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NITROGEN RATES ON GROWTH, YIELD AND NITRATE FOLIAR CONTENT OF ARUGULA[1]

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ABSTRACT - Nitrogen is the second nutrient most demanded by arugula. However, the positive effect it has on yield can negatively impact the quality of this vegetable, with negative effects on human health. Two experiments (summer and autumn) were carried out to evaluate the effect of N rates (0, 50, 100, 150, 200 and 250 kg ha^{-1}) on the arugula growth, yield and quality (nitrate content). Each experiment was set up in a randomized block design with four replications. Increasing rates of N reflected positively on leaf N content and thus, higher and desirable values of height, leaf number, leaf area and plant dry mass were obtained in both growing seasons. Consequently, the yield had significant increase, which N was maximized in summer (4.9 kg m^{-2}) and autumn (2.6 kg m^{-2}) and leaf N of 29.4 and 27.0 g kg^{-1}, respectively, at rates of 250 and 213 kg ha^{-1} and both growing seasons. The higher rate the higher foliar nitrate content of arugula, in summer (2931.3 mg kg^{-1}) and autumn (4218.4 mg kg^{-1}). The foliar nitrate content in both crop season do not reach the risk level to human being heath.

Keywords: *Eruca sativa* Mill. Leaf vegetable. Nitrate. Quality.

DOSES DE NITROGÊNIO NO CRESCIMENTO, PRODUTIVIDADE E TEOR FOLIAR DE NITRATO DA RÚCULA

RESUMO - O nitrogênio (N) é o segundo nutriente com maior demanda pela rúcula. No entanto, o efeito positivo que ele tem sobre o rendimento pode afetar negativamente a qualidade desse vegetal, com efeitos negativos para saúde humana. Essa resposta da planta depende da cultivar, do ambiente de cultivo e do manejo da colheita, cuja interação não é clara na literatura. Dois experimentos em campo (verão e outono), estabelecidos em delineamento de blocos casualizados com quatro repetições, foram realizados com o objetivo de avaliar o efeito das doses de N (0, 50, 100, 150, 200 e 250 kg ha^{-1}) no crescimento, produção e qualidade da rúcula. As doses crescentes de N refletiram positivamente no teor de N nas folhas e, portanto, foram obtidos valores mais elevados e desejáveis de altura, número de folhas, área foliar e massa seca das plantas em ambas as épocas de cultivo. Consequentemente, foi observado aumento significativo da produtividade, o qual foi maximizado no verão (4,9 kg m^{-2}) e no outono (2,6 kg m^{-2}) e N foliar de 29,4 e 27,0 g kg^{-1}, respectivamente, nas doses de 250 e 213 kg ha^{-1} e ambas épocas de cultivo. Quanto maior a dose, maior o teor de nitrato foliar da rúcula, no verão (2931,3 mg kg^{-1}) e outono (4218,4 mg kg^{-1}). Nas duas estações, quanto maior a dose, maior o teor de nitrato, contudo, nas duas safras não atingiu o nível de risco para a saúde humana.

Palavras-chave: *Eruca sativa* Mill. Hortaliça folhosa. Nitrato. Qualidade.

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INTRODUCTION

Nitrogen (N) is the second most demanded nutrient for arugula (*Eruca sativa* Miller) (GRANGEIRO et al., 2011). As in any other crop, when in adequate levels, N stimulates vegetative growth, expands the photosynthetically active area, and provides more attractive and succulent coloured leaves (AGUIAR JÚNIOR et al., 2010; NASCIMENTO et al., 2017).

In arugula crops, N deficiency is rarely observed as it is a species considered very responsive to nutrient supply (PURQUERIO et al., 2007; STEINER et al., 2011; SILVA et al., 2020) which leads producers to apply high rates. In excess, due to the high capacity of N to modify morphological and consequently, physiological aspects of the plant, it favours an imbalance between shoot and root system growth, with negative implications on processes such as respiration, nutrition, photosynthesis, mechanisms of growth, defense to biotic and abiotic factors, among others. Of course, these and other implications lead to lost productivity; however, this may not be so quickly affected, as leafy vegetables have a high response and ability to accumulate nutrients without toxicity.

