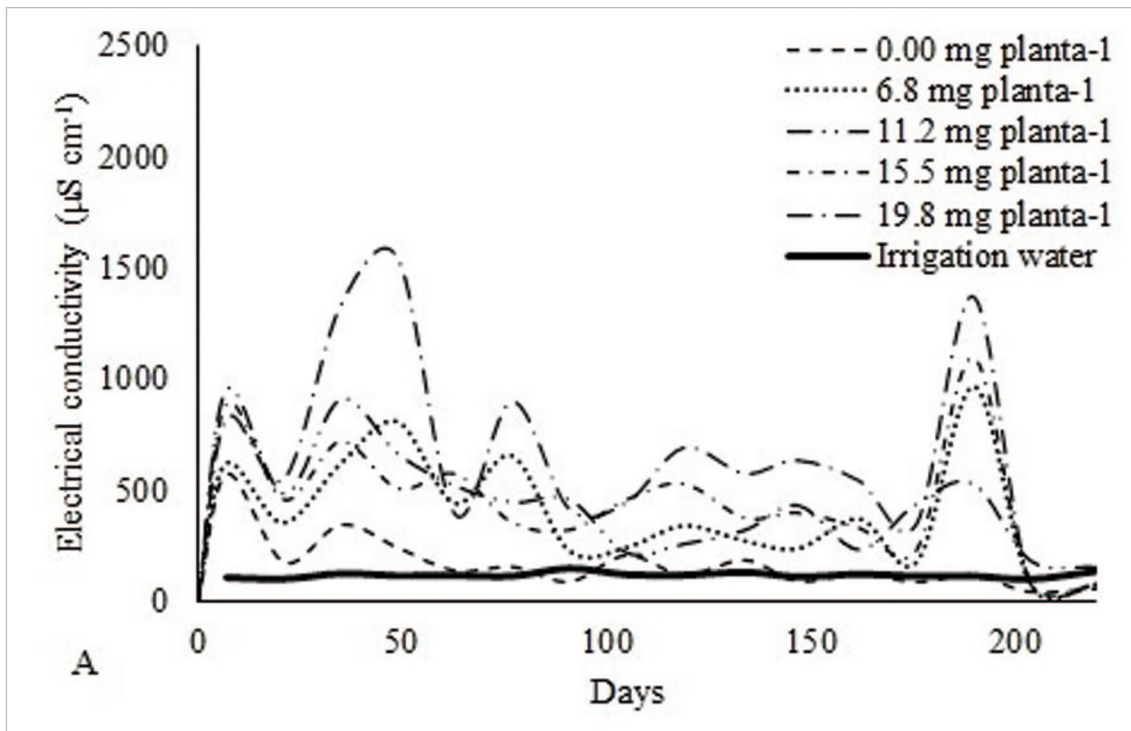
	Significance level	Average	CVe (%)	DF <sup>f</sup>
APDM <sup>a</sup> (g)	p<0.001	4.11	24.32	15
RDM <sup>b</sup> (g)	00p=0.5741 <sup>ns</sup>	02.04	35.17	15
H <sup>c</sup> /APDM	p<0.001	10.88	16.72	15
APDM/RDM	p<0.010	02.06	20.56	15
DQI <sup>d</sup>	00p=0.2543 <sup>ns</sup>	00.63	34.09	15

<sup>a</sup>APDM = aerial part dry mass; <sup>b</sup>RDM = root dry matter; <sup>c</sup>H = height; <sup>d</sup>DQI = Dickson quality index; <sup>e</sup>CV = coefficient of variation; <sup>f</sup>DF = degree of freedom; <sup>ns</sup> = not significant at 5% error probability.

For shoot dry mass, there was a quadratic trend as nitrogen concentrations in the fertirrigation solution were increased, with a maximum N use point of 14.45 mg plant<sup>-1</sup> (*Fig. 1*). From this point on, seedling aerial mass reduced, therefore demonstrating that the increase in nitrogen supply is beneficial up to an optimal dose, and after that, the nutrient can be harmful, negatively influencing plant growth and development. Despite the reduction in the dry weight of the aerial part of the plants with the highest N rates tested, nitrogen fertilization provided greater mass increase when compared to the absence of this nutrient, since the smallest average occurred in treatment with zero N dose.

Plants have an adequate range of each nutrient for their growth, which also varies according to species development (Taiz *et al.*, 2017). In the case of nitrogen, the lack impairs the formation of the primary plant molecules, mainly affecting the photosynthetic apparatus and, therefore, there is less formation of carbohydrate required for mass growth, which justifies the minimal growth of *M. myrtoides* plants in low doses of N. At higher doses, its absorption occurs by low-affinity transporters, which culminates in a higher than necessary nitrogen flow, causing toxicity to cellular metabolism and, consequently, reduction in growth (Marschner, 2012).

