

## GROWTH AND PHENOLIC COMPOUNDS IN CILANTRO AS A FUNCTION OF THE IONIC STRENGTH OF THE NUTRIENT SOLUTION

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### ABSTRACT

Cilantro (*Coriandrum sativum* L.) is widely used in culinary, pharmaceutical, and cosmetic industries. Proper nutritional management is essential for the growth and quality of plants in hydroponic cultivation. This study evaluated the growth, macronutrient content, and phenolic compounds in cilantro under different concentrations of nutrient solutions. The experiment, conducted in a greenhouse, followed a randomized block design with six treatments (25% to 150% of Hoagland and Arnon ionic strength) and five replicates. Plants were harvested 60 days after sowing for analysis of height, dry mass, leaf area, macronutrients, and total phenols. Maximum growth occurred between 80% and 100% ionic strength. Nitrogen (N) and potassium (K) showed higher concentrations at 85% and 63.5%, respectively, while phosphorus (P) increased up to the maximum concentration, corresponding to 150% of the ionic strength, and calcium (Ca), magnesium (Mg), and sulfur (S) decreased at higher ionic strength. The content of phenolic compounds decreased with increasing ionic strength. It is concluded that ionic strength directly influences the growth, nutrition, and quality of cilantro, requiring a nutritional balance to maximize production and the quality of secondary metabolites.

**Additional Keywords:** *Coriandrum sativum* L., electrical conductivity, hydroponics, secondary metabolites

### RESUMEN

#### **Crecimiento y compuestos fenólicos en el cilantro en función de la fuerza iónica de la solución nutritiva**

El cilantro (*Coriandrum sativum* L.) posee amplio uso doméstico, así como en las industrias farmacéutica y cosmética. Por tanto, un manejo nutricional adecuado es esencial para el crecimiento y la calidad de las plantas en cultivos hidropónicos. Se evaluó el crecimiento, los contenidos de macronutrientes y de compuestos fenólicos del cilantro bajo diferentes concentraciones de solución nutritiva. El experimento fue realizado en un invernadero siguiendo un diseño de bloques al azar con seis tratamientos (25 a 150 % de la fuerza iónica de Hoagland y Arnon) y cinco repeticiones. Las plantas fueron cosechadas a los 60 días después de la siembra para analizar la altura, la masa seca, el área foliar, los macronutrientes y los fenoles totales. El crecimiento máximo se observó entre el 80 y 100 % de la fuerza iónica (FI). Por su parte, el N y el K presentaron mayores contenidos al 85 y 63,5 %, respectivamente, mientras que el P aumentó hasta la concentración máxima, correspondiente al 150 % de FI; y el Ca, Mg y S disminuyeron en los valores más altos. El contenido de compuestos fenólicos se redujo con el aumento de FI. Se concluyó que esta variable influye directamente en el crecimiento, la nutrición y la calidad del cilantro, siendo necesario un equilibrio nutricional para maximizar la producción y la calidad de los metabolitos secundarios.

**Palabras clave adicionales:** *Coriandrum sativum* L., conductividad eléctrica, hidroponía, metabolitos secundarios

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### INTRODUCTION

*Coriandrum sativum* L. is a perennial herbaceous plant belonging to the Apiaceae family, with nutritional properties (Guimarães *et al.*, 2024). Originating from the eastern Mediterranean and Black Sea regions, it has rapidly spread to various areas due to its hardiness

and natural propagation. Records of its cultivation date back thousands of years, owing to its aromatic, aphrodisiac, medicinal, and culinary properties (Sharma and Sharma, 2012; Laribi *et al.*, 2015; Önder, 2018). In gastronomy, the seeds are used to flavor fish, meat, bread, and desserts, while the fresh leaves, known as cilantro or Chinese parsley, are widely used in Oriental and

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Indian cuisines to enhance flavors or mask unpleasant odors (Laribi *et al.*, 2015).

Cilantro has attracted significant scientific attention due to its rich phytochemical composition and diverse biological activities (Matasyoh *et al.*, 2009). With the growing promotion and application of traditional Chinese medicine worldwide, herbs like cilantro are still present in 40 % of prescribed medications (Wei *et al.*, 2024).