Excess of N has other implications as important as that mentioned above on productivity. Firstly, the excessive use of N fertilizer results in low N use efficiency and has no yield benefits (AHMED et al., 2017). The cost of fertilizer and the operation of its application is about 30-35% of the operating production cost of the arugula (REZENDE et al., 2005; REZENDE et al., 2009). Secondly, there is a strong indication that excess rates of N result in higher nutrient losses, which, in turn, have greater potential for eutrophication of water resources and increase the emissions of the greenhouse gases (WARD, 2009; LIU et al., 2014; WANG et al., 2014). Thirdly, are possible negative effects on food quality. When the absorption by the plant is greater than actual demand, characterising the excess, the nutrient is stored as nitrate (NURZYŃSKA-WIERDAK, 2009). Nitrate is not harmful to man, but under potentiating conditions present in the human organism, nitrate can combine with amines, and producing nitrosamines that are mutagenic and carcinogenic, or possibly achieving the bloodstream and cause methaemoglobinaemia (KHAN et al., 2013; OLLÚA; LOGEGARAY; CHIESA, 2016).

Arugula is a short-cycle leafy vegetable and belongs to a group that has a high capacity to accumulate nitrate (NURZYŃSKA-WIERDAK, 2009; BARROS JÚNIOR et al., 2020). According to Qadir et al. (2017), studies show that vegetables provide approximately 40 - 92% of the average daily intake of nitrate by humans, and according to Liu et al. (2014), the arugula, spinach and lettuce are some of the richest sources of nitrate in the human diet. However, nitrate accumulation is not only genotype dependent but highly influenced by the environment and crop management (LIU et al., 2014; STAGNARI; GALIENI; PISANTE, 2015; AHMED et al., 2017).

Consequently, because the amount of N supplied to the arugula affects its productivity and quality (NURZYŃSKA-WIERDAK, 2009), it is pertinent to study locally the rationalization of the N fertilization to arugula and not extrapolate the results obtained under different conditions of management, weather and cultivar. According to Liu et al. (2014), rationalizing fertilizer application is an important issue for sustainable agriculture.

In this context, the objective was to evaluate the agronomic performance and quality of arugula as a function of N rates.

MATERIALS AND METHODS

Experimental site

Two experiments were conducted under field conditions (21°15'22”S, 48°18'58”W and altitude of 575 m a.s.l.) in Jaboticabal, Brazil, from 24 January to 3 March 2017 (summer, Experiment 1) and from 10 May to 15 June 2018 (autumn, Experiment 2).

The climate of the region is classified as subtropical, rainy during the summer and relatively dry in the winter. The annual means for precipitation and temperature and maximal and minimal temperatures are 1424.6 mm, 22.2 ºC, 28.9 ºC and 16.8 ºC, respectively. The table 1 shows the climatic parameters along the two crops. The data were obtained from the agrometeorological station at UNESP-FCAV, Jaboticabal - SP.

Table 1. Temperature (Temp.), relative humidity (RH), rainfall (R) and solar radiation (SR) in Summer (S) and Autumn (A) experiments.

	Temp. (ºC)		RH (%)		R (mm)		SR (MJ m^{-2})	
	S	A	S	A	S	A	S	A
Maximum	31.1	27.7	92.8	87.5	-	-	-	-
Average	24.4	20.4	74.1	64.1	-	-	22.5	14.7
Minimum	19.6	14.3	45.1	36.3	-	-	-	-
Total	-	-	-	-	276 (18)[1]	7.2 (1)	902	529

[1]The number in parentheses corresponds to rainy days.

