Research Article

Leaf inoculation of *Azospirillum brasilense* and *Trichoderma harzianum* in hydroponic arugula improve productive components and plant nutrition and reduce leaf nitrate[1]

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ABSTRACT

Using beneficial fungi and bacteria to plant growth may reduce the leaf nitrate content and improve the quality of produced food. This study aimed to evaluate the isolated and combined effect of inoculation with *Azospirillum brasilense* and *Trichoderma hazianum* at two electrical conductivities on the nutrition and production of hydroponic arugula cultivation. The experiment was designed in randomized blocks, in a 4 x 2 factorial scheme, with five replications. The treatments consisted of inoculations (non-inoculated, *A. brasilense*, *T. harzianum* and co-inoculation) and two electrical conductivities (1.4 and 1.6 dS m^{-1}). The isolated inoculation of *T. harzianum* and *A. brasilense* produced a higher root fresh mass, while the leaf chlorophyll index was higher with the inoculation of *A. brasilense*, concerning the other treatments. The inoculation of *A. brasilense* reduced the nitrate content in the arugula leaves. The inoculations and co-inoculation of *A. brasilense* and *T. harzianum* improved the yield components and plant nutrition, reduced the leaf nitrate content and promoted the biofortification of arugula leaves with Zn and Fe. In addition, the inoculation with *T. harzianum* increased the P and S leaf content.

KEYWORDS: *Eruca sativa*, growth-promoting microorganisms, nutrient film technique.

RESUMO

Inoculação foliar de *Azospirillum brasilense* e *Trichoderma harzianum* em rúcula hidropônica melhoram os componentes produtivos e nutrição das plantas e reduzem o nitrato foliar

O uso de fungos e bactérias benéficos ao crescimento das plantas pode reduzir a concentração de nitrato nas folhas e melhorar a qualidade dos alimentos produzidos. Objetivou-se avaliar o efeito isolado e combinado da inoculação com *Azospirillum brasilense* e *Trichoderma hazianum*, em duas condutividades elétricas, na nutrição e produção do cultivo hidropônico de rúcula. O experimento foi delineado em blocos casualizados, em esquema fatorial 4 x 2, com cinco repetições. Os tratamentos consistiram de inoculações (não inoculado, *A. brasilense*, *T. harzianum* e coinoculação) e duas condutividades elétricas (1,4 e 1,6 dS m^{-1}). A inoculação isolada de *T. harzianum* e *A. brasilense* produziu maior massa fresca de raiz, enquanto o índice de clorofila foliar foi maior com a inoculação de *A. brasilense*, em relação aos demais tratamentos. A inoculação de *A. brasilense* reduziu a concentração de nitrato nas folhas de rúcula. As inoculações e coinoculação de *A. brasilense* e *T. harzianum* melhoraram os componentes produtivos e a nutrição das plantas, reduziram a concentração de nitrato foliar e promoveram a biofortificação das folhas de rúcula com Zn e Fe. Além disso, a inoculação com *T. harzianum* promoveu concentração foliar de P e S.

PALAVRAS-CHAVE: *Eruca sativa*, microrganismos promotores de crescimento, técnica de filme de nutrientes.

INTRODUCTION

Arugula (*Eruca sativa* L.) is one of the leafy vegetables most produced in the hydroponic system (Soares et al. 2018). This vegetable is mainly consumed in the form of fresh salad, due to its richness in vitamin C, iron and calcium, and has detoxifying characteristics for the healthy functioning of the body, provided by the presence of antioxidant compounds (antigenotoxic, polyphenols and glucosinolates) (Aguiar et al. 2014).

Arugula and other leafy vegetables highly demand nitrogen (N) for a greater mass gain and leaf growth. However, increasing doses of N fertilization also increase the leaf nitrate content (Cavarianni et al. 2008). Increasing the efficiency of absorption and accumulation of nutrients available to plants may lead to a reduced use of chemical products and fertilizers in nutritious food, and that is currently a major challenge in horticulture (Oliveira et al. 2017).

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The use of beneficial microorganisms is a low-cost technology that improves plant growth and nutrient acquisition. The inoculation of *Azospirillum brasilense* increases the nitrate reductase activity in leaves and reduces the conversion of nitric N into ammoniacal N, alternately contributing to the reduction of leaf nitrate content (Pereira-Defilippi et al. 2017). Its inoculation has also increased the N use efficiency by reducing in 25 % the N fertilizer application (Galindo et al. 2020, Jalal et al. 2021). In addition, the inoculation of *Trichoderma* fungus contributes to overcome phytopathogens (Kumar et al. 2021) and modify the root architecture by stimulating auxin signaling. It also increases enzymatic activities, production of secondary metabolites, nutrient acquisition and use efficiency by roots (Meng et al. 2019). Furthermore, strains of *Trichoderma* spp. have been used as phosphate solubilizers through the release of organic acids (Bonini et al. 2020).

The inoculation of beneficial microorganisms is an alternative strategy to reduce the fertilizer application, while increasing enzymatic activities, nutrient absorption and phytohormones production to reach the leaf nitrate content for producing quality food. *A. brasilense* and *T. harzianum* have been little studied as growth promoters in arugula plants under hydroponic cultivation. Therefore, this study aimed to verify the beneficial effects of this microorganisms and electrical conductivities on the nutritional status of arugula cultivation in a hydroponic system.