Cilantro is rich in vitamins A, B1, B2, and C, as well as minerals such as Ca and Fe (Filgueira, 2013). Another important characteristic is the presence of phytochemicals, which act as antioxidants, enzyme inducers/inhibitors, bactericides, and antivirals (Ashraf *et al.*, 2018), in addition to providing its characteristic flavor and aroma (Mallik *et al.*, 2020).

One of the main groups of phytochemicals is phenolic compounds derived from secondary metabolism (Arnoso *et al.*, 2019). These compounds are known for their antioxidant properties and preventive effects against various diseases, including cancer, heart disease, pathogenic infections, diabetes, and muscle disorders (Tungmunnithum *et al.*, 2018).

According to Deus *et al.* (2019), the demand for natural antioxidants for use in food products or pharmaceuticals has increased significantly since the 1980s.

Phenolic compounds are formed from the secondary metabolism of plants and contain a phenol group, i.e., a functional hydroxyl group attached to an aromatic ring (Isah *et al.*, 2018). They play a crucial role in plant defense mechanisms under stress conditions (Borges and Amorim, 2020). Environmental factors such as temperature, humidity, light, water availability, minerals, and carbon dioxide concentration, CO<sub>2</sub>, influence plant growth and directly impact biochemical pathways affecting secondary metabolite production (Akula and Ravishankar, 2011). Thus, the accumulation of these metabolites can be enhanced in response to abiotic stress, either independently or in combination (Lu *et al.*, 2017).

Recent studies on various crops grown hydroponically have reported that the availability and concentration of nutrient solutions not only affect growth but also alter the concentration of secondary metabolites such as terpenes and phenolic compounds. Examples include studies on *Diplotaxis tenuifolia* (L.) DC (Bonasia *et al.*,

2017), *Solanum lycopersicum* (Moya *et al.*, 2017), *Perilla frutescens* (Lu *et al.*, 2017), *Perilla frutescens* var. *crispa* cv. (Nguyen *et al.*, 2021), *Ocimum basilicum* L. var. *basilicum* L. cv. Genovese (Ren *et al.*, 2022), *Agastache rugosa* (Lam *et al.*, 2020), and *Acmeella oleracea* L. (Carmo *et al.*, 2024).

The use of hydroponic cultivation for medicinal and aromatic plants is a common practice. It serves as an alternative to facilitate the control and availability of nutrients in the nutrient solution provided during cultivation (Ferreira *et al.*, 2017), promoting vigorous, efficient, and uniform development, longer productive cycles, and water savings of 80-90 % compared to conventional cultivation (Sharma *et al.*, 2018).

According to Scandar *et al.* (2023), more than 3,000 articles on coriander have been published since the beginning of this century, with over 2,000 published in the last 12 years. Most of them address topics related to essential oils, whereas only 343 focus on polyphenols. Despite increasing research on the discovery, evaluation, and quantification of active compounds in aromatic and medicinal plants, more studies are needed on nutrient solution management to enhance the productivity and quality of these plants. Therefore, this study aimed to evaluate the effect of ionic strength concentrations in the nutrient solution on the growth, nutrition, and phenolic compound content in cilantro plants.

## MATERIALS AND METHODS

The experiment was conducted in a greenhouse covered with low-density polyethylene film (100 µm) and Sombrite® 50 % at the Research Support Unit of the Center for Agricultural Sciences and Technologies, located at the State University of Northern Rio de Janeiro Darcy Ribeiro, in Campos dos Goytacazes, RJ (21°19'23" S, 41°10'40" W, 14 m altitude). During the experiment, maximum and minimum temperatures and relative humidity were recorded using a HOBO® Data Logger Pro v2 (Onset Headquarters, Bourne, MA, USA), with an average maximum temperature of 27.2 °C and minimum of 17.9 °C, and an average maximum relative humidity of 99.3 % and minimum of 54.4 %.