Nitrogen is one of the highly required macronutrients by plants because it has constitution functions of amino acid, protein, nucleic acid, nucleotide, coenzyme, among others (Taiz *et al.*, 2017). Plants absorb this nutrient in the form of ammonium (NH<sup>+</sup><sub>4</sub>) and nitrate (NO<sup>-3</sup>), which is displaced to meristematic tissues, functionally acting in structural expansion and growth (Kerbaudy, 2013; Taiz *et al.*, 2017).



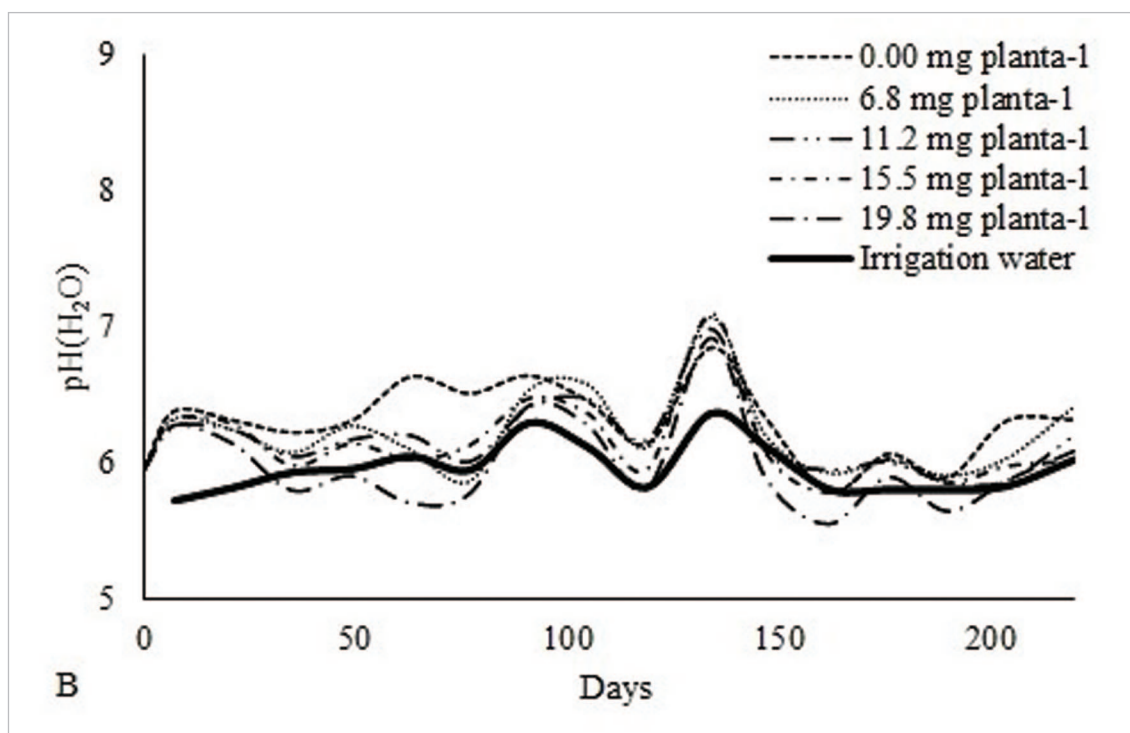
**Figura 1.** Dry mass of the aerial part of *Myrceugenia myrtooides* seedlings treated with different doses of nitrogen fertilization.

Thus, balancing nutrient concentration and the balance in nutrient solutions is of utmost importance for plant development (Vasconcelos *et al.*, 2014).

The variations in the growth of *M. myrtooides* found in this study may be a result of the general nutritional change and not just the availability of N, since the availability of a nutrient may alter the nutrition of the species in general, even if no symptoms of excess or lack of other nutrients have been visually detected. Dias *et al.* (2012), when evaluating the accumulation of other nutrients in response to nitrogen fertilization in guava (*P. guajava* cv. Paluma), observed that the doses of N employed in the study influenced the concentrations of other nutrients such as phosphorus, potassium, calcium, magnesium, zinc and sulfur. For the same species, Dantas Neto *et al.* (2013) reported the influence of nitrogen fertilization on leaf nutrient levels, mainly for calcium and potassium.