MATERIAL AND METHODS

The trail on hydroponic arugula nutrient film technique cultivation was developed in a greenhouse with 30 % shading, at the Universidade Estadual Paulista (Unesp), in Ilha Solteira, São Paulo State, Brazil (20º25'07"S, 51º20'31"W and altitude of 376 m), from June 18 to July 20, 2021. The meteorological data were collected from an automatic station at Unesp (Figure 1).

The Atro arugula cultivar was used, which is characterized by early maturity, moderate resistance to early pinning, vigorous plants, broad leaves with less cut area, high yields and a bundle of visual qualities (well-formed and wide leaves). The seedling nursery was developed in phenolic foam for 15 days and then transplanted into permanent benches of the NTF system, where they remained for 31 days until harvest. The nutrients solution was composed of concentrated Hidrogood Fert Nacional (HFN) fertilizers with the following contents of nutrients (ppm): 0.01 of N, 0.009 of P, 0.028 of K, 0.0043 of S, 0.0033 of Mg, 0.00006 of B, 0.00001 of Cu, 0.00109 of Fe, 0.00007 of Mo, 0.00005 of Mn and

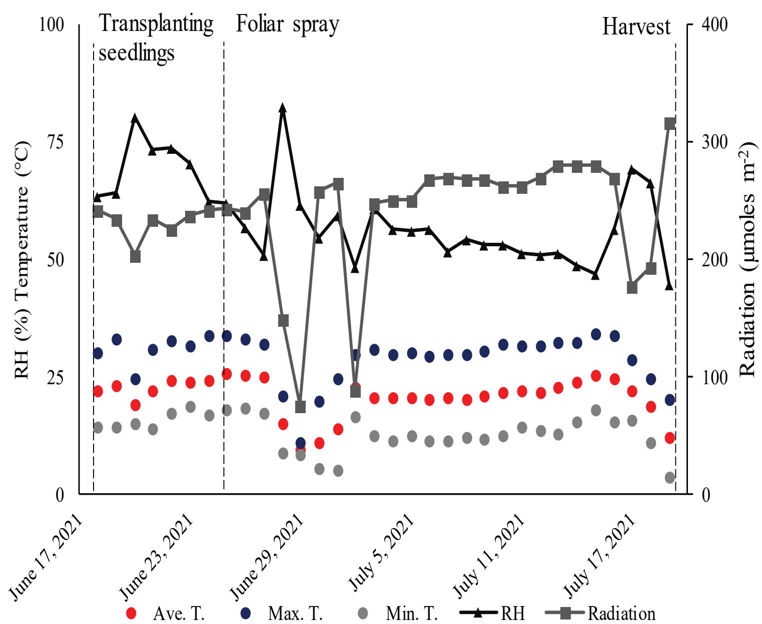

Figure 1. Relative air humidity (RH), average temperature (Ave. T.), maximum temperature (Max. T.), minimum temperature (Min. T) and radiation during the experiment conduction (June 18 to July 20, 2021).

0.00002 of Zn, besides calcium nitrate $[Ca(NO_3)^2]$ fertilizer (0.0155 ppm of N and 0.0265 ppm of Ca), as well as Hidrogood Fert Ferro (HFF) EDDHA fertilizer with 0.006 ppm of Fe content. To reach the electrical conductivity (EC) of 1.4 dS m^{-1}, the nutrients solution was added with 0.622 g L^{-1} of HFN in water, 0.462 g L^{-1} of $Ca(NO_3)_2$ and 0.028 g L^{-1} of HFF, while, to attain the electrical conductivity of 1.6 dS m^{-1}, 0.710 g L^{-1} of HFN in water, 0.528 g L^{-1} of $Ca(NO_3)_2$ and 0.032 g L^{-1} of HFF were added.

The measurement and correction of pH and conductivity were performed daily in the morning. The EC was readjusted as determined for each cultivation bench with the replacement of fertilizers, if necessary, and the pH was maintained between 5.5 and 6.5, using phosphoric acid (85 %) for a pH above 6.5 and sodium hydroxide (25 %) for a pH below 5.5 (Figure 2).

The experiment was designed in randomized blocks, in a 4 x 2 factorial scheme, with five replications, with each experimental unit being composed by 8 plants. The factors consisted of leaf microorganisms inoculation [not inoculated; *Azospirillum brasilense* - Ab-V5 and Ab-V6 strains, with guarantee of 2×10^8 colony forming units (CFU) mL^{-1} and 300 mL of inoculant sprayed in 300 L ha^{-1} of water; *Trichoderma harzianum* - ESALQ-1306 strains, with guarantee of 2×10^9 CFU mL^{-1} and 500 mL of inoculant sprayed in 300 L ha^{-1} of water; and co-inoculation of these two microorganisms]. The application was carried out in the morning, at a temperature of 21 ºC and relative humidity of 80 %, using an 18-L backpack sprayer, at 7 days after the seedlings transplantation. The second factor consisted of two electrical conductivities of nutrients solution (1.4 and 1.6 dS m^{-1}).