According to the classification of Santos et al. (2018), the soil of the area is a typical Oxisol with clayey texture (660 g kg^{-1} of clay), and the soil chemical analysis at the 0 to 20 cm profile, were: pH $_{(CaCl2)}$ = 5.3 and 5.6; organic matter = 27 and 17 mg dm^{-3}; $P_{(Resin)}$ = 9 and 31 mg dm^{-3}; S = 12 and 9 mg dm^{-3}; Ca = 42 and 22 $mmol_c$ dm^{-3}; Mg = 15 and 10 $mmol_c$ dm^{-3}; K = 5.9 and 3.3 $mmol_c$ dm^{-3}; H+Al = 23 and 15 $mmol_c$ dm^{-3}; and base saturation (V) = 73 and 71%.

Experimental design and treatments

The two experiments differed as to the season of cultivation (summer and autumn). In both, the same six N rates (0, 50, 100, 150, 200 and 250 kg ha^{-1}) were evaluated, in a randomised block design, with four replications. The N source used was urea (45% N).

Each experimental unit was 3.0 m in length (12 transversal lines in relation to the length of bed) and 1.0 m wide, giving a total area of 3 m^2. The useful area for data collection corresponded to the eight central lines (2 m²).

Crop management

According to the base saturation index, there was no need to apply limestone prior to both experiments. Planting fertilization was performed based on soil analysis, following the recommendation for fertilization and liming in the state of São Paulo (TRANI; PASSOS; AZEVEDO FILHO, 1997), thus were applied 400 kg ha^{-1} of P_2O_5 (simple superphosphate) and 50 kg ha^{-1} of K_2O (potassium chloride). Additionally, boric acid was sprayed onto the bed soil to provide 1 kg ha^{-1} of B. Except in plots with a rate of 0 kg ha^{-1} of N, was applied 50 kg ha^{-1} of N as planting fertilization for all experimental units of others treatments. It were incorporated fertilizers into the soil and the 'Folha Larga' arugula was sown in grooves approximately 0.015 m deep, spaced 0.25 m apart. Seedling emergence occurred three days after sowing for both experiments. Two thinning processes were performed to adjust the spacing between plants to 0.05 m, one at four days after emergence (DAE) and other at 10 DAE. The topdressing was only N (except for the 0 and 50 kg ha^{-1} N treatments) in an amount to complete the total established in the treatments, discounting the 50 kg ha^{-1} of N applied at planting fertilization. The remainder of N fertilization was sliced equally and the amounts applied at 10 and 17 DAE. Irrigation was performed by spraying, according to the water needs of the crop, in order to maintain soil moisture close to field capacity.

Evaluated characteristics

At arugula harvest were evaluated, the plant height (beginning of petiole up to the top of highest leaf), number of leaves, leaf area (leaf area integrator LI-COR 3100) and shoot dry mass. In order to obtain the dry mass of the aerial part, the plants were washed, dried in an oven with forced air circulation at 65 °C to 70 °C until a constant mass was obtained. Then, the dry matter was measured accurately to two decimal places. Yield, expressed in kg m^{-2}, was estimated based on the fresh mass of arugula plants obtained immediately after harvesting between 6 a.m. and 7 a.m.

The foliar N content was measured according to the methodology of Miyazawa et al. (2009). After washing the leaves in deionized water, they were dried in an oven with forced air circulation from 65 °C to 70 °C until a constant mass. After drying, the material was ground and weighed (0.1 g). Sulphur digestion was performed and the N content determined.

To evaluate the nitrate content in the shoot, six plants were randomly collected in the useful area of each experimental unit, in the morning between 5.30 a.m. and 6.30 a.m. Nitrate content was determined according to Mantovani et al. (2005), using the following procedure. In 50 mL Falcon tubes, 0.2 g of leaf dry matter and 20 mL of deionised water were placed. The tubes were kept in a water bath at 60 ± 5 °C for one hour, during which time shaking was performed every 15 minutes. The samples were filtered on fast filter paper. From this extract, 5 mL was taken and 20 mL deionized water and 0.2 g MgO were added to it. A first distillation was made to convert ammonium to ammonia and the distillate was discarded. Then, in the same extract, 0.4 g of Devarda alloy was added to reduce nitrate into ammonium, which in an alkaline medium is converted into ammonia, which is entrained by the vapours, and collected in a beaker containing 10 mL of H_3BO_3 20 g L^{-1} solution. Then, the quantification of ammonium N was performed by automatic titrator (848 Titrino plus) with a standardised solution of 0.00263 mol L^{-1} H_2SO_4. The nitrate content obtained in the dry mass of leaves was converted to mg kg^{-1} of NO_3^- in the fresh mass.