The experimental design was randomized blocks with six treatments and five replicates. The

treatments corresponded to six ionic strengths of the nutrient solution proposed by Hoagland and Arnon (1950), with an electrical conductivity (EC) of 2.0 mS cm<sup>-1</sup> in its original composition (100 %). The treatments were: 0.5 mS cm<sup>-1</sup> (25 %), 1.0 mS·cm<sup>-1</sup> (50 %), 1.5 mS cm<sup>-1</sup> (75 %), 2.0 mS cm<sup>-1</sup> (100 %), 2.5 mS cm<sup>-1</sup> (125 %), and 3.0 mS cm<sup>-1</sup> (150%). The pH of the solutions was maintained between 5.5 and 5.8, adjusted with 1N NaOH and HCl. The solutions were replaced when the EC fell below the treatment levels. Each experimental unit consisted of a 16 L pot containing four plants.

The propagative material used was cilantro seeds cv. Verdão SF 177. Sowing was performed in phenolic foam trays washed with deionized water. After sowing, the seeds were irrigated only with deionized water to maintain moisture until germination and emergence of the first pair of leaves. From this point, a nutrient solution with 25 % ionic strength was applied.

To ensure the plants reached the necessary size for transplantation, they were grown in trays using polystyrene foam (7 cm in diameter) as support. Five days after sowing, the foam cells containing cilantro seedlings were placed in small polystyrene rings and placed in trays containing a solution with 50 % ionic strength, aerated with aquarium pumps (SKRW, model CA-46, 127 V, 60 Hz, 5 W, flow rate 2-4 L min<sup>-1</sup>). Eight days after sowing, the concentration was increased to 75 %, and after 12 days, to 100 %. The concentration was increased until the respective treatment levels were reached in each tray.

The pots were painted white to prevent heating of the nutrient solution. Each pot contained a circular polystyrene support with a thickness of 30 mm for the plants. Four equidistant holes were made in the supports, where the cilantro plants were later placed. A small hole near the edge allowed for the passage of a silicone hose with a porous stone at its end, connected to an aquarium pump which operated continuously to aerate the nutrient solution during the experiment.

At 60 days after sowing, when the plants reached commercial size (25-40 cm; Luz *et al.*, 2012), the experiment was harvested. Biometric parameters such as height, leaf area, and dry mass of the aerial part (commercial portion) were evaluated. Measurements were taken using a ruler (cm), a precision scale (g) (OHAUS, model Adventurer, accuracy 0.1 g), and a leaf area meter

(LI-COR, model LI-3100C, range 0-1000 cm<sup>2</sup>, resolution 1 mm<sup>2</sup>). It was measured immediately after harvest, and dry mass was obtained after drying at 45 °C in a forced-air (MARCONI, model MA035/B 4.2 kW) oven for 72 hours.

The plant material was ground in a Wiley knife mill (MARCONI, model MA340) equipped with a 1-mm mesh sieve, resulting in particles smaller than 1 mm for quantification of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) content.

For N determination, the plant material (0.1 g) was subjected to sulfuric digestion, and nitrogen was determined using the Nessler method (Jackson, 1965). The other nutrients (P, K, Ca, Mg, and S) were quantified using ICP-OES after digestion with concentrated HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> in an open digestion system. The ICP conditions were: plasma gas 8.0 L min<sup>-1</sup>, auxiliary gas 0.70 L min<sup>-1</sup>, and carrier gas 0.55 L min<sup>-1</sup> (Peters, 2005).

For total phenol determination in the aerial part of cilantro plants (leaves and stems), 5.0 g of plant tissue with 12 % moisture and a particle size between 60 and 80 mesh were weighed. 20 mL of methanol were added, and the mixture was agitated for one hour on a magnetic stirrer or shaker. After a 15-minute pause, 5 mL of methanol was added, and agitation resumed for another 30 minutes. The homogenate was filtered through Whatman No. 1 filter paper into a 25 mL volumetric flask, and the volume was completed with methanol. The extract was then transferred to a separatory funnel and washed three times with 10 mL of hexane.