The arugula was harvested after 31 days of transplantation. The root and shoot fresh matter were determined by harvesting 8 plants, placing them on a table, and separating them into shoot and roots. The material was placed in an air-forced oven at 60 ºC, for 72 h, to obtain the root, shoot and total dry matter. The leaf chlorophyll index was recorded by a portable chlorophyll meter (ClorofiLOG® - model CFL - 1030 Falker), while the leaf yield was calculated via the following equation: yield (g m^{-2}) = shoot fresh weight (g) × plant population m^{-2} (19.5 plants m^{-2}).

The dried materials were weighed and grounded in a Willey-type mill to determine the contents of N, P, K, Ca, Mg, S, Fe, Mn and Zn in the arugula shoots (Malavolta et al. 1997). The determination of nitrate and ammonium contents in leaves and roots was carried out according to Cataldo et al. (1975).

The data of all variables presented normal distribution and homogeneous variances; therefore, they were submitted to analysis of variance. The F-test measured the significance of the mean squares of the analysis of variance at 5 % of probability. The means for inoculation were compared by the Tukey test at 5 % of probability. The means for the electrical conductivity levels were compared by the Fisher test at 5 % of probability.

RESULTS AND DISCUSSION

There was a significant interaction ($p < 0.01$) between electrical conductivities and foliar inoculations for root fresh and dry matter, shoot fresh and dry matter, leaf chlorophyll index and leaf yield (Table 1).

The highest root fresh matter was obtained with the inoculation of *T. harzianum* at the electrical

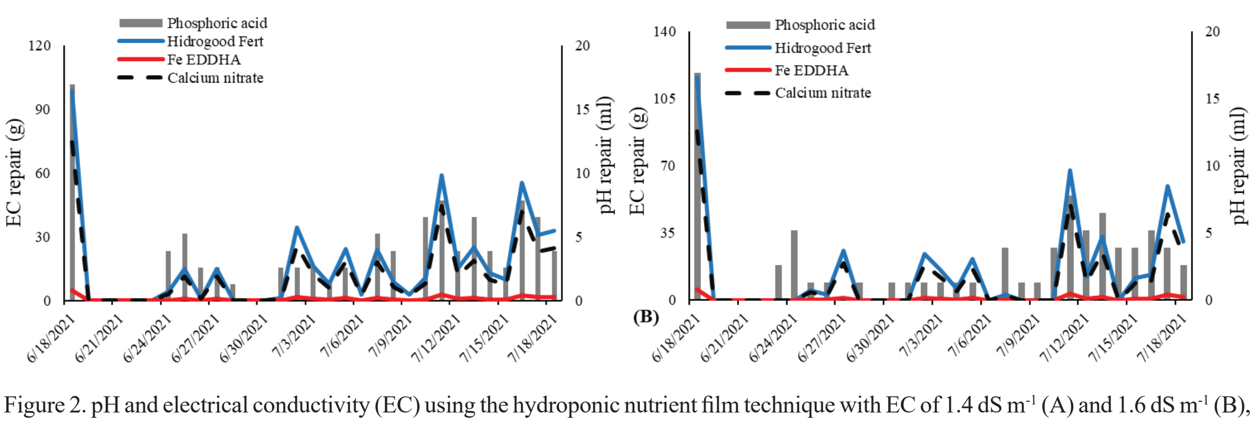

Figure 2. pH and electrical conductivity (EC) using the hydroponic nutrient film technique with EC of 1.4 dS m^{-1} (A) and 1.6 dS m^{-1} (B), during the experiment.

Table 1. Analysis of variance and probability of shoot (SFM) and root fresh matter (RFM), shoot (SDM) and root dry matter (RDM), leaf chlorophyll index (LCI) and leaf yield (YIELD).

Inoculations	SFM	RFM	SDM	RDM	LCI	YIELD
	g plant^{-1}					g m^{-2}
Non-inoculation	213.00	29.33	13.67	3.67	33.33	4,153.50
Azospirillum brasilense	232.17	48.50	14.33	4.33	36.92	4,527.25
Trichoderma hazianum	265.00	51.17	16.00	5.67	42.28	5,167.50
Co-inoculation	215.00	45.00	14.67	4.67	37.63	4,192.50
EC						
1.4 dS m^{-1}	229.50	41.67	14.08	4.08	35.53	4,475.30
1.6 dS m^{-1}	233.08	45.33	15.25	5.08	39.55	4,545.10
Variation factor	Probability					
Block	0.27^{ns}	0.56^{ns}	0.41^{ns}	0.14^{ns}	0.70^{ns}	0.27^{ns}
EC	0.00**	0.00**	0.00**	0.00**	0.06^{ns}	0.00**
Inoculation	0.00**	0.00**	0.03*	0.00**	0.00**	0.00**
EC * INO	0.00**	0.01**	0.00**	0.00**	0.00**	0.00**
CV (%)	11.91	7.97	5.67	8.91	5.23	7.72

^{ns} Not significant; ** significant at 1 %; * significant at 5 % of probability by the F-test. CV: coefficient of variation; EC: electrical conductivities; INO: inoculations.

conductivity (EC) of 1.4 dS m^{-1} and *A. brasilense* at 1.6 dS m^{-1}, as compared to non-inoculation and co-inoculation (Figure 3A). In addition, all inoculations at the highest EC (1.6 dS m^{-1}) showed a greater root dry matter for the arugula plants. However, the inoculation with *A. brasilense* showed a greater root dry matter accumulation at the lowest electrical conductivity (Figure 3B). The shoot fresh mass and leaf yield were increased with the inoculation of *A. brasilense* at both EC, as compared to the other inoculations (Figures 3C and 3F). All inoculations provided a greater shoot dry mass accumulation than without leaf inoculation in both EC, highlighting the inoculation with *A. brasilense*, which was higher than all the others (Figure 3D).

