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Nitrogen Sources and Doses in Arugula Development

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Abstract

Leafy vegetables have a high demand for nitrogen availability; however, excessive nitrogen supply causes economic, environmental and agronomic losses, compromising food security. Given the above, the objective was to assess the agronomic responses of arugula that are associated with different nitrogen sources and doses. The experiment was run under greenhouse conditions. A randomized block design was employed; the blocks were arranged in a factorial scheme (2 × 4), using two sources (urea and calcium nitrate) and four nitrogen doses (0, 40, 120 and 360 mg kg^{-1}), with four replications. Thirty-five days after transplanting, the following were assessed: plant height, number of leaves, shoot fresh mass, root fresh mass, shoot dry mass, root dry mass, shoot/root dry matter ratio, leaf area, and leaf nitrogen content. It was found that nitrogen fertilization optimizes crop development and yield. Doses of 100 to 272 kg ha^{-1} promote increase in plant height and leaf number, respectively. Under the conditions studied, 200 kg ha^{-1} of N is recommended as a dose of maximum economic efficiency in arugula production. Calcium nitrate is indicated as the best nitrogen source for the production of the crop.

Keywords: *Eruca sativa*, nitrogen fertilization, urea, calcium nitrate

1. Introduction

The arugula (*Eruca sativa*) market has been constantly expanding in recent years. The vegetable's predominance is widely observed among those chosen by consumers, mainly for its nutritional value (Chun et al., 2017; Jasper, Wagstaff, & Bell, 2020). Due to the diversification in the consumption of leafy vegetables, an increasing production and use of arugula has been seen across the country, especially in the south and southeast regions. According to the IBGE Agricultural Census (2017), 40,527 tons of arugula were produced in 20,567 agricultural establishments in the country in 2016, which shows, based on the extension of the cultivated area, its economic relevance. In 2017, 4,104 tons of the crop were sold in the country (Ceagesp, 2017).

Vegetables in general present a high soil fertility demand. Because of their short cycle, leafy ones require a large amount of fertilizer per unit of area. Studies show yields of up to 46 t/ha (fresh matter) in adequate mineral fertilization management, as well as overall increases in agronomic performance (Lima, Carvalho, Souza, Guerra & Ribeiro, 2009; Souza, 2014). Furlani and Purquerio (2010) warn that each vegetable species, including cultivars, has different production cycle and nutritional requirements, making it necessary to characterize the nutritional management for the production system.

The arugula crop is responsive to nitrogen fertilization, with nitrogen being the second nutrient most accumulated by the species (Morais et al., 2020). The responses observed from fertilization with N directly reflect on yield, as it favors vegetative growth, expansion of the photosynthetically active area, development of leaves with more attractive color and that are more succulent, and increased production potential (Nascimento et al., 2017).

However, an excessive supply of N can cause environmental, economic and agronomic damage, such as excessive shoot growth in relation to the root system, rendering the plant susceptible to water and nutritional deficiencies, especially when it comes to the phosphorus and potassium macronutrients. Excessive doses of nitrogen also have a strong relationship with the accumulation of nitrate (NO_3^-) in crop harvest, compromising food security and the quality of the product supplied (Nascimento, Nascimento, & Cecílio Filho, 2021). According to Schiattone, Viggiani, Venere, and Sergio (2018), arugula, just as other green leafy vegetables, is a nitrate-hyperaccumulating species. It is noteworthy that excessive nitrate in plant tissues, when ingested by humans, can be converted through biochemical reactions to carcinogenic substances harmful to health (Mengel & Kirkby, 1987, Mancin, 2012).

Essential information for arugula production, such as the practice of fertilizing and nourishing the vegetable, is still little explored in the literature (Oliveira et al., 2016; Pereira et al., 2020). In addition, fertilization programs for vegetable production are old and have not kept up with the changes made in the production system and with the introduction of new technologies (Morais et al., 2017). Despite knowing that proper fertilization management is one of the main factors that maximize the productive efficiency of crops, and that unspecific recommendations can lead to insufficient responses in terms of yield and product quality, studies addressing the nutritional management of arugula are scarce, especially with regard to the optimization of nitrogen fertilization, an extremely relevant factor that raises production costs by up to 16.6% (Nascimento, Nascimento, & Cecílio Filho, 2018).

In this context, the objective was to assess the agronomic responses of arugula that are associated with different nitrogen sources and doses.