The aqueous extract was clarified with 5 mL of 0.3 mol·L<sup>-1</sup> barium hydroxide and 5 mL of 5 % zinc sulfate solution. After 20 minutes of rest, it was centrifuged. Subsequently, a 2 mL aliquot of the clarified extract was taken, and 2 mL of 2 % sodium carbonate in 0.1 mol·L<sup>-1</sup> NaOH and 1 mL of Folin-Ciocalteu reagent (1:2 dilution) were added. After 10 minutes at 37 °C, the samples were read in a spectrophotometer (ANALYTIC JENA, model Specord 210) at 660 nm. A standard curve of gallic acid with concentrations ranging from 2 to 20 mg mL<sup>-1</sup> of methanol was used to quantify phenol content (Furlong *et al.*, 2003).

After meeting the assumptions of normality and homogeneity, the data were subjected to analysis of variance (ANOVA) using the F-test

and regression analysis at a 5 % probability level, using the SANEST program (Zonta *et al.*, 1984).

## RESULTS AND DISCUSSION

The summary of the analysis of variance (ANOVA) for growth, phenolic compounds, and nutrient accumulation in cilantro plants is presented in Table 1. This table shows the significance of the effects of treatments, as well as

the coefficients of variation (CV) and overall means for each evaluated variable.

**Growth of *C. sativum* L.** Cultivation under different ionic strengths of the Hoagland and Arnon nutrient solution influenced growth variables, except for plant height, which averaged 36.87 cm at 60 days after sowing, a parameter generally less sensitive to nutrient availability in cilantro.

**Table 1.** Summary of the analysis of variance (ANOVA) for plant growth, phenolic compounds, and macronutrient contents (N, P, K, Ca, Mg, and S) of cilantro plants cultivated under different ionic strengths of the nutrient solution.

| Variable                                 | Treatment ( <i>p</i> >F) | CV (%) | Mean   |
|--|--------------------------|--------|--------|
| Plant height (cm)                        | 0.34772 ns               | 10.46  | 36.87  |
| Shoot dry mass (g)                       | 0.00236 *                | 14.86  | 2.67   |
| Leaf area (cm <sup>2</sup> )             | 0.00393*                 | 10.78  | 715.22 |
| Phenolic compounds (g kg <sup>-1</sup> ) | 0.00001*                 | 4.15   | 9.65   |
| N (g kg <sup>-1</sup> )                  | 0.00001*                 | 5.15   | 57.20  |
| P (g kg <sup>-1</sup> )                  | 0.00275*                 | 7.68   | 8.34   |
| K (g kg <sup>-1</sup> )                  | 0.03695*                 | 5.55   | 89.72  |
| Ca (g kg <sup>-1</sup> )                 | 0.00001*                 | 5.15   | 17.63  |
| Mg (g kg <sup>-1</sup> )                 | 0.00001*                 | 2.34   | 3.24   |
| S (g kg <sup>-1</sup> )                  | 0.00621*                 | 8.48   | 3.6    |

ns=not significant; \*=significant at 5% probability by the F-test.

Silva *et al.* (2020), in an experiment with the 'Verdão' cultivar under 100 % ionic strength of the Furlani *et al.* (1999) solution (EC 2.27-2.37 dS·m<sup>-1</sup>), obtained an average height of 25.42 cm at 35 days after sowing in summer. Oliveira *et al.* (2016) and Luz *et al.* (2012) reported similar results, with maximum heights of 28.98 cm at 87.2 % and 27.9 cm at 95 % ionic strength of the Furlani *et al.* (1999) solution, respectively, in hydroponic cultivation of cilantro cv. Verdão. These values are lower than those found in the present study, likely due to the shorter harvest time in the mentioned studies, as cilantro has a wide commercial size range (25-40 cm) depending on market demands (Luz *et al.*, 2012).

The leaf area followed quadratic regression model (Figure 1A), with maximum value of 792.5 cm<sup>2</sup> estimated at 87.4 % ionic strength. Leaf area represents the main site for photoassimilate production in plant metabolic processes and is also economically important, since leaves constitute the main commercial product in cilantro cultivation.