Crop yield can be easily altered by the interaction between microorganisms and plants (Emmett et al. 2017). Root mass gains by *Trichoderma* sp. are related to the ability of the microorganism to increase auxin contents in the root region through modulation by jasmonate biosynthesis during fungal colonization in roots (Meng et al. 2019). Under inoculation with *A. brasilense*, which stimulates an increase in the production of auxins in the root zone, and in previous studies, it was explained that there is a change in root architecture, increasing the lateral roots and thus the efficiency of the roots in absorbing water and nutrients (Spaepen et al. 2007, Averkina et al. 2021). Therefore, one of the reasons why the inoculation with *A. brasilense* and *T. harzianum* can increase shoot mass is the increase in water and nutrient absorption. However, it may occur due to increased photosynthetic activity, which increases the carbon accumulation in plant tissues (Moreira et al. 2022).

The foliar inoculation of *A. brasilense* and *T. harzianum* in arugula increased the leaf chlorophyll index by 36 and 20 % at the EC of 1.4 dS m^{-1}, respectively, and by 18 and 4 % at 1.6 dS m^{-1}, respectively (Figure 3E). It was reported that the inoculation with *A. brasilense* increased the leaf chlorophyll content and photosynthetic efficiency in several crops, favoring a greater plant growth and dry matter accumulation in plant tissues (Alvarez et al. 2019). The inoculation with *A. brasilense* via leaves increased the leaf content of chlorophyll a, chlorophyll b and carotenoids (Bulegon et al. 2016). The foliar inoculation of *A. brasilense* responded better to the plants with a short cycle. In addition, the inoculation with *Trichoderma* increased the shoot and root growth, with a greater crop yield (Uddin et al. 2016). The *Trichoderma* can stimulate and increase the production of auxins in primary and secondary roots and increase the shoots growth, with a higher chlorophyll content (Nieto-Jacobo et al. 2017).

A significant ($p < 0.01$) interaction between electrical conductivities and inoculations was observed for the contents of ammonium, nitrate and nitrogen in the shoots and roots of the arugula plants (Table 2).

The highest leaf NH_4^+ content in hydroponic arugula was noted with inoculating *A. brasilense*