2. Material and Methods

The experiment was run under greenhouse conditions, with average temperatures of 25 °C, at the State University of Montes Claros/Campus Janaúba. A randomized block design was used; the blocks were arranged in a factorial scheme (2 × 4), referring to two sources (urea and calcium nitrate) and four N doses (0, 40, 120 and 360 mg kg^{-1}), with each treatment having four replications. The experimental plots consisted of plastic pots with a capacity of 4 kg of soil, containing two plants. The fertilizers were applied in solid form.

Topsoil (0-20 cm), classified as Oxisol, was used. It was broken up, air dried and sieved through a sieve measuring 2 mm in diameter. For fertilizer recommendation, the chemical characterization of the soil was performed (Table 1). A total of 60 mg kg^{-1} of P (H_3PO_4), 12 mg kg^{-1} of Zn ($ZnSO_4$), 23 mg kg^{-1} of Fe ($FeCl_3{\cdot}6H_2O$), 16 mg kg^{-1} of Cu ($CuSO_4$) and 5 mg kg^{-1} of B (H_3BO_3) were applied. The fertilizations referring to the treatments were divided into three applications, 7, 14 and 21 days after seedling transplanting.

Table 1. Chemical characterization of the soil used in the experiment

pH[1]	P[2]	K[2]	Na[2]	Ca[3]	Mg[3]	Al[3]	H+Al[4]	SB	t	T	V	m	B	Cu[3]	Fe[3]	Mn[3]	Zn[3]
H_2O	-- mg kg^{-1} --		------------------------ cmolc kg^{-1} ------------------------								--- % ---		-------------- mg kg^{-1} --------------				
7.1	19.4	219	0.2	5.0	0.9	0	1.0	6.7	6.7	7.6	87	0	0.4	0.5	22.4	36.5	1.8

Note. [1] pH in water; [2] Mehlich-1; [3] KCl 1 mol L^{-1}. [4] Ca acetate at pH 7.0; [5] water; t: effective CEC; T: CEC at pH 7; V: Base saturation; m: Aluminum saturation.

The seeds of broadleaf arugula were sown in polystyrene trays containing commercial substrate. Three to four seeds were used per cell. After seedling emergence, thinning was performed, with one seedling being kept per cell. Ten days after sowing (DAS), the seedlings were transplanted into the pots. Manual irrigation was performed twice a day, keeping soil moisture close to field capacity.

Thirty-five days after transplanting, the following variables were assessed: plant length (PL), number of leaves (NL), leaf area (LA), shoot fresh mass (SFM), shoot dry mass (SDM), root fresh mass (RFM), root dry mass (RDM), shoot/root dry matter ratio (S/RR) and leaf nitrogen content (LNC).

PL assessments were conducted with the aid of a graduated ruler; the measuring started at 0.5 cm above the plant's neck and ended at the highest leaf. NL was obtained by counting leaves per plant. After the plants were cut and had their morphological characteristics assessed, SFM and RFM were determined. The samples were labeled, put in paper bags and placed in an air circulation oven at 65 °C until constant weight. They were weighed on a semi-analytical balance for SDM and RDM determination. To calculate the S/RR, the ratio between total shoot dry mass and total root dry mass was used. LA was obtained in accordance with the descriptions of Kemp's (1960) method. LNC was determined through the Kjeldahl method.

Data were subjected to analysis of variance using the F test. A regression study was carried out to assess the fit of the means obtained as to the increase in nitrogen doses. Statistical analyses were processed using the R statistical software.

3. Results and Discussion

The results of the analysis of variance showed a significant interaction of the N source and dose factors for the SFM and SDM characteristics. As for the PL, NL and S/RR variables, isolated effect was observed only for dose. LNC showed isolated effect for source and dose. The LA and RDM variables showed no significant difference between treatments at a 5% significance level (Table 2).