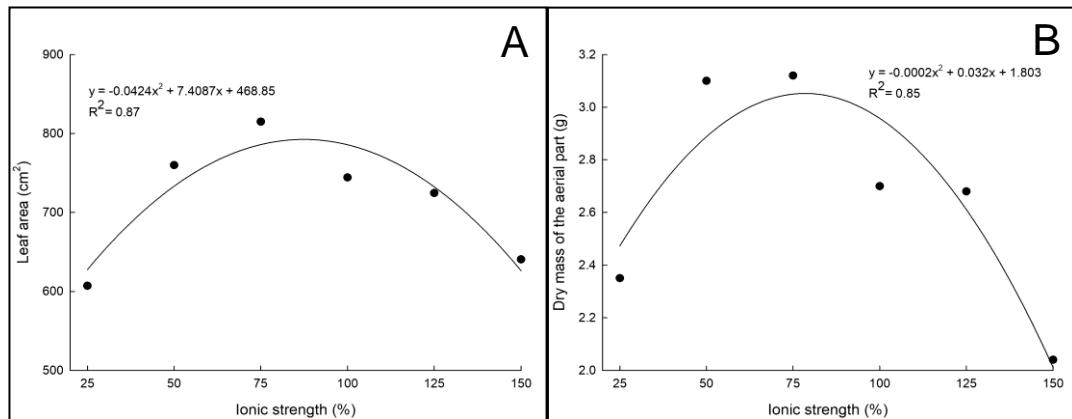
Calori *et al.* (2014) observed a linear increase in leaf area in lettuce up to 75 % ionic strength. Conversely, Rebouças *et al.* (2013) reported a decrease in leaf area and height in cilantro under salt stress, possibly as a plant strategy to maintain photosynthetic activity and development. Souza *et al.* (2020a) observed a quadratic response in leaf area in watercress, like the present study, with the best results at 1.7 dS·m<sup>-1</sup>.

The increase in ionic strength of the nutrient solution led to an initial increase followed by a decrease in dry mass of the aerial part. The maximum value was 3.0 g at 79.8 % ionic strength. (Figure 1B). Luz *et al.* (2012) also observed a quadratic response in cilantro, with maximum dry mass of 10.93 g·plant<sup>-1</sup> at 95.48 % ionic strength. Differences in values may be related to varying cultivation conditions, such as climate and hydroponic systems (DFT and NFT), as well as cultivation time.

Calori *et al.* (2014) reported an increase in dry mass of lettuce up to 75 % ionic strength. In contrast, Filgueiras *et al.* (2002) found no

significant differences in this variable in lettuce under increasing nutrient solution concentrations. Luz *et al.* (2012) observed an increase in dry mass

of parsley, with a maximum at 91.4 % ionic strength.



**Figure 1.** (A) Leaf area; (B) Dry mass of the aerial part of cilantro plants (leaves and stems) grown in a hydroponic system under different ionic strengths of the nutrient solution, Campos dos Goytacazes-RJ, Brazil.

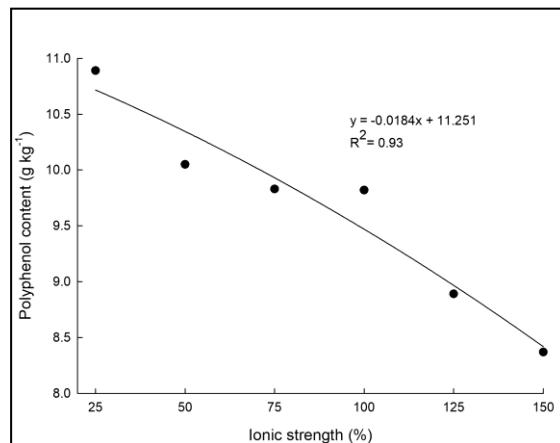
Vasconcelos *et al.* (2014) reported similar results for cilantro under different concentrations of the Furlani *et al.* (1999) solution, with the highest dry mass at  $2.14 \text{ dS} \cdot \text{m}^{-1}$ . In arugula under salt stress, Silva *et al.* (2013) observed a linear decrease in dry mass with increasing EC.

