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Nicotinamide and *Azospirillum brasilense* improves the quality of *Coffea arabica* seedlings[1]

Nicotinamida e *Azospirillum brasilense* melhoram a qualidade de mudas de *Coffea arabica*

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HIGHLIGHTS:

The combined use of Azospirillum brasilense *and nicotinamide has a synergistic effect on the growth of* Coffea arabica *seedlings.*
The growth and quality of coffee seedlings were favored by nicotinamide doses between 23.6 and 61.8 mg L^{-1}.
The dose of 120 mg L^{-1} of nicotinamide caused damage to the growth characteristics of coffee seedlings.

ABSTRACT: The use of biostimulants in coffee seedlings can promote gains in their growth and quality. Thus, this study aimed to evaluate the growth and quality characteristics of *Coffea arabica* seedlings under the effect of the nicotinamide and *Azospirillum brasilense* application. The experimental design was randomized blocks with treatments arranged in a 5 × 2 factorial scheme, with four replicates. The treatments resulted from the use of five doses of nicotinamide (0, 30, 60, 90, and 120 mg L^{-1} of water) combined with the absence and presence of *Azospirillum brasilense* applied to *Coffea arabica* seedlings from Catuaí Vermelho 144 cultivar. Plant height, stem diameter, number of leaves, leaf area, shoot dry mass, root dry mass, plant height:stem diameter ratio, shoot:root dry mass ratio, plant height:shoot dry mass ratio, and Dickson quality index were evaluated. The combined or isolated use of *A. brasilense* and nicotinamide, up to a dose of 33.5 mg L^{-1}, increased the biometric characteristics and dry mass accumulation. However, using nicotinamide doses between 30 and 61.8 g L^{-1} increased the quality of *Coffea arabica* seedlings. The synergistic effect of the use of *A. brasilense* and nicotinamide was verified for the growth and quality of *Coffea arabica* seedlings.

Key words: *Coffea arabica* L., diazotrophic bacteria, vitamin B3, niacin

RESUMO: O uso de bioestimulantes em mudas de café pode promover ganhos em seu crescimento e na sua qualidade. Assim, o objetivo desse trabalho foi avaliar as características de crescimento e qualidade de mudas de café arábica sob o efeito da aplicação dos bioestimulantes nicotinamida e *Azospirillum brasilense*. O delineamento experimental utilizado foi o de blocos casualizados, em esquema fatorial 5 × 2, com quatro repetições. Os tratamentos resultaram da utilização de cinco doses de nicotinamida (0, 30, 60, 90 e 120 mg L^{-1} de água) combinadas com a ausência e presença de *Azospirillum brasilense* aplicadas em mudas de *Coffea arabica* da cultivar Catuaí Vermelho 144. Foram avaliadas a altura de planta, diâmetro do caule, número de folhas, área foliar, massa seca da parte aérea, massa seca da raiz, razão entre altura e diâmetro do caule, razão entre massa seca da parte aérea e massa seca da raiz, razão entre altura e massa seca da parte aérea e índice de qualidade Dickson. A utilização conjunta ou isolado do *A. brasilense* e da nicotinamida, até a dose de 33.5 mg L^{-1}, aumentou características biométricas e acúmulo de massa seca. No entanto, a utilização de doses de nicotinamida entre 30 e 61,8 mg L^{-1} aumentou da qualidade de mudas de *Coffea arabica*. Foi verificado efeito sinérgico do uso de *A. brasilense* e nicotinamida para crescimento e qualidade de mudas.

Palavras-chave: *Coffea arabica* L., bactérias diazotróficas, vitamina B3, niacina

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Introduction

Brazil is the largest producer and exporter of coffee in the world, with an estimated production of *Coffea arabica* in 2022 harvest of 32.4 million processed bags, occupying an area of 1.4 million hectares in production and 364.6 thousand ha in formation (CONAB, 2022). This entire area of coffee in formation requires quality seedlings for the producer to succeed. In the production of *C. arabica* seedlings, there is an increase in the initiatives aimed at improving or shortening the process, such as the use of biostimulants, plant growth regulators, inoculation with symbiotic microorganisms, among others, which are alternatives promising for plant growth (Coelho et al., 2018; Ferreira et al., 2018; Costa et al., 2022).