The calculated ratio between the dry matter of the aerial part and its root system is considered as an efficient and safe index to express the quality of the seedlings, used for plant growth measurements in ecological and physiological studies (Gomes and Paiva, 2013). In this study, the ratio between dry mass of the aerial part and root system showed that the aerial part of the seedling was superior to the root system as the concentration of N applied to the substrate increased, with a maximum point at the highest tested dose tested ( $19.8 \text{ mg planta}^{-1}$ ) (Fig. 2).

Plants submitted to fertirrigation without nitrogen application had the lowest APDM/RDM ratio (Fig. 2). When the rhizosphere is poor in nutrients, root growth



**Figura 2.** Ratio between aerial part dry mass and root dry mass (APDM/RDM) of *Myrceugenia myrtooides* seedlings treated with different doses of nitrogen fertilization.

amplitude becomes smaller and, consequently, the translocation of essential minerals to the aerial system will be reduced, which will reduce plant growth (Taiz *et al.*, 2017), which explain the results obtained in *M. myrtooides* seedlings not fertirrigated with nitrogen. Such plants had their initial growth supported by ions already present in the substrate at transplanting and also in the seed, which can be corroborated by the electrical conductivity of  $0.51 \text{ mS cm}^{-1}$  showed by it (Tab. 1). By depleting the nutrients present in the substrate, the seedlings in the treatment without complementary fertirrigation had their development affected due to the absence, particular of nitrogen, a fundamental component of plant biomolecules necessary for cell multiplication.

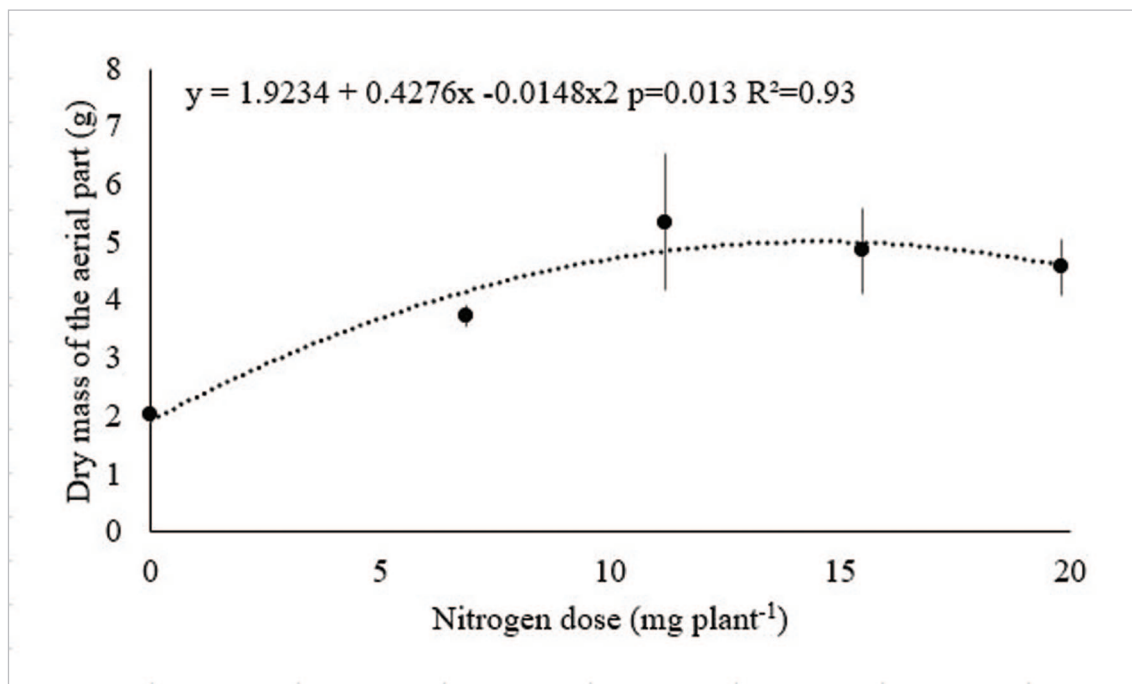
The highest N concentrations provided superiority significantly in APDM/RDM ratio of *M. myrtooides* seedlings. Thus, the plants allocated more energy to the aerial part, increasing the photosynthetic area, consequently the carbohydrate production. In the early stages of development of most plants, a rapid growth is observed, such as seedling formation. In this situation, the rates of nutrient reduction such as nitrogen and sulfur and amino acid synthesis are high, which are mainly used in the synthesis of Rubisco, chlorophyll and other proteins in the aerial part. Thus, growth of the aerial part is prioritized over the root system, as this is the local of energy production for plant growth and development (Floss, 2006; Taiz *et al.*, 2017).