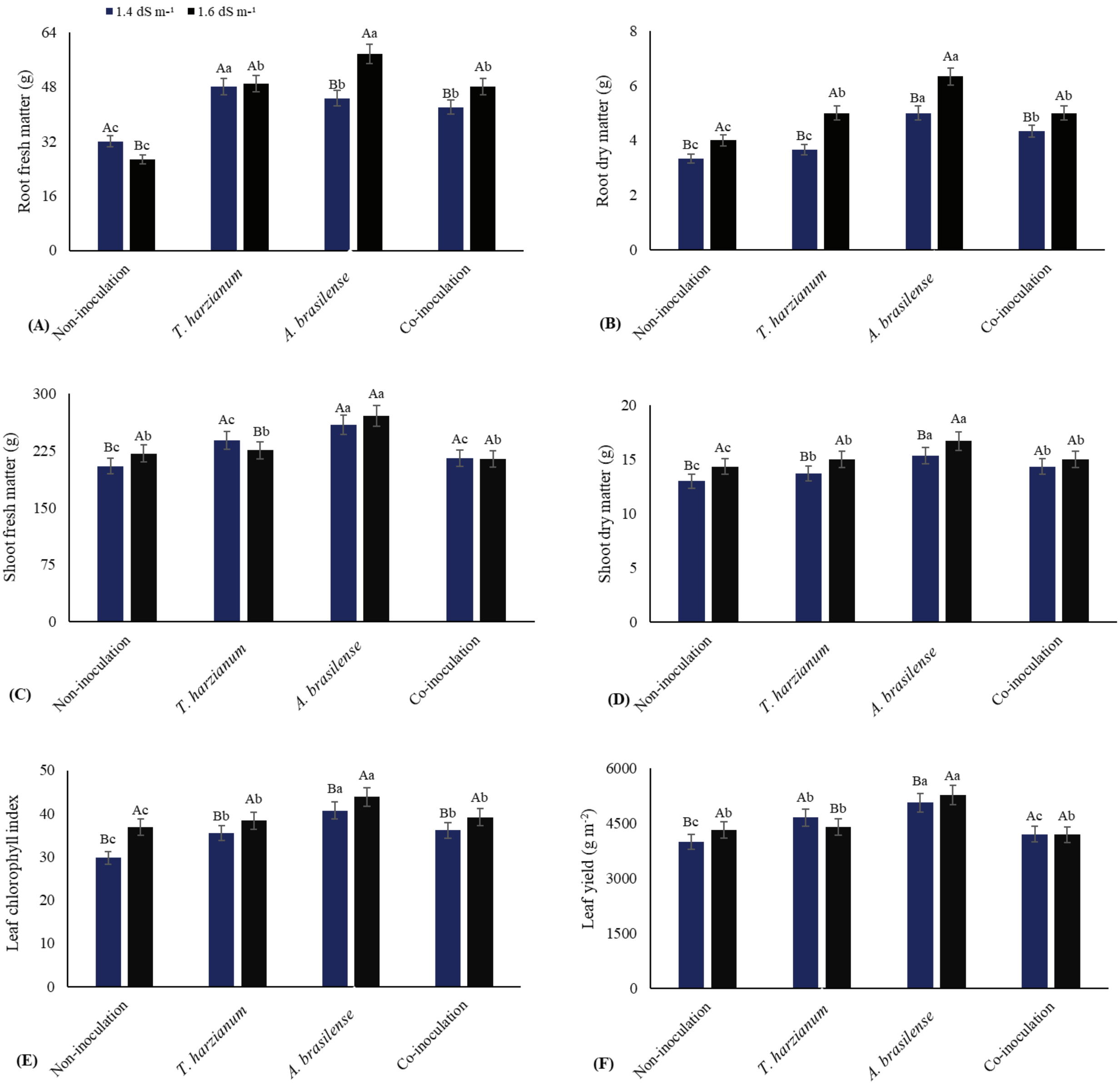

Figure 3. Interaction between foliar inoculations and electrical conductivity (EC) rates on root fresh (A) and dry matter (B), shoot fresh (C) and dry matter (D), leaf chlorophyll index (E) and leaf yield (F) of hydroponic arugula. The uppercase letters indicate the difference between the EC of the nutrition solution and lowercase letters among inoculations. Error bars indicate the standard deviation.

at both EC, while the lowest root NH_4^+ content was observed with inoculation of *A. brasilense* and *T. harzianum* at 1.4 dS m^{-1} (Figures 4A and 4D). The inoculation with *A. brasilense* and co-inoculation increased the total-N contents in shoot and roots of hydroponic arugula (Figures 4C and 4F). The highest leaf N and NH_4^+ contents were noted with inoculation of *A. brasilense* (Figures 4A and 4C). The foliar inoculation with *A. brasilense* and co-inoculation with *A. brasilense* + *T. harzianum* provided lower leaf and root NO_3^- contents at both EC (Figures 4B and 4E).

Inoculation with *A. brasilense* stimulates the nitrate reductase enzyme activity in plant leaves, thus reducing nitrate to nitrite (Pereira-Defilippi et al. 2017). Inoculation with *Trichoderma* increases the nitrogen cycle, production of secondary metabolism, amino acids, root nutrients acquisition and nutrient use efficiency by plants (Malmierca et al. 2015). Nitrate reduction occurs in the cytosol, through the

Table 2. Analysis of variance and probability of shoot accumulation of ammonium (NH_4^+-shoot), nitrate (NO_3^--shoot) and nitrogen (N-shoot), and root accumulation of ammonium (NH_4^+-root), nitrate (NO_3^--root) and nitrogen (N-root).

Inoculations	NH_4^+-shoot	NO_3^--shoot	N-shoot	NH_4^+-root	NO_3^--root	N-root
	mg m^{-2}		g m^{-2}	mg m^{-2}		g m^{-2}
Non-inoculation	255.63	678.11	10.18	140.89	93.70	2.37
Azospirillum brasilense	641.53	367.66	13.78	164.24	49.38	3.33
Trichoderma hazianum	710.67	202.22	15.45	200.37	46.42	4.66
Co-inoculation	972.32	213.92	19.91	244.45	41.78	6.53
EC						
1.4 dS m^{-1}	965.03	170.18	19.79	211.53	35.69	3.46
1.6 dS m^{-1}	325.04	560.78	9.87	163.44	79.95	4.99
Variation factor			Probability			
Block	0.93^{ns}	0.32^{ns}	0.68^{ns}	0.23^{ns}	0.30^{ns}	0.22^{ns}
EC	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
Inoculation	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
EC * INO	0.00**	0.00**	0.01**	0.00**	0.00**	0.00**
CV (%)	7.44	13.64	6.35	9.42	14.11	8.17

^{ns} Not significant; ** significant at 1 % of probability by the F-test. CV: coefficient of variation; EC: electrical conductivities; INO: inoculations.

activity of the nitrate reductase enzyme, resulting in nitrite, which enters the root plastids or leaves chloroplasts and is reduced to ammonia, which is fixed via glutamate synthase/glutamine synthase into amino acids (Yoneyama & Suzuki 2020). The glutamine and glutamate serve as substrate for transamination reactions to produce amino acids necessary for the protein synthesis (Yoneyama & Suzuki 2020).