Biostimulants are synthetic substances formed by a set of plant regulators or by compounds of nutrients, vitamins, amino acids, and other products, which can perform similar effects to those obtained by natural plant hormones (Prieto et al., 2017; Mohamed et al., 2020). In *Coffea arabica* of the cultivar Catuaí Amarelo, the application of biostimulant promoted better performance for germination, height, and the number of pairs of leaves, increasing its precocity for transplanting (Ferreira et al., 2018). Nicotinamide and diazotrophic bacteria such as *Azospirillum brasilense* are promising biostimulants for agricultural use.

Nicotinamide, also known as vitamin B3 or niacin, is a biostimulant that has stood out for its importance within the biological processes of plants, such as enzyme synthesis (Berglund et al., 2017), growth regulation (Mohamed et al., 2020) and enzymatic oxidation-reduction reactions in cells (El-Lethy et al., 2019). This vitamin can improve plant growth and yield and increase photosynthetic pigments, carbohydrate content, indoleacetic acid (IAA), GA3, and cytokinins (El-Lethy et al., 2019; Mohamed et al., 2020).

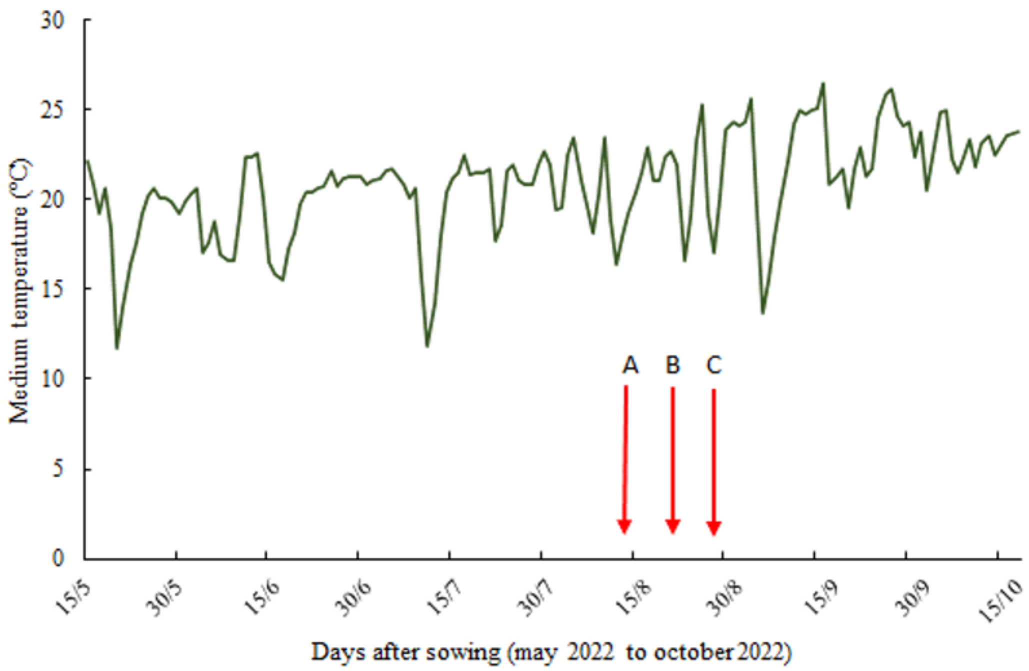
The associative endophytic diazotrophic bacteria of the genus *Azospirillum* act in the biological fixation of nitrogen and the biosynthesis of growth-regulating hormones such as IAA, auxins, gibberellins, and cytokinins, releasing them onto the root system, increasing dry mass and N accumulation, increasing the plant growth (Galeano et al., 2019; Vega et al., 2020). In *Coffea arabica* seedlings, Silva et al. (2020) found that the application of *A. brasilense* bacteria promoted greater growth of the pivoting root and fresh matter of the root system. Although these biostimulants have specific individual plant functions, their joint use has not been evaluated, opening the way for new research.