The increase in the APDM/RDM ratio may have a negative effect on seedling quality as this condition shows that there is a reduction in root growth. An adequa-



te root system has been placed as fundamental for a proper survival and growth in the field (Fracaro and Pereira, 2004; Mafia *et al.*, 2005; Oliveira *et al.*, 2006; Simões *et al.*, 2012). However, this result may also be linked to the size of the container (0.4 L) because according to Cruz *et al.* (2016), the size of the container directly influences the growth and development of seedling root system. Thus, it is observed that for *M. myrtooides* seedlings there was a limitation on the root growth of the seedlings due to the smaller space imposed by the cultivation pot.

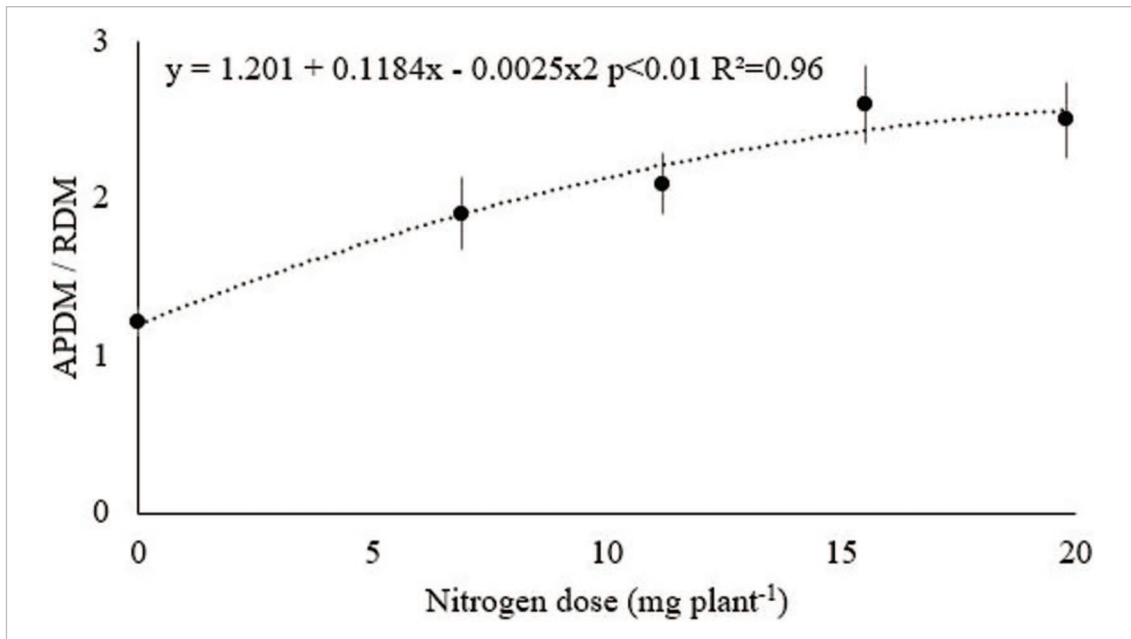
The ratio between height and dry mass of the aerial part of the plants is very useful for predicting the survival potential of the seedlings in the field. The lower this index, the more lignified the seedlings and the greater their survival capacity in the field (Gomes and Paiva, 2013). The results of this study showed a quadratic tendency proportional to the increase in nitrogen fertilization concentrations, with a minimum point of 16.1 mg plant<sup>-1</sup> (Fig. 3). The mean for treatment in the absence of N was 16.1, with an average decrease of 40% for the treatments that received nitrogen fertilization. Thus, the highest concentrations used in the *M. myrtooides* study are indicated to obtain seedlings with a higher chance of survival in the field.