Table 2. ANAVA summary on the development of arugula subjected to N sources and doses

Sources of Variation	**DF**	**Mean squares**								
		PL (cm)	**NL**	**SFM (g)**	**RFM (g)**	**SDM (g)**	**RDM (g)**	**S/RR**	**LNC**	**LA (cm^2)**
Source	1	0.0301^{ns}	1.377^{ns}	128.858^{ns}	3.720^{ns}	2.07*	0.897^{ns}	0.216^{ns}	4.284*	35.79^{ns}
Dose	3	33.728*	28.606*	151.718*	51.506^{ns}	1.866*	2.854^{ns}	0.543*	6.170*	1285.24^{ns}
S × D	3	0.962^{ns}	4.461^{ns}	120.820*	35.642^{ns}	1.313*	0.359^{ns}	0.032^{ns}	0.337^{ns}	471.14^{ns}
Block	2	6.175*	0.323^{ns}	171.279*	163.648^{ns}	6.479*	0.526^{ns}	0.065^{ns}	1.332*	2600.37^{ns}
Residue	14	0.846	1.493	27.539	28.699	0.270	1.025	0.097	0.237	439.31
CV (%)		3.26	11.24	16.28	33.68	6.9	21.23	19.01	13.15	15.88

Note. PL: Plant length; NL: Number of leaves; SFM: Shoot fresh mass; RFM: Root fresh matter; SDM: Shoot dry mass; RDM: Root dry mass; S/RR: Shoot/root dry matter ratio; LNC: Leaf N content; LA: Leaf area; *: Significant at 5% probability by F test; ns: not significant.

After regression analysis of the PL variable, data were found to fit the quadratic model to describe the effect promoted by the doses on arugula growth (Figure 1). Estimated maximum height (32.11 cm) was found at a dose of 194.81 mg kg^{-1} of N, with an increase of 23.85% compared to the control treatment. Guimarães, Souza, Silva, and Bittar (2019), when assessing nitrogen fertilization in arugula production, observed a maximum height of 23.5 cm with the application of 200 kg ha^{-1} of N, meeting the recommendation for the marketing of plants with a height of 20 cm after harvest (Cecílio Filho, Costa, Rezende, & Leeuwen, 2008). N has a large participation in the chlorophyll synthesis and significantly interferes in the photosynthetic process (Reis, Furlani Júnior, Buzetti, & Andreotti, 2006). Plant growth is directly influenced by this process, which optimizes the production and use of photoassimilates. Thus, nitrogen fertilization favors vegetative development and shoot expansion, as observed in the arugula crop of the present study.

An excessive supply of nitrogen fertilizers can cause some damage to crops, as evidenced by Nascimento Nascimento and Cecílio Filho (2021), who also observed a significant reduction in plant growth associated with N doses greater than 200 mg dm^{-3}. Possibly, this reduction occurred due to the plants being under osmotic stress, because of the high salinity in the root environment, caused by excess nitrogen fertilizers. Under these conditions, plants have their water demand increased, but have difficulty absorbing water through the roots, so they quickly adapt metabolically to water stress conditions, having their shoot growth reduced.

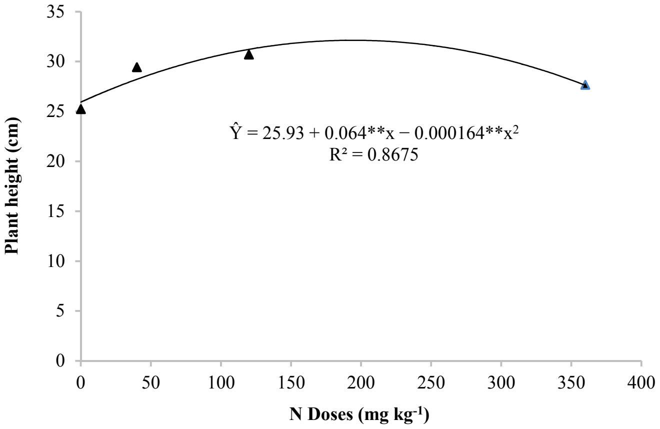

Figure 1. Height of arugula plants subjected to N doses

The NL characteristic was also described by the quadratic regression model (Figure 2). Maximum economic efficiency (MEE) was observed at an N dose of 137.58 mg kg^{-1}, with an average of 24.65 leaves/plant, and with NL 47% higher than that of the control treatment. It is worth highlighting that doses above 280 mg kg^{-1} did not promote an increase in NL. Guimarães et al. (2019), when applying 100 mg dm^{-3} of N, reached maximum NL means 48% lower than the means observed in this study. The authors stated that N promotes physiological changes in the plant that favor vegetative development and increase the number of leaves grown. It is noteworthy that PL and NL are parameters of great relevance for consumers, since the crop is sold in bundles (Cavallaro Junior, Trani, Passos, Kuhn neto & Tivelli, 2009).