The hypothesis tested is that the application of nicotinamide and *Azospirillum brasilense* as a biostimulant improves the growth and quality of *Coffea arabica* seedlings. Thus, this study aimed to evaluate the growth and quality characteristics of *Coffea arabica* seedlings under the effect of nicotinamide and *A. brasilense* application.

Material and Methods

The experiment was carried out from May 2022 to October 2022 in a protected environment, belonging to the Departamento de Engenharia Florestal, from the experimental area of the Universidade Federal de Mato Grosso do Sul, Chapadão do Sul campus (18° 46’ 17.8” S, 52° 37’ 27.7” W, and altitude of 813 m). The seedling production environment had micro-sprinkler irrigation, 100-micron plastic film coverage and 50% shading overlay on top, and a side coating with 50% shading. The floor was made of soil coated with gravel to facilitate drainage. During the experiment, microclimatic data were recorded daily inside the protected environment (Figure 1).

The experimental design was randomized blocks with treatments arranged in a 5 × 2 factorial scheme, with four replicates. The treatments resulted from the use of five doses of nicotinamide (0, 30, 60, 90, and 120 mg L^{-1} of water) combined with the absence and presence of *Azospirillum brasilense*



A - End of seed emergence; B - Application of treatment with *Azospirillum brasilense*; C - Application of nicotinamide treatment

Figure 1. Average air temperature inside the protected environment during the experiment

applied to *Coffea arabica* seedlings of the Catuaí Vermelho 144 cultivar. The plots were composed of polyethylene trays of 96 tubes with conical shapes, with a capacity of 120 cm^{-3}.

To produce seedlings, the tubes were placed in supports for 96 units, filled with a substrate in a 1:1:1 ratio (soil: cotton residue: sugarcane bagasse), and two coffee seeds were placed in each tube at a depth of 2 cm. The soil used was obtained from a ravine, eliminating the upper layer. The cotton residue was obtained from a cotton processing company, with a minimum time of two years of composting. The sugarcane bagasse came from an alcohol plant, from material obtained in the current harvest, and used to promote greater aeration of the substrate mixture. The substrate was enriched by applying 6 kg m^{-3} of Osmocote fertilizer (NPK 19-19-19) with a controlled release (seven-nine months).

The chemical attributes of the compounds used in the substrate were: soil - pH in $CaCl_2$ = 4.6, organic matter = 8.0 g dm^{-3}, P and S (in mg dm^{-3}) = 4.0 and 2.0, respectively, Ca, Mg, K, Al and H + Al ($mmol_c$ dm^{-3}) = 5.0, 1.0, 0.1, 1.0, and 19, respectively, cation exchange capacity (CEC) = 25,1 and sum of bases (BS) = 6.1; cotton residue - pH in $CaCl_2$ = 6.6, moisture = 73.64%, organic carbon = 34.65%, N = 3.48%, C/N ratio = 9.96, P = 5.4 g kg^{-1}, K^{2+} = 20.2 g kg^{-1}, Ca^{2+} = 35.2 g kg^{-1}, Mg^{2+} = 5.8 g kg^{-1}, Cu = 16 mg kg^{-1}, S = 4.9 g kg^{-1}, Fe = 10,8 g kg^{-1}, Mn = 148 mg kg^{-1}, Zn = 72 mg kg^{-1}, and B = 87 mg kg^{-1}; sugarcane bagasse – pH = 5.7, moisture, organic matter, organic carbon, N, P_2O_5, K_20, CaO, and MgO (in %) = 27.4, 93.2, 54.1, 0.6, 0.3, 0.1, 1.8, and 0.6, respectively.

After the establishment of the seedlings, which occurred ten days after the completion of germination, thinning was carried out, leaving only one seedling per container. Germination occurred from 40 to 90 days after sowing. Due to the variation in the size of coffee seedlings, 12 seedlings were considered per plot, selecting five uniform seedlings for the evaluations.

After thinning the seedlings, *A. brasilense* was applied at a dose of 1 mL per plant using the commercial product Biomax®, which contains the strain Ab-V5 (2 × 10^8 CFU mL^{-1}). The product was diluted in distilled water and then applied with a syringe directly to the substrate of each tube with the coffee seedlings.

Nicotinamide was applied with an electric sprayer with a constant pressure of 300 kPa and a flow rate of 136 L ha^{-1} using an empty cone jet nozzle. The product was diluted in distilled water and applied 120 days after sowing.