Statistical analysis

Data analysis of variance, F-test, was performed for each experiment and also joint analysis of the experiments. The difference between the experiments was verified by the significance of the F-test. When the N rate factor was significant, a regression study was performed, adopting the equation with the highest significance and coefficient of determination. Statistical analyses were processed using the AgroEstat statistical program (BARBOSA; MALDONADO JÚNIOR, 2015).

RESULTS AND DISCUSSION

There was no interaction of N and season on plant height and leaf area, which were influenced only by the factors individually, except for the number of leaves, which had no influence of the evaluated factors (Table 2).

The N is a nutrient known to determine, in general, major changes in plants, both in terms of growth, productivity and quality of the product to be consumed. In the present study, all these effects of N on arugula were observed.

Plant height and leaf area increased up to 210 and 198 kg ha^{-1} of N, when plants were 33.9 cm and 468.0 cm^2, respectively, while non-N fertilized plants were 26.0 cm and 251.6 cm^2 (Figure 1A and 1B).

Table 2. Summary of joint variance analysis for the variables plant height (PH), number of leaves (NL), leaf area (LA), shoot dry mass (SDM), nitrate content (NO_3^-), nitrogen content (N) by plant and yield (Y) of arugula as a function of nitrogen rates in summer and autumn seasons.

Sources of variation	PH	NL	LA	SDM	N	Y	NO_3^-
	F values						
Treatments (T)	28.03**	4.66^{ns}	11.29**	2.76^{ns}	1.51^{ns}	10.38*	6.87*
Seasons (S)	117.37**	4.52^{ns}	42.59**	4.25^{ns}	0.38^{ns}	42.24**	41.52**
T x S	0.69^{ns}	1.42^{ns}	1.11^{ns}	11.31**	7.13**	2.71*	6.70**
	CV (%)						
Summer	8.7	16.1	19.9	13.4	8.8	16.3	17.7
Autumn	4.3	7.3	8.9	9.7	3.4	14.3	11.8
	Averages						
	cm	leaves/pl	cm^2/pl	g/pl	g kg^{-1}	kg m^{-2}	mg kg^{-1}
Summer	28.6	10.0	466.9	4.3	24.4	3.5	3347.5
Autumn	34.0	9.1	329.7	3.2	25.2	2.1	1663.1

** and * Significant to 1% and 5% of probability by F-test, respectively; ^{ns} non significant.

Increasing rates of N to arugula reflected positively on N leaf content and thus, higher and desirable values of plant height and leaf area and shoot dry mass were obtained. A great promoter effect on these characteristics was noted between the non-supply of N and rates that provided their maximum values, which showed positive impacts on height (30%) and leaf area (86%), shoot dry mass increased by 133% and 140% in summer and autumn, respectively. But the arugula yield answered differently as the season. In the autumn, an increment of only 10% was observed when the N rate increased from 125 to 213 kg ha^{-1} of N (maximum yield), while, in the summer, for the same rate range it was observed 40% of increment on arugula yield.

The shoot dry mass of the arugula was influenced by the interaction of the factors, N rates and growing seasons (Table 2). In response to the increased N supply to the arugula, in summer, there was a linear increase in shoot dry mass, while in the autumn there was a quadratic equation adjustment with a maximum of 168 kg ha^{-1} N (Figure 1C).