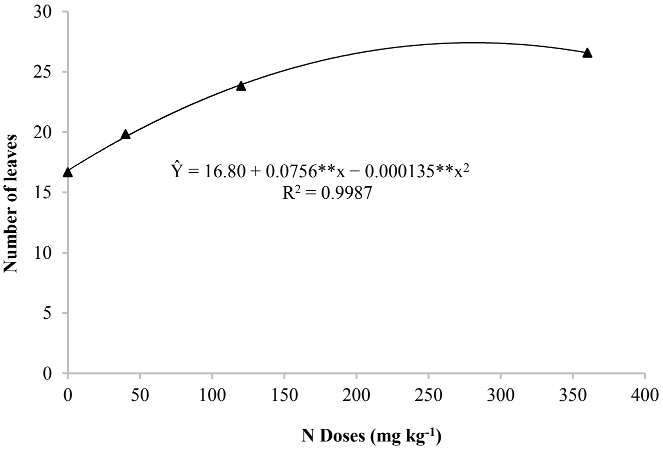

Figure 2. Number of leaves of arugula plants subjected to N doses

There was interaction ($p < 0.05$) between N source and dose for SFM. Significant difference for source was found at the 360 mg kg^{-1} dose, with statistical superiority shown in the source of calcium nitrate (Table 3).

Table 3. Breakdown of the interaction between nitrogen source and dose for arugula shoot fresh mass (SFM)

Source	**Dose (mg kg^{-1})**			
	0	**40**	**120**	**360**
Calcium nitrate	26.00 aB	32.66 aAB	36.34 aAB	43.13 aA
Urea	25.61 aB	29.76 aAB	38.92 aA	25.51 bB
CV (%)	16.28			

Note. Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ from each other by Tukey's test at 5% probability.

The SFM response as a function of the calcium nitrate doses fitted the increasing linear regression model, with the highest production mean (43.13 g) being observed at the dose of 360 mg kg^{-1}. There was a 17.12 g increase in production compared to the control treatment, with an SFM mean 66% higher. The coefficient referring to the dose of calcium nitrate reveals that each 2 kg of fertilizer added to 1 ha promoted an increase of 0.0415 g of arugula SFM (Figure 3). These results evidenced a direct influence of N on the physiological metabolism of the plant, on the formation of nitrogen compounds and proteins that are essential for the plant to express its agronomic potential (Nascimento et al., 2017).

Guimarães et al. (2019) observed a linear effect between N dose and arugula SFM, with greater fresh mass at the dose of 100 mg dm^{-3}. Linear increments proportional to the increase in calcium nitrate doses show a shoot formation strategy in the initial phase of the plant's development, as a higher shoot growth promotes an increase in photosynthetic tissue and accumulation of carbohydrates in the roots, favoring the final production of the crop (Grangeiro, Freitas, Negreiros, Marrocos, Lucena, & Oliveira, 2011).

Regarding the assessed urea doses, it was found that the curve that best fitted the trend was a second-order polynomial one, with $R^2 = 0.98$, evidencing a maximum SFM of 40.17 g at the dose of 181.18 mg kg^{-1} however, the MEE dose for arugula SFM was 88 mg kg^{-1} of urea, with an increase in production 45% greater than that of the control treatment (Figure 3).

A significant reduction in SFM was seen at urea doses greater than 181 mg kg^{-1}. This reduction can be attributed to phytotoxicity caused by ammonium accumulation, as observed by Ferreira, Rocio, Lauer, Rossoni, and Nicoulaud (2001), who verified a reduction in the yield of leafy vegetables subjected to a dose of 200 mg dm^{-3} of N. The authors reiterate that high N doses cause phytotoxicity due to the release of ammonium by urea hydrolysis, raising the ammonium levels in the medium. The ammonium being absorbed in excess by the plant is toxic due to the dissipation of pH gradient across the cytoplasmic membrane, directly reflecting in the production of fresh mass.

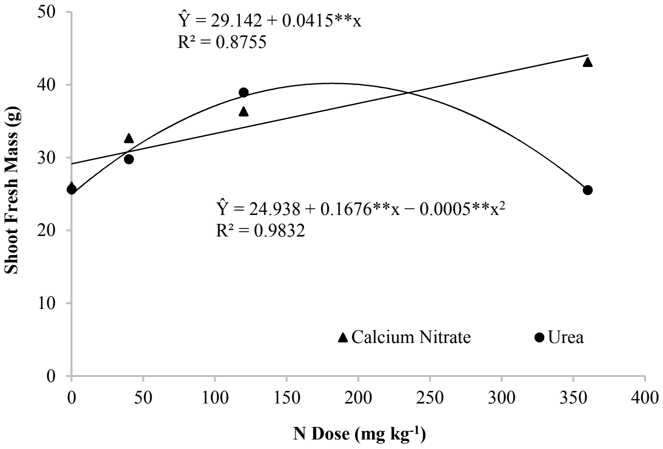

Figure 3. Shoot fresh mass of arugula plants subjected to N sources and doses.