At 150 days after sowing, the plant height (PH) variables were measured, expressed in cm, measured with a millimeter ruler, considering the region between the stem and the apical bud; stem diameter (SD) expressed in mm, using a digital caliper, measured just above the substrate line; the number of leaves (NL); leaf area (LA) in cm^2, using the leaf area meter LI-COR model LI-3000; shoot dry mass (SDM) and root dry mass (RDM) both expressed in grams and determined after drying in an oven with forced air circulation at 65 °C, kept in drying until reaching constant mass.

The seedling quality was evaluated by the Dickson quality index (DQI), described by Dickson et al. (1960), using Eq. 1.

$$DQI = \frac{\text{Total dry mass}}{(RHD + RSR)} \quad (1)$$

where:

DQI - Dickson quality index;

RHD - plant height:stem diameter ratio; and,

RSR - shoot:root dry mass ratio.

Before proceeding with the analysis of variance, the homogeneity of variance and normality of errors were verified using the Cochran and Lilliefors tests, respectively. Data were subjected to analysis of variance (p ≤ 0.05). The means of *A. brasilense* were compared by F-test at p ≤ 0.05. The means from nicotinamide doses were submitted to regression analysis at p ≤ 0.05. Statistical analyzes were performed using the Sisvar 5.1 software (Ferreira, 2019). Canonical variables were analyzed using the Rbio program (Bhering, 2017).

Results and Discussion

The interaction of nicotinamide application with *A. brasilense* influenced variables PH, NL, SDM, RSR, and RHS. The RHD variable was not affected by the application of the factors alone or together. In contrast, the variables SD, LA, RDM, and DQI were influenced by one of the two factors (Table 1).

The application of *A. brasilense* provided greater seedling growth and quality of seedlings, as indicated by RDM and DQI (Table 1). The gains resulting from diazotrophic bacteria in coffee seedlings were 14.5 and 12.9% for RDM and DQI, respectively.

It was observed that the use of *A. brasilense* always provided a greater height of coffee seedlings compared to the absence of application of the bacteria. With the use of *A. brasilense*, the dose of 33.53 mg L^{-1} of nicotinamide resulted in the highest

Table 1. Summary of analysis of variance for plant height (PH), stem diameter (SD), number of leaves (NL), leaf area (LA), shoot dry mass (SDM), root dry mass (RDM), plant height:stem diameter ratio (RHD), shoot:root system dry mass ratio (RSR), plant height:shoot dry mass ratio (RHS) and Dickson quality index (DQI) according to the application of nicotinamide and *Azospirillum brasilense* in *Coffea arabica* seedlings, cultivar Catuaí Vermelho 144

SV	DF	Mean squares				
		PH	SD	NL	LA	SDM
Block	3	0.2544^{ns}	0.0227^{ns}	0.1403^{ns}	0.6317^{ns}	0.0074^{ns}
Azo	1	2.0783^{**}	0.0926^{*}	0.0012^{ns}	256.8564^{**}	0.0284^{**}
Nic	4	7.3473^{**}	0.3250^{**}	1.1684^{**}	2031.3088^{**}	0.1948^{**}
Azo × Nic	4	0.4621^{*}	0.0187^{ns}	0.5101^{*}	16.8959^{ns}	0.0163^{**}
Residue	27	0.1515	0.0141	0.1723	18.6176	0.0037
CV%		3.9	4.1	5.8	2.7	4.7
		RDM	RHD	RSR	RHS	DQI
Block	3	0.0021^{ns}	0.0660^{ns}	0.0736^{ns}	0.6306^{ns}	0.0001^{ns}
Azo	1	0.0764^{**}	0.0195^{ns}	0.4699^{**}	0.0002^{ns}	0.0129^{**}
Nic	4	0.0649^{**}	0.0679^{ns}	0.7473^{**}	1.0211^{**}	0.0133^{**}
Azo × Nic	4	0.0019^{ns}	0.0949^{ns}	0.1032^{*}	0.9202^{**}	0.0002^{ns}
Residue	27	0.0009	0.0379	0.0292	0.1765	0.0003
CV%		5.2	5.6	7.7	5.4	5.1
Azo		SD	LA	RDM	DQI	
Presence		2.94 a	162.12 a	0.63 a	0.35 a	
Absence		2.84 a	157.05 a	0.54 b	0.31 b	