The effects of ionic strength on plant growth vary among species, but extreme concentrations (low or high) provide insights into the necessary balance for optimal development. Grattan and Grieve (1999) highlighted that low salt

concentrations reduce growth and productivity due to nutrient deficiency, while high concentrations inhibit nutrient absorption through competition at absorption sites, leading to water stress and reduced photosynthetic efficiency (Taiz and Zeiger, 2013; Safdar *et al.*, 2019).

#### Polyphenol Content in *C. sativum* L.

Polyphenol production in cilantro showed a linear decrease with increasing nutrient solution concentration, with a 23.1 % reduction between the lowest and highest doses (Figure 2).



**Figure 2.** Polyphenol content in the aerial part of cilantro plants (leaves and stems) grown in a hydroponic system under different ionic strengths of the nutrient solution, Campos dos Goytacazes-RJ, Brazil.

Polyphenols, including flavonoids and isoflavonoids, are secondary metabolites that play a role in plant growth and stress responses (Ashraf *et al.*, 2018). The response of secondary metabolism to environmental stimuli varies among species. Bonasia *et al.* (2017) observed higher total phenols and antioxidant capacity in arugula under higher salinity ( $2.5 \text{ dS} \cdot \text{m}^{-1}$  vs.  $3.5 \text{ dS} \cdot \text{m}^{-1}$ ) in autumn-winter cultivation. In tomato cultivation, Moya *et al.* (2017) reported an 8.3 % increase in total phenols and 11.1 % in antioxidant activity at higher EC ( $4.5 \text{ dS} \cdot \text{m}^{-1}$ ).

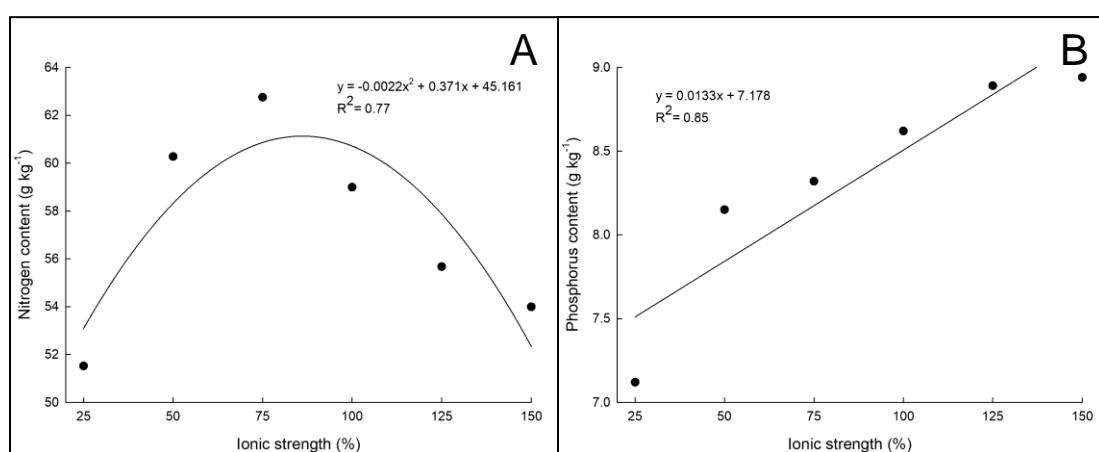
Conversely, Ren *et al.* (2022) found that lower EC increased total phenols and antioxidant capacity in basil. Similarly, Portela *et al.* (2012) reported increased phenolic compound production in strawberries at EC levels below  $1.7 \text{ dS} \cdot \text{m}^{-1}$ , highlighting the role of abiotic factors such as nutrient concentration, temperature, and solar radiation in secondary metabolite synthesis.

**Macronutrient Content in *C. sativum* L.** The Hoagland and Arnon (1950) nutrient solution is suitable for leafy vegetables harvested at early

growth stages, despite being originally designed for more nutrient-demanding crops like tomatoes (Alberici *et al.*, 2008).