A significant interaction effect ($p < 0.01$) between electrical conductivities and inoculations was observed for leaf contents of P, K, S, Zn, Mn and Fe. The effects of electrical conductivity and interaction were not significant for leaf Ca and Mg content. The single foliar inoculation with *A. brasilense* and *T. harzianum* increased the leaf Ca content, in relation to the other treatments. The inoculation with *A. brasilense* increased the leaf Mg content, concerning the co-inoculation of both microorganisms (Table 3).

Inoculation favors the acquisition of Ca and Mg by plants, which make up the cell wall structure of leaves and roots, since Ca and Mg are components of photosynthesis (Teixeira Filho et al. 2017). The

Table 3. Analysis of variance and probability of shoot accumulation of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), manganese (Mn) and iron (Fe).

Inoculations	P	K	Ca	Mg	S	Zn	Mn	Fe
			g kg^{-1}				mg kg^{-1}	
Non-inoculation	8.2	64.7	13.6 b	5.3 ab	17.0	126.0	92.8	79.5
Azospirillum brasilense	16.2	86.8	16.4 a	5.4 ab	23.4	179.8	95.8	82.8
Trichoderma hazianum	10.8	91.3	16.1 a	5.6 a	19.4	135.7	99.5	72.2
Co-inoculation	7.7	92.2	13.9 b	5.0 b	16.2	158.8	92.0	83.2
EC								
1.4 dS m^{-1}	9.7	79.9	14.8 A	5.4 A	18.3	141.4	102.7	79.4
1.6 dS m^{-1}	11.7	87.5	15.2 A	5.2 A	19.7	158.8	87.4	79.4
Variation factor				Probability				
Block	0.26^{ns}	0.16^{ns}	0.56^{ns}	0.22^{ns}	0.42^{ns}	0.13^{ns}	0.78^{ns}	0.37^{ns}
EC	0.00**	0.00**	0.40^{ns}	0.08^{ns}	0.00**	0.00**	0.02*	0.03*
Inoculation	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
EC * INO	0.00**	0.00**	0.16^{ns}	0.11^{ns}	0.01**	0.00**	0.00**	0.00**
CV (%)	7.97	9.12	11.91	9.72	9.72	8.75	9.89	17.57

^{ns} Not significant; ** significant at 1 %; * significant at 5 % of probability by the F-test. CV: coefficient of variation; EC: electrical conductivities; INO: inoculations. Uppercase letters indicate differences between EC and lowercase letters among inoculations by the Tukey test.