ns - Not significant (p > 0.05); * - Significant (p ≤ 0.05); ** - Significant (p ≤ 0.01); Azo - *Azospirillum brasilense*; Nic - Nicotinamide; CV - Coefficient of variation; SV - Source of variation; and DF - degree of freedom. Means followed by the same lowercase letter in the column do not indicate a significant difference between them by the F-test at p ≤ 0.05

plant height, 11.04 cm (Figure 2A). For stem diameter (Figure 2B), the dose of 32 mg L^{-1} of nicotinamide allowed the highest value. It is important to consider that the use of higher doses of nicotinamide was harmful to the growth in height and diameter of the seedlings, decreasing these variables by 19.2 and 13.4%, respectively, when using the dose of 120 mg L^{-1} of nicotinamide compared to the treatment without its application.

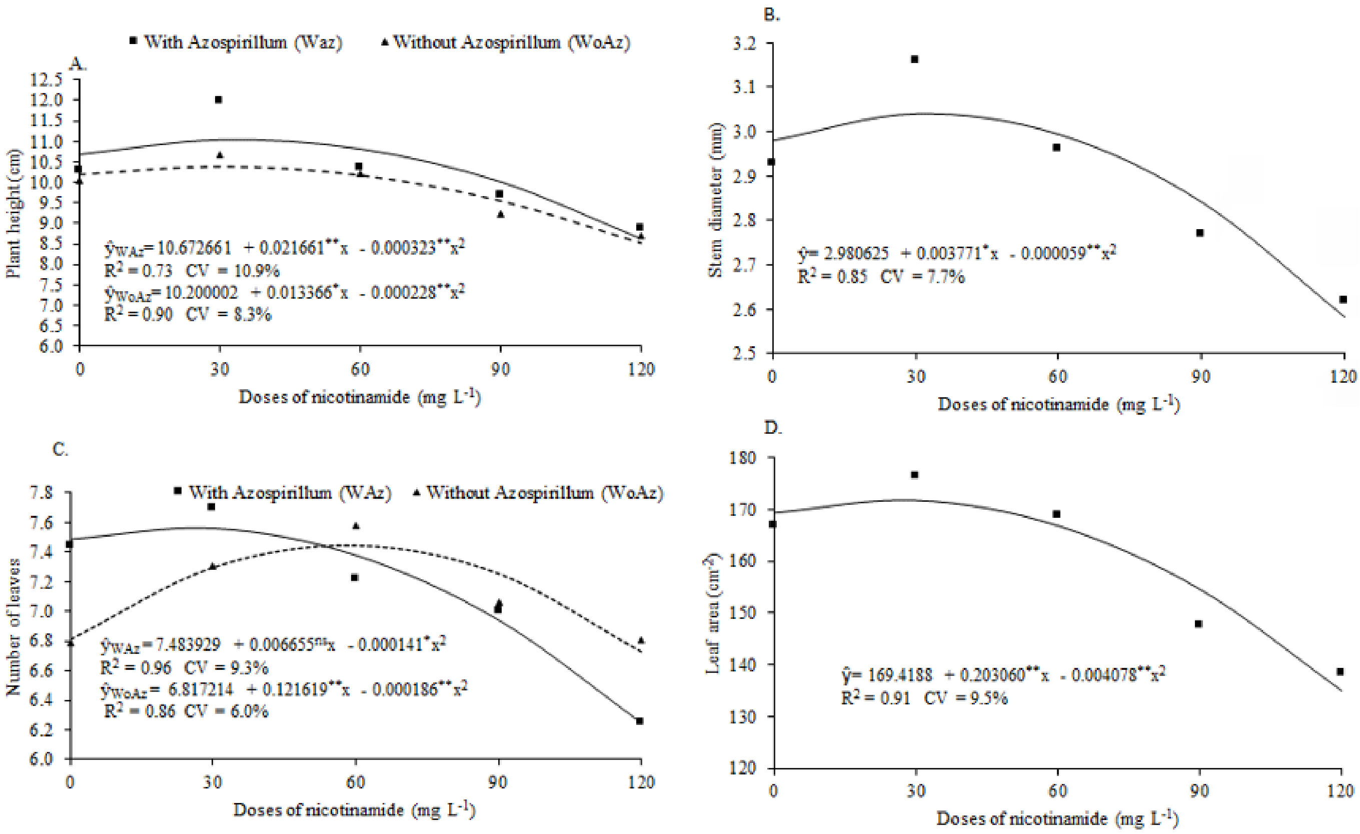
Diazotrophic bacteria, such as *A. brasilense*, in addition to having the potential to fix atmospheric nitrogen in the soil when in association with plant species, produce growth hormones, such as auxin, 3-indoleacetic acid, cytokinin, and gibberellin, regulating gene expression, plant development and signaling against environmental stresses (Fukami et al., 2018; Galindo et al., 2020). Gibberellin is essential in cell elongation and may contribute to plant stem growth (Dawood et al., 2019; Mohamed et al., 2020). Thus, it can be verified that the application of *A. brasilense* promoted an increase in the height of *Coffea arabica* seedlings (Figure 2A), probably due to its potential for producing gibberellin. In addition, *Azospirillum* sp. can colonize a diversity of plant species, inducing beneficial effects, as it brings together several mechanisms to promote plant growth, which makes it a valuable candidate as a biofertilizer (Vendruscolo & Lima, 2021).