The SDM response of the arugula plants under nitric fertilization fitted the increasing linear polynomial regression model, with maximum mean at the dose of 360 mg kg^{-1} (Figure 4). The variation between the maximum and minimum means was 2 g, indicating greater leaf volume and thickness as a result of calcium nitrate. The coefficient of this source revealed better SDM performance, with an increment of 0.00457 g for each 2 kg ha^{-1} of calcium nitrate applied.

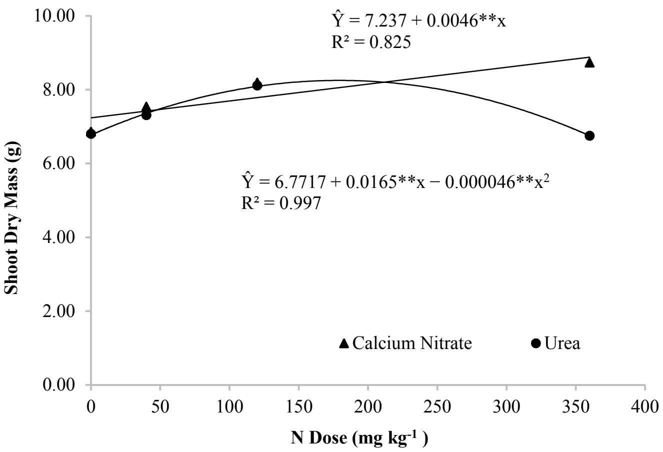

Figure 4. Shoot dry mass of arugula plants subjected to N sources and doses

Using urea, SDM showed a quadratic behavior, with an average of 7.43 g reached at the MEE dose (45.41 mg kg^{-1}). An increase of approximately 20% was observed in relation to the control treatment (Figure 4). Sardeiro, Santana, Felix, Souza, and Costa (2015) found the same equation fit in response to nitrogen fertilization in field arugula cultivation, with an SDM increase of 17706 kg ha^{-1} at a dose of 137 kg ha^{-1}.

SDM had a behavior similar to that of SFM in relation to the applied doses. At urea doses greater than 200 mg kg^{-1}, there was a considerable decrease in arugula SFM and SDM production, possibly due to nutritional imbalance, or due to toxicity from excess of the nutrient (N), which occurs mainly in urea-based fertilization (Araujo et al., 2012). Another hypothesis would be critical stress due to excess nitrogen fertilization, which triggers intense vegetative growth and, consequently, self-shading, thus compromising the photosynthetic efficiency of lower leaves, affecting dry matter production and causing production losses (Larcher, 2004).

All assessed variables showed a correlated behavior in response to nitrogen fertilization, with a relevant reduction in increments under excessive doses of N. Increasing doses of a nutrient initially promote a high production increase, with successive decrease, based on the law of maximum (Alvarez, 1985), which shows that an excess of nutrients limits yield to the highest level of those nutrients that are being antagonized by this same excess.

The best trend-fit curve for the S/RR variable was a second-order polynomial one with $R^2 = 0.99$ (Figure 5). The highest S/RR (2.04:1) occurred at the dose of 242.34 mg kg^{-1}, with an increase of 59.23% compared to the control treatment. It showed that the higher the ratio, the more the photoassimilates will be aimed at shoot production. This is a supposedly positive mechanism, since arugula is a leafy vegetable whose shoot is of economic importance and requires good visual and production quality (Cecilio Filho et al., 2008); however, under these conditions, its root system tends to be underdeveloped, leaving the plants susceptible to water deficit, toppling and nutritional deficiency due to reduced soil exploration (Merida, 2013).