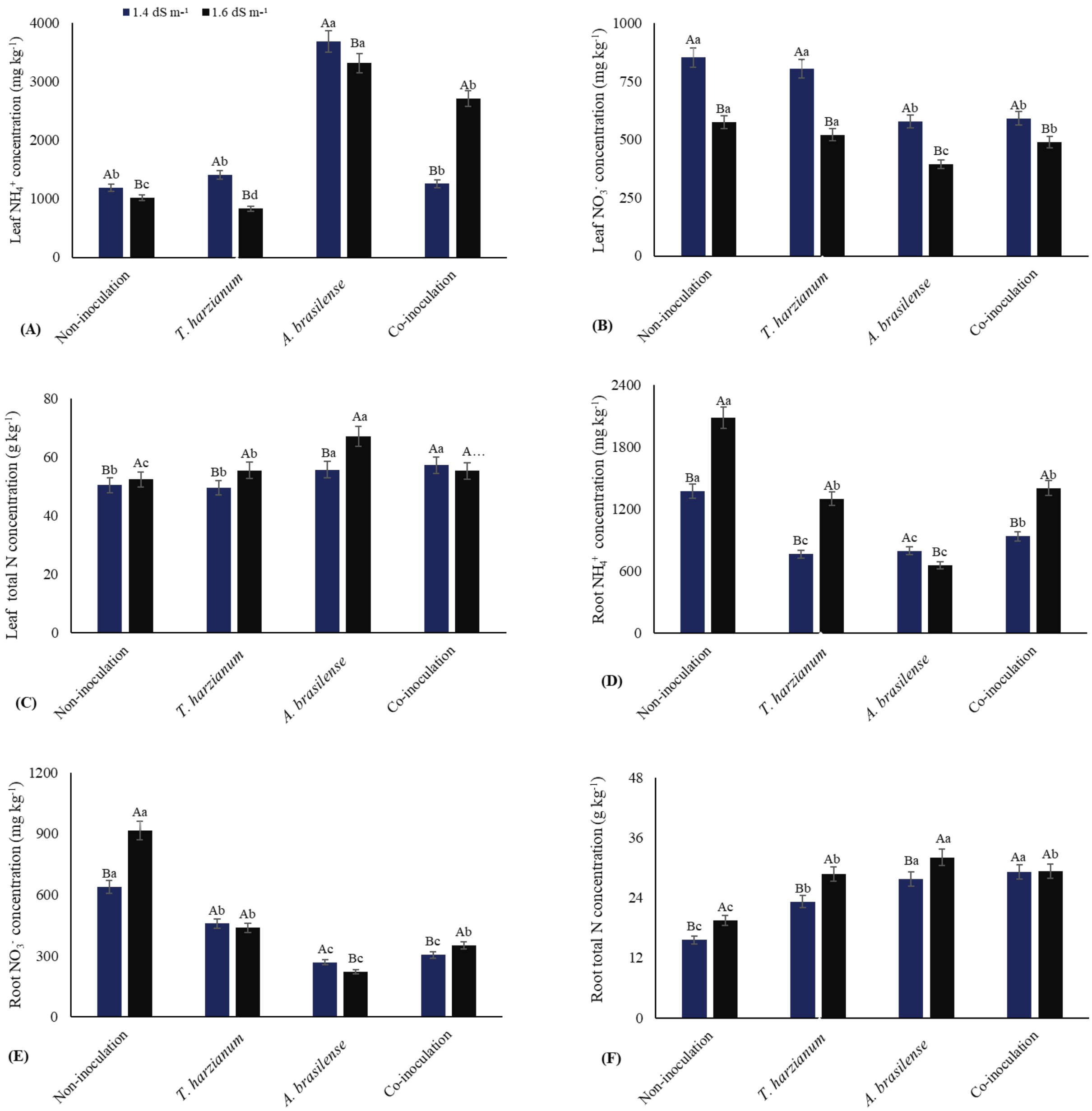

Figure 4. Interaction of foliar inoculation and electrical conductivity (EC) rates on arugula leaf contents of ammonium (A), nitrate (B) and nitrogen (C), and root contents of ammonium (D), nitrate (E) and nitrogen (F). The uppercase letters indicate the difference between the EC of the nutrition solution and lowercase letters among inoculations. Error bars indicate the standard deviation.

Ca_2^+ binding-proteins are located in the chloroplast; however, transporter-like s-adenosylmethionine is located in the chloroplast membrane, where Ca_2^+ acts as a cofactor for redox activation of the photosystem II (Wang et al. 2019). This Ca_2^+ also regulates the fructose-1,6-bisphosphatase (FBPase) and sedoheptulose-1,7-bisphosphatase (SBPase) enzymes, which are key factors of the Calvin cycle, as a pathway of carbon assimilation in the chloroplast stroma (Wang et al. 2019). Foliar and/or seed inoculation with *A. brasilense* in wheat improved the plant nutrition by increasing the absorption and translocation of Ca and Mg, consequently contributing to a higher content of Ca and Mg in the shoot (Teixeira Filho et al. 2017).

Inoculations and co-inoculation increased the leaf K content in the arugula plants, with the highest leaf K content being observed at both EC of the cultivation medium (Figure 5A). The better plant nutrition and water status occurred due to K, which is a functional element in osmoregulation and controls the stomatal opening and closing, thus enhancing the plant growth in harsh conditions. It was also reported that the stomata regulation was improved with fertilizer application, which maintained the carbohydrate synthesis and thus enhanced the plant growth (Oliveira et al. 2022).

The leaf P and S contents increased with the inoculation of *T. harzianum* at both EC of the cultivation solutions (Figures 5B and 5C). The inoculation with *A. brasilense* provided higher contents of N, P and K in maize shoot, as it provided a