The increase observed in the root system of *Coffea arabica* seedlings inoculated with *A. brasilense* (Table 1) is possibly due, according to Mehnaz (2015), to the fact that the root system of plants inoculated with *Azospirillum* sp. show response, in which the application of the inoculant increases the number and length of root hairs, the number of lateral roots, the diameter and length of lateral roots and adventitious roots, consequently improving plant nutrition, as well as their growth vegetable. In addition, with a positive effect on the growth and mass accumulation of shoots and roots, the application of *A. brasilense* provides a balanced growth between plant organs, reflected in the increase in the IQD (Table 1).

The increase observed in the height of coffee seedlings with the application of nicotinamide, and *A. brasilense* (Figure 2A) may be related to the action of plant hormones produced by the bacteria associated with the action of nicotinamide. The results obtained by Dawood et al. (2019) show that the foliar application of nicotinamide on fava bean plants increased plant height at 75 days after sowing. The authors also describe that the height of the plants gradually increased according to the concentration of nicotinamide (5 mg L^{-1} = 37.50 cm, 10 mg L^{-1} = 38.00 cm, and 20 mg L^{-1} = 38.50 cm).

It is also possible to observe that the isolated use of nicotinamide increased the stem diameter of coffee seedlings (Figure 2B). For Berglund et al. (2017), nicotinamide is a substance that regulates growth, which can alter some physiological aspects of plants in small amounts. Also, according to the same authors, this statement can be reinforced because nicotinamide is recognized as a growth regulator that influences many physiological processes, such as the biosynthesis of enzymes, nucleic acids, and proteins, acting as an activator of enzymes that catalyze redox reactions, such as nicotinamide adenine dinucleotide phosphate ($NADP^+$), a reducing agent for carbon fixation in the Calvin-Benson Cycle, and nicotinamide adenine dinucleotide (NAD^+/NADH).

The greatest number of leaves (7.6) and leaf area (172 cm^2) were obtained with the doses of nicotinamide of 23.6 and 24.9



* or ** or ns - Significant at $p \leq 0.05$ and ≤ 0.01, or not significant by the F-test, respectively