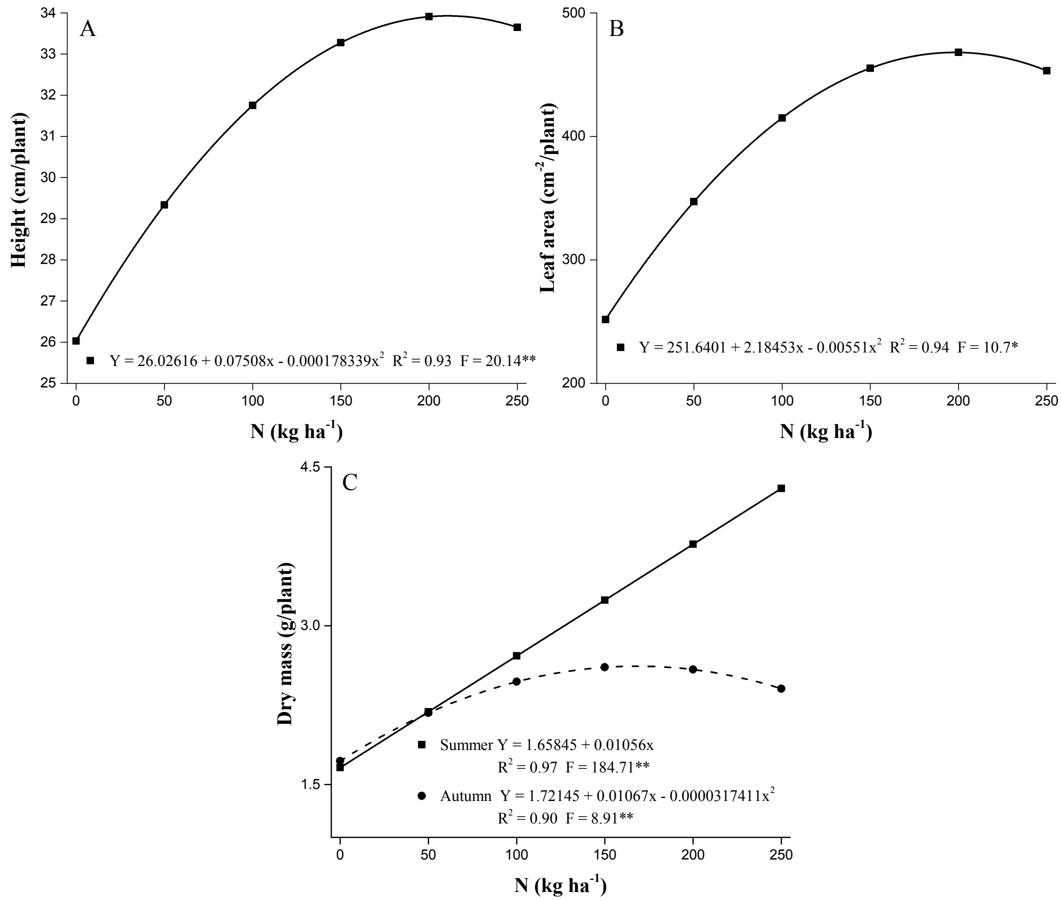

Figure 1. (A) Height and (B) leaf area as a function of nitrogen rate and (C) dry mass of 'Folha Larga' arugula as a function of the growing season and nitrogen rate interaction. (*, ** correspond to significance by F-test at 5% and 1% of probability, respectively).

For leaf N content and arugula yield, there was an interaction between N rate and growing season (Table 2). In both growing seasons, maximum N content was verified with the highest rate evaluated (250 kg ha^{-1} de N) (Figure 2A). Regarding yield, maximums of 4.9 and 2.6 kg m^{-2} were recorded with 250 and 213 kg ha^{-1} of N in summer and autumn, respectively. When not fertilized with N, the yields were 2.1 and 1.1 kg ha^{-1} in the both growing seasons respectively (Figure 2B).