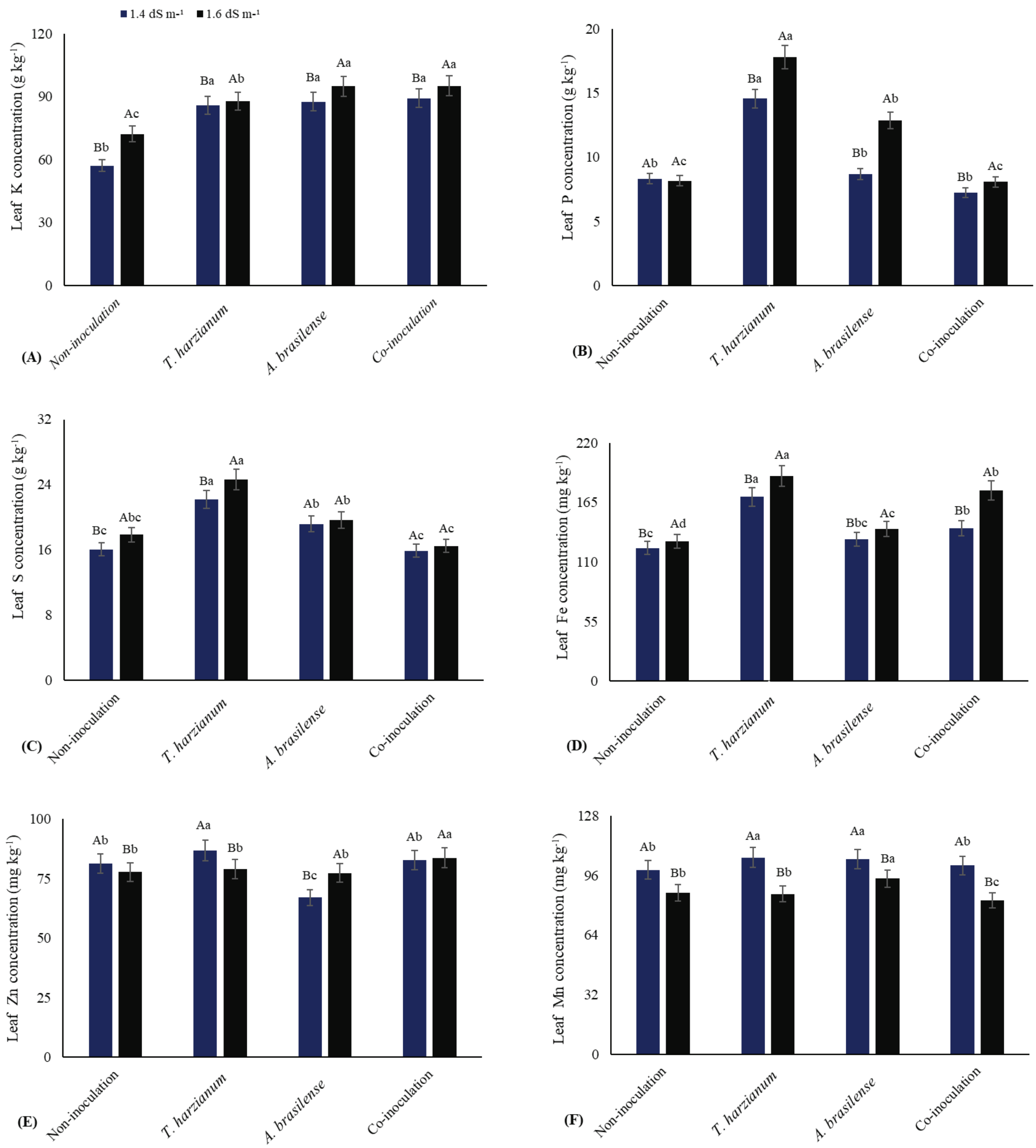

Figure 5. Leaf contents of potassium (A), phosphorus (B), sulfur (C), iron (D), zinc (E) and manganese (F) on arugula plants under foliar inoculation and electrical conductivity (EC) rates. The uppercase letters indicate the difference between the EC of the nutrition solution and lowercase letters among inoculations. Error bars indicate the standard deviation.

greater use efficiency of nutrients to plants (Marques et al. 2020). In addition, *Azospirillum* increased the P and protein contents in the shoot of tomato seedlings (Mangmang et al. 2015) and was also able to mobilize S and P by increasing their uptake and translocation to the shoot of plants (Fox et al. 2014).

The highest leaf Fe content was observed with inoculation of *T. harzianum*, concerning the other treatments, regardless of the EC (Figure 5D). The highest leaf content of Zn was noted with inoculation of *T. harzianum* at the EC of 1.4 dS m^{-1}, while at 1.6 dS m^{-1} the highest content was under co-inoculation (Figure 5E). There was a higher leaf Mn content for all the foliar inoculations at the EC of 1.4 dS m^{-1}, concerning the EC of 1.6 dS m^{-1}. The isolated inoculation of *A. brasilense* and *T. harzianum* provided a higher leaf content of Mn at the EC of 1.4 dS m^{-1}, and the inoculation with *A. brasilense* provided the highest leaf content of Mn at 1.6 dS m^{-1} (Figure 5F).

The inoculation with *T. harzianum* increased the leaf content of Zn by 6 and 2 %, and the leaf content of Fe by 38 and 46 %, respectively at the EC of 1.4 and 1.6 dS m^{-1}. In addition, the inoculation with *A. brasilense* increased the Fe content by 6 and 8 %, respectively at the EC of 1.4 and 1.6 dS m^{-1}, concerning the non-inoculation (Figure 5), being a viable biofortification strategy in arugula plants for human consumption. The inoculation with *A. brasilense* favors the food biofortification with an increasing absorption and translocation of Zn and Fe in edible parts (Jalal et al. 2021). This effect was also observed with the inoculation of *T. harzianum*, that increased the contents of Zn, Fe, vitamin A, carotenoids, proteins and total soluble solids in tomato fruits (Singh et al. 2018).

Microorganisms can produce secondary metabolites that act as Fe and Zn carriers, facilitating the nutrient absorption by roots and, consequently, the transport to shoots (Goswami et al. 2016). Biofortification via beneficial bacteria and fungi are effective ways to improve food quality with reduced use of fertilizers under the current global environmental concern (Moreira et al. 2022). Arugula plants with high nutritional quality may be an alternative strategy to eliminate medicines supplementation. Arugula plants consumed freshly provide a diet rich in Fe, Ca and vitamin C, along with antioxidant characteristics that improve the human health (Aguiar et al. 2014).

The present study provided a new perspective on inoculation and co-inoculation of *A. brasilense* and *T. harzianum* to increase yield, nutrients absorption and agronomic biofortification of arugula plants in a hydroponic system with reduced fertilizer application.

CONCLUSIONS

1. Inoculation with *Azospirillum brasilense* increases root and shoot growth, fresh mass yield, nitrogen, ammonium and potassium content, and decreases the leaf nitrate content in arugula plants, while the inoculation with *Trichoderma harzianum* increases the leaf contents of P, S, Zn and Fe;
2. The electrical conductivity of 1.6 dS m^{-1} improves yield components, plant nutrition and biofortification of micronutrients with inoculation and co-inoculation of *A. brasilense* and *T. harzianum*, while inoculation with *T. harzianum* increases the yield of arugula leaves at the electrical conductivity of 1.4 dS m^{-1} and allows a reduction of 18 % in the use of fertilizers.

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