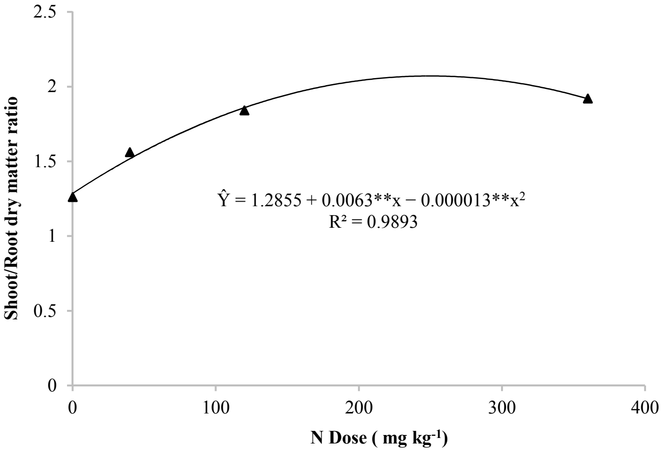

Figure 5. Shoot/root dry matter ratio of arugula plants subjected to N sources and doses

Nitrogen fertilization reflected positively in the arugula LNC (Figure 6). At the dose of 280 mg kg^{-1}, a higher LNC was obtained (4.95 dag kg^{-1}), but this value is higher than that found for maximum yield (43.13 g kg^{-1}), characterizing luxury consumption. Silva (2017) assessed the effect of N doses on arugula growth and yield and found similar results, with maximum LNC at a dose of 250 mg dm^{-3} (3.05 dag kg^{-1} in the plant).

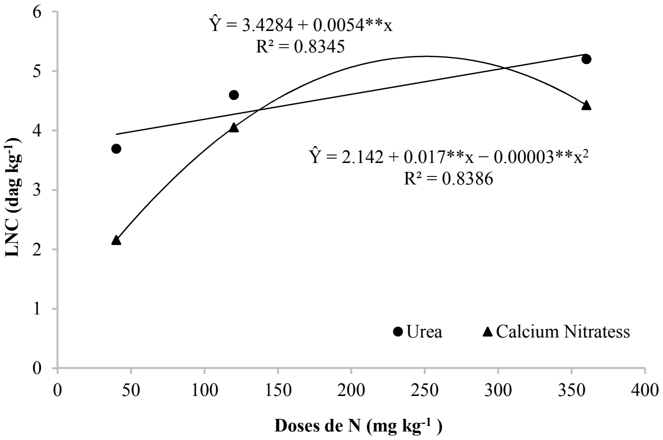

Figure 6. Leaf Nitrogen Content (LNC) of arugula plants subjected to N sources and doses

When fixing doses, there were no statistical differences between the sources for each dose, but urea was superior as to the overall means of LNC (Table 4). This was an expected result, since the absorption of urea is optimized due to its dissociation and transformations into ammonium and, finally, nitrate. Fernandes and Rossielo (1986) state that the absorption of ammonium (NH_4^+) is easier because it is less energy-dependent, while for nitrate (NO_3^-) it occurs against an electrochemical potential gradient, being therefore less efficient (Cruz, Pelacani, & Araújo, 2006). It is estimated that nitrate reduction and assimilation can consume up to 25% of the processes associated with photosynthesis and with the electron transport that occurs at the mitochondrial level (Bloom, Caldwell, Finazzo, Warner, & Weissbart, 1989).

Another relevant hypothesis to elucidate the results would be the lower SDM production in plants fertilized with urea, a characteristic that favored the high concentration of N in the arugula tissues, leading to superior LNC related to this treatment. N losses may explain the inferior results observed in the calcium nitrate treatment, since the pots used in the experiment had holes, favoring the leaching of the nitric source, which is the most susceptible to leaching, followed by the amidic and ammoniacal sources, corroborating with the results found by Bono et al., 2008.

Table 4. Leaf nitrogen content (LNC) in arugula crop subjected to N sources and doses

Source	**Dose (mg kg^{-1})**				
	0	**40**	**120**	**360**	**Mean**
Calcium nitrate	2.51	2.16	4.05	4.43	3.29 b
Urea	3.04	3.69	4.58	5.2	4.14 a
CV (%)			13.15		

Note. Means followed by the same lowercase letter in the column do not differ from each other by Tukey's test at 5% probability.

4. Conclusions

Nitrogen fertilization optimizes arugula development and yield.

N doses of 100 to 272 kg ha^{-1} promote greater increases in plant height and number of leaves, respectively.

The dose of maximum economic efficiency for arugula production under the studied conditions is 180 kg ha^{-1} of N.

Calcium nitrate is the best source for fertilizing arugula under the studied conditions.

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