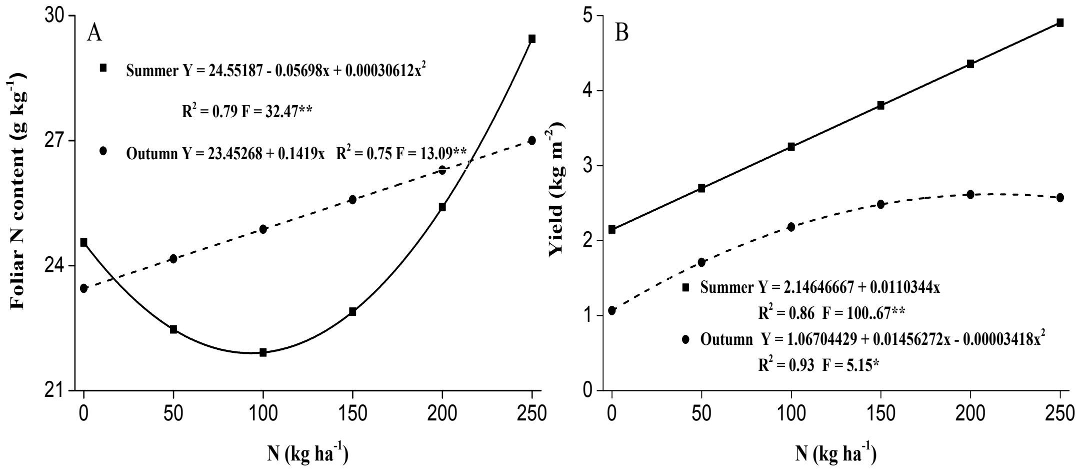

Figure 2. (A) Foliar N content and (B) yield of 'Folha Larga' arugula as a function of the growing season and nitrogen rate interaction. (*, ** correspond to significance by F-test at 5% and 1% of probability, respectively).

The rates found to maximize arugula yield were close to the 248.2 (PURQUERIO et al., 2007) and 240 (BENETT et al., 2019) kg ha^{-1} N to maximize the productivity of arugula, when it was cultivated in the field. The results corroborate information that arugula is very responsive to N fertilization (SILVA et al., 2020; STEINER et al., 2011). On the other hand, the N rates evaluated in our study to get great arugula performance are higher than those recommended by Trani et al. (2018) which have range of 110 - 190 kg ha^{-1} of N total (planting and covering) for arugula crop. According to this recommendation of fertilization for many vegetables crops, the arugula yields would be 3.3 to 4.2 kg m^{-2} in the summer and 2.3 to 2.6 kg m^{-2} in the autumn. Thus, the losses in relation to the maximum obtained which could reach 32% in the summer would be only of 11% in the autumn.

The maximum yields were related with 29.4 and 27.0 g kg^{-1} of N in the shoot dry mass. The difference in polynomial adjustment for the averages of the arugula N foliar content in the two growing seasons can be explained by the effect of dilution of the nutrient phenomenon (ANDRIOLO et al., 2006), in addition to the arugula response in the accumulation of shoot dry mass (Figure 1C). When not fertilized, the arugula produced a small amount of shoot dry mass. As increased the N rate, the shoot dry mass produced was greater per accumulated N unit. This occurred for rates between 0 and around 100 kg ha^{-1} of N reducing at higher rates. Trani et al. (2014) considered N contents between 40 and 50 g kg^{-1} as suitable for arugula; however, these contents were obtained between half and two thirds of the cycle. However, in both growing seasons, no visual symptoms of deficiency of yellow old leaves were observed, even in the treatment with no N application.

The increase in N supply had a positive effect on arugula yield, on the other hand, there was also a promoter effect on nitrate increase in the plant, in agreement with that observed by Nurzyńska-Wierdak (2009).

An interaction of the growing season and N rate in the nitrate content in the shoot fresh mass of the arugula was verified (Table 1). In summer, leaf contents were much lower than those observed in autumn, regardless of N rate. However, in both seasons, it was found that the higher the N rate, the higher the nitrate content in the arugula leaves (Figure 3).