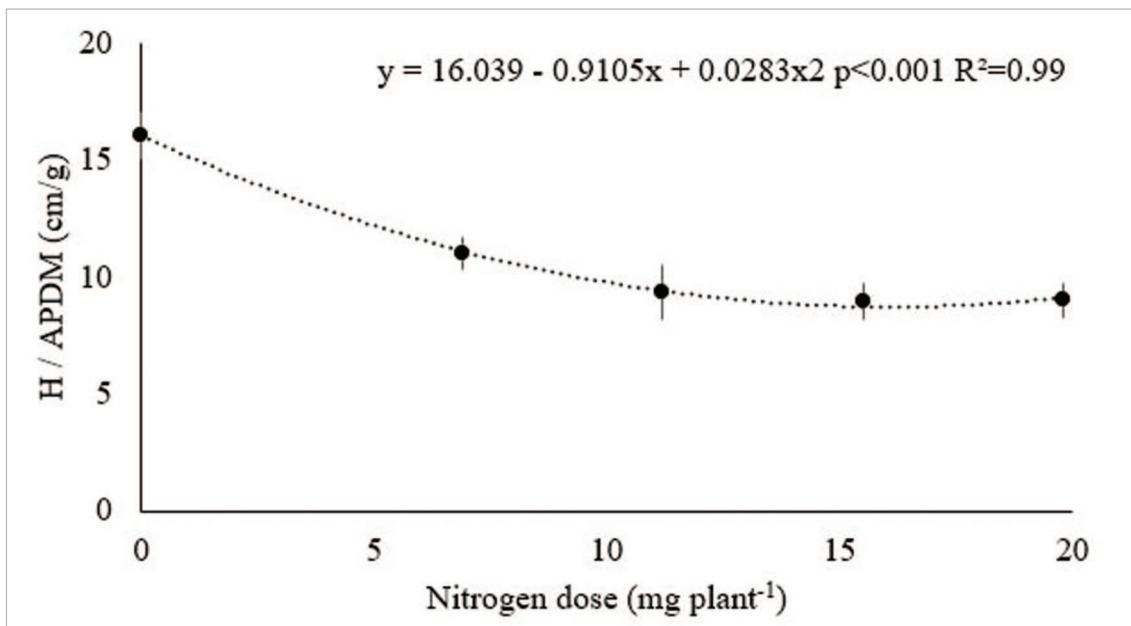
**Figura 3.** Ratio between height and aerial part dry mass (H/APDM) of *Myrceugenia myrtooides* seedlings as a function of the different doses of nitrogen fertilization.

Appropriate use of nitrogen doses in plant fertilization has also shown satisfactory results in the production and quality of seedlings of other species of the *Myrtaceae* family, as it was found in works in protected environment with *Campomanesia aurea* O. Berg (Emer *et al.*, 2019) and *Campomanesia xanthocarpa* O. Berg (Carnevali *et al.*, 2017).

Plant nutrition in the nurseries is managed by using base and complementary fertilizers (Kämpf, 2005). Therefore, knowledge about the chemical and physical properties of substrates is of fundamental importance as they are key factors in crop management and quality control (Schmitz *et al.*, 2002). The electrical conductivity values of substrates provide an estimate of the soluble salt content of the medium, as ions dissolved in water conduct electric current in a direct proportion to their concentration (Fermino, 2014). In this context, the monitoring data of substrate electri-



**Figure 4.** Electrical conductivity (A) and hydrogen potential (pH) (B) over *Myrceugenia myrtooides* seedlings growing period as a function of the different doses of nitrogen fertilization.



cal conductivity over *M. myrtooides* seedling cultivation period showed that there was no substrate salinization with fertirrigation. Since, soon after the increase in conductivity values, the treatments with the highest doses of nitrogen fertilization (15.5 and 19.8 mg planta<sup>-1</sup>) showed a decrease (*Fig. 4A*), indicating that the excess of nutrients was absorbed by the plants.

The pH refers to the alkalinity or acidity reaction of the culture medium, and its knowledge is paramount as they influence nutrient availability and have effects on plant physiological processes (Kämpf, 2005). For substrates with predominance of organic matter, the recommended pH range is 5.0 to 5.8; and for mineral-based materials, between 6.0 and 6.5 (Kämpf, 2005). Some factors may cause changes in their values during the cultivation period, among which the alkalinity of irrigation water and the fertilization adopted by the management of the nurseryman can be highlighted (Fochesato *et al.*, 2008; Ceccagno *et al.*, 2019). As a result, the pH variation verified throughout this study is due to the irrigation water alkalinity, that is, the similar variation between the different concentrations of nitrogen fertilization oscillated according to the pH values of the irrigation water (*Fig. 4B*).

In this context, nitrogen is one of the main nutrients for plants as its accumulation in plant tissues triggers the regulation of carbon flow towards more intense protein synthesis (high N content) or carbohydrate (low N content). (Pinheiro *et al.*, 2017). Moreover, it acts in the formation of important molecules for plant metabolism, such as proteins, amino acids, chlorophyll, among others (Taiz *et al.*, 2017). Therefore, nitrogen is decisive for plant growth because it influences the physiological processes responsible for the energy supply of plants (Marschner, 2012). Thus, the use of nitrogen fertilization is an important supply for the formation of quality seedlings, because, according to Marinho *et al.* (2010), nitrogen increases production and yield over cultivation.

#### **4. Conclusions**

Nitrogen fertilization on *Myrceugenia myrtooides* seedlings at concentrations between 15.5 and 19.8 mg per plant provided higher morphological quality in plants grown in pots with the composted pine bark substrate.

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