Nitrogen (N) content in cilantro followed a quadratic response, with a maximum of  $60.8 \text{ g} \cdot \text{kg}^{-1}$  at 85 % ionic strength (Figure 3A). Oliveira *et al.* (2016) observed a linear increase in N content up to  $26.7 \text{ g} \cdot \text{kg}^{-1}$  at 125 % ionic strength in fertigated substrate in northeastern Brazil. In jambu cultivation, Carmo *et al.* (2024) reported a linear increase in N content up to  $14.59 \text{ %}$  at  $4 \text{ mS} \cdot \text{cm}^{-1}$ .

Regarding the phosphorus (P) content in the aerial parts of cilantro, a positive linear response was observed with increasing nutrient solution concentration, up to the maximum level studied, showing a 25.6 % increase at the highest dose compared to the lowest (Figure 3B). Daflon *et al.* (2014) demonstrated that nitrogen (N) and phosphorus (P) are the most limiting nutrients in cilantro production, with plants deficient in these nutrients reducing their biomass by 50 and 49.6 %, respectively.



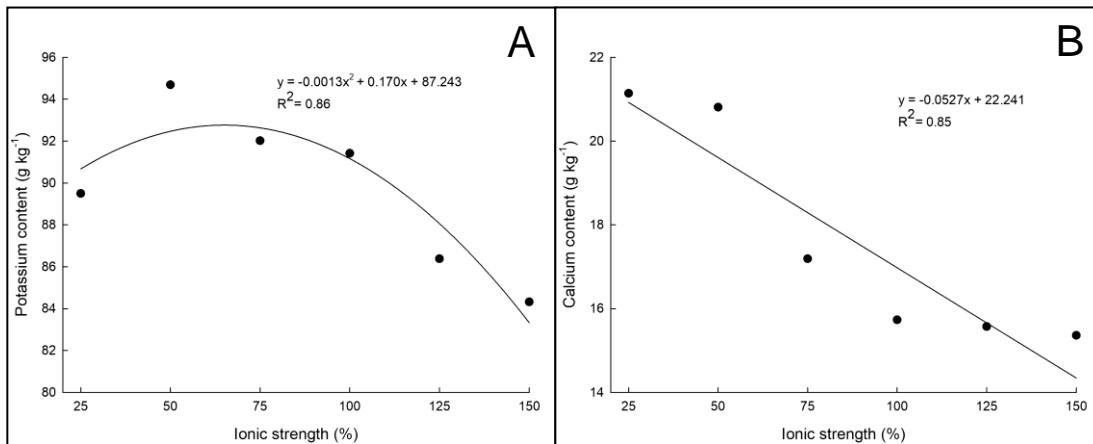
**Figure 3.** (A) Nitrogen content in the aerial part; (B) Phosphorus content in the aerial part of cilantro plants (leaves and stems) grown in a hydroponic system under different ionic strengths of the nutrient solution, Campos dos Goytacazes-RJ, Brazil.

Potassium (K) levels were influenced by the ionic strength of the solution, fitting a quadratic regression model, with the highest content of  $92.8 \text{ g kg}^{-1}$  estimated at an ionic strength of 65.3 % (Figure 4A). Corroborating the present study, Oliveira *et al.* (2016) observed a quadratic behavior in K levels in cilantro cultivation under different ionic strengths of the nutrient solution, with a maximum content of  $53.6 \text{ g kg}^{-1}$  estimated