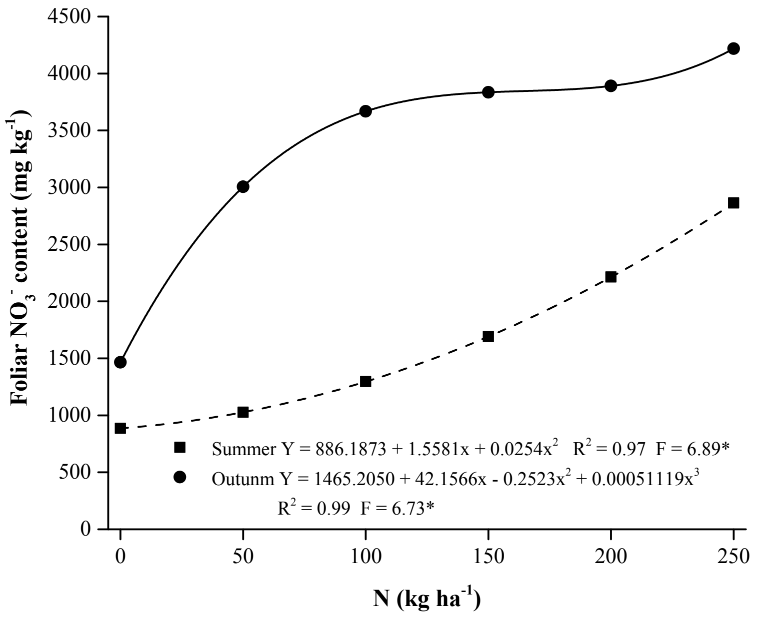

Figure 3. Nitrate content in leaves of 'Folha Larga' arugula as a function of the growing season and nitrogen rate interaction. (* significant by the F-test at 5%).

Nitrate content in arugula is an attribute that may compromise the quality of the vegetables, since, according to Maynard et al. (1976), Khan et al. (2013) and Ollúa, Logegaray and Chiesa (2016), that this compound is associated with the possibility of formation of carcinogenic and mutagenic compounds in the human organism.

The maximum nitrate content (4218.4 mg kg^{-1}) in shoot fresh mass of arugula was achieved with 250 kg ha^{-1} of N, in autumn. In summer, the maximum level was 2931.3 mg kg^{-1} also with the highest N. Purquerio et al. (2007)

evaluated N rates in arugula (0 to 240 kg ha^{-1} N) and also reported increases in nitrate accumulation up to the highest evaluated rate, being 1360 and 1290 mg kg^{-1} in shoot fresh mass of arugula cultivated in field and protected environments, respectively. The result corroborates the claim that crop management is an important factor in the quality of vegetables, especially related to nitrate accumulation (LIU et al., 2014; STAGNARI; GALIENI; PISANTE, 2015; AHMED et al., 2017). N rate is one of the main factors that promotes higher accumulation of nitrate (MAYNARD et al., 1976; CEYLAN et al., 2002). Higher solar radiation and temperature present in summer growing (Table 1) favoured lower N accumulation than in autumn cultivation. Cometti et al. (2011), evaluating shading and N rates in lettuce, found that the lower the radiation, the greater the nitrate accumulation by lettuce.

The results agree with those obtained by Steiner et al. (2011), who also found low nitrate content in arugula (430 to 1400 mg kg^{-1}) and with Cometti et al. (2011) for lettuce. Thus, although the increase in N supply increased by 223% in summer and 187% in autumn the nitrate content of arugula leaves compared to plants not fertilized with N, the observed content is below the limit (7000 mg kg^{-1}) proposed by European Union (2011). Even with the high N rate, the nitrate levels observed were below the proposed limit and can be explained by the climatic conditions of the experimental periods (Table 1).

Higher summer temperatures, solar radiation and precipitation favour plant metabolism and nitrate assimilation (MAYNARD et al., 1976; COMETTI et al., 2011). Thus, from the point of view of the quality of arugula, it is possible to recommend the maximum N rate evaluated (250 kg ha^{-1}) in summer and 213 kg ha^{-1} of N in autumn for fertilization of the crop, giving maximum yields in these seasons without compromising the quality of the vegetables.

CONCLUSION

The supply of 250 kg ha^{-1} of N to arugula in summer and 213 kg ha^{-1} of N in autumn provides substantial increases in height, leaf area, shoot dry mass and yield without compromising quality evaluated by nitrate content in leaves.

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