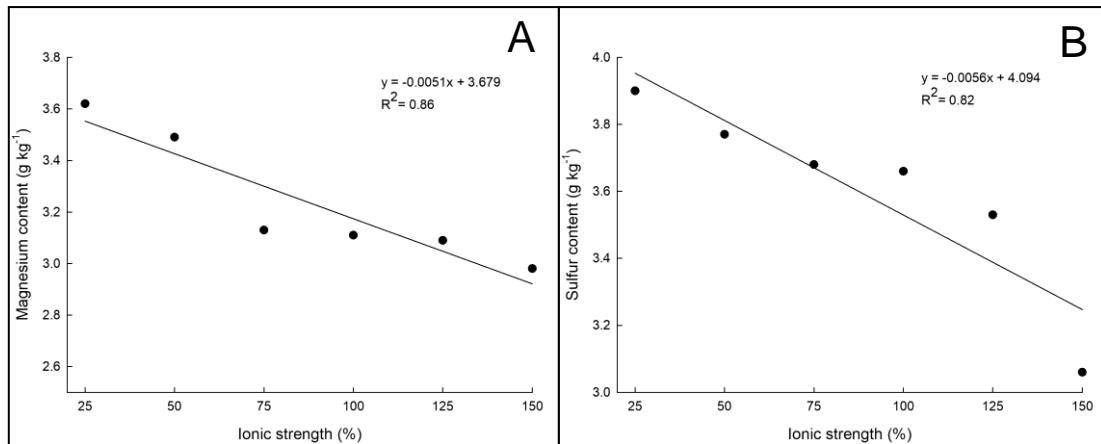
at an ionic strength of 110 %. Carmo *et al.* (2024) reported a linear increase in K content up to an electrical conductivity of  $4 \text{ mS} \cdot \text{cm}^{-1}$  in jambu cultivation, with a maximum content of  $58.7 \text{ g kg}^{-1}$ . This highlights the higher demand for potassium in cilantro compared to jambu, even though the latter was cultivated in Hoagland and Arnon (1950) solution, which has a higher K concentration than Furlani *et al.* (1999) solution.

For the macronutrients calcium (Ca), magnesium (Mg), and sulfur (S), a linear reduction in their content in the aerial parts of cilantro was observed due to the increase in salt concentration in the nutrient solution, with reductions of 27.3 %, 21.5 % and 17.7 %, respectively (Figures 4B, 5A, and 5B). Except for Mg, which exhibited a quadratic behavior with a decrease starting at an electrical conductivity of

2.0 mS·cm<sup>-1</sup>, the Ca and S levels in the study by Carmo *et al.* (2024) decreased linearly up to the maximum conductivity, consistent with the present study. This observed reduction in potassium absorption after the peak, as well as in magnesium, calcium, and sulfur, may be related to the inhibition of nutrient absorption caused by high salt concentrations in the solution (Grattan and Grieve, 1999).



**Figure 4.** (A) Potassium content in the aerial part; (B) Calcium content in the aerial part of cilantro plants (leaves and stems) grown in a hydroponic system under different ionic strengths of the nutrient solution, Campos dos Goytacazes-RJ, Brazil.



**Figure 5.** (A) Magnesium content in the aerial part; (B) Sulfur content in the aerial part of cilantro plants (leaves and stems) grown in a hydroponic system under different ionic strengths of the nutrient solution, Campos dos Goytacazes-RJ, Brazil.

Freitas *et al.* (2020) demonstrated the importance of potassium and sulfur in the composition of cilantro fruits and the production

of essential oils. In their study, these authors observed that the potassium and sulfur composition in the fruit showed a significant

response to increasing potassium availability up to the maximum level studied. Similarly, the essential oil content responded directly to the increase in potassium availability in the medium. The same study also showed how increased potassium availability affects phosphorus and calcium absorption in the plant, linearly compromising the concentration of both nutrients in the plant.

Vasconcelos *et al.* (2014) found that potassium, calcium, and magnesium reach their maximum concentration in the plant at an ionic strength of around 100 % in Furlani *et al.* (1999) solution, followed by a sharp decline with further increases in ionic strength. In contrast, sulfur reached its maximum concentration in the plant at the highest ionic strength studied, 125 %.

Souza *et al.* (2020b), under saline stress conditions induced by the addition of  $\text{Na}^+$  and  $\text{Cl}^-$  to Furlani *et al.* (1999) solution and two circulation frequencies (2 and 3 times per day), observed that the concentration of potassium, calcium, magnesium, and sulfur in green onion plants decreased linearly with increasing salinity of the nutrient solution.

## CONCLUSIONS

Cilantro growth was reduced at high ionic strengths of the nutrient solution. Increased ionic strength caused nutritional imbalances, impairing nutrient absorption and reflecting in reduced plant growth. Polyphenol levels in the aerial parts of cilantro decreased with increasing ionic strength of the nutrient solution. Therefore, under low nutrient availability, the production of these secondary compounds is stimulated.

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