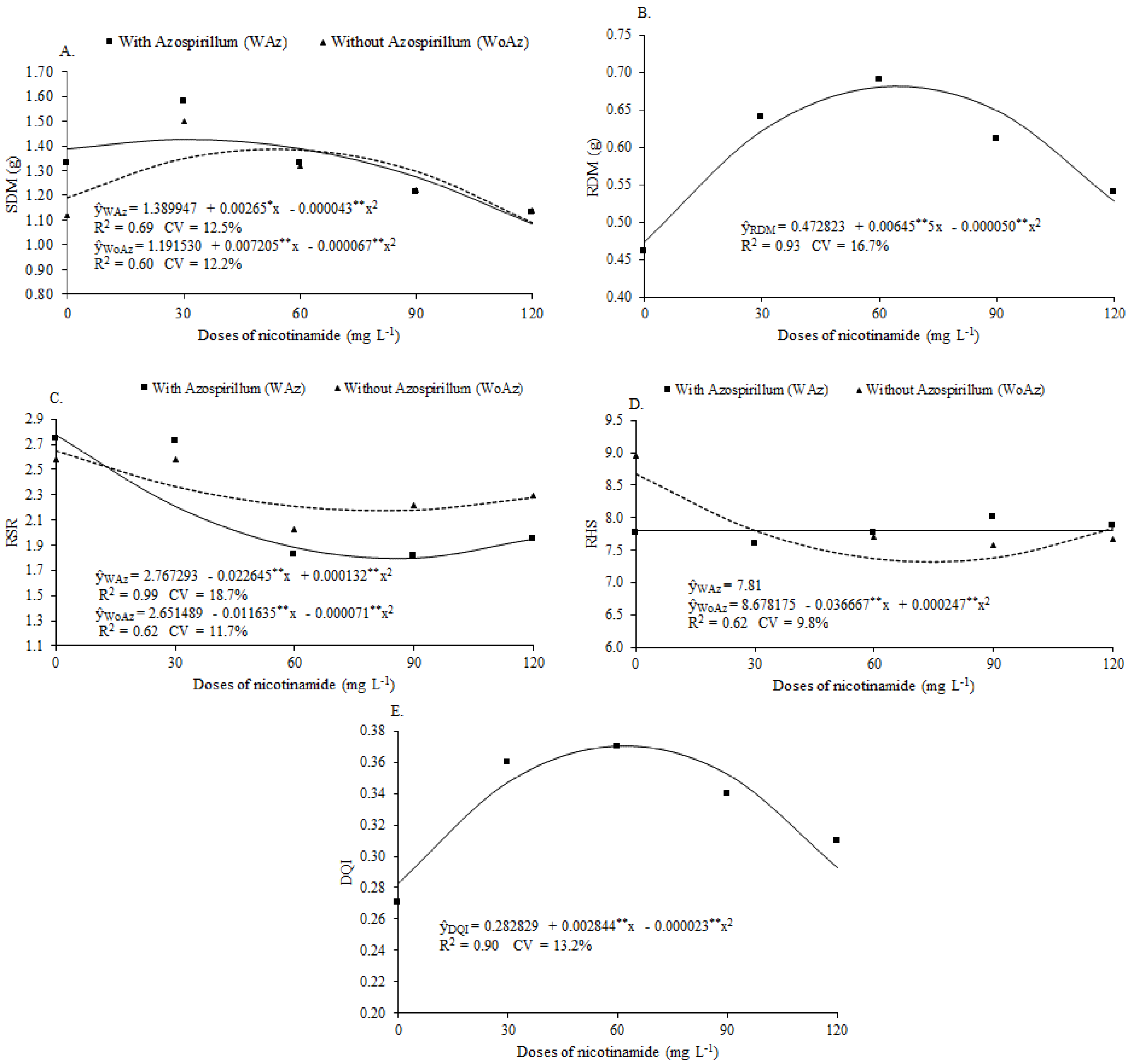
Figure 2. Seedling height (A), stem diameter (B), number of leaves (C), and leaf area (D) of *Coffea arabica* seedlings according to the nicotinamide doses and *Azospirillum brasilense*

mg L^{-1}, respectively, with the greatest number of leaves being achieved with the *A. brasilense* application (Figures 1C and D). Higher doses of nicotinamide caused a reduction in the number of leaves and the leaf area of the seedlings by 17.3 and 20.2%, respectively, with a dose of 120 mg L^{-1}, compared to the absence of application of the vitamin.

The application of nicotinamide increases the leaf area of vegetables, as observed for the corn crop (Colla et al., 2021). This increase is related to the activity of the photosynthetic system (Vendruscolo et al., 2019), which increases energy reserves (Abdelhamid et al., 2013). These reserves can be used during periods when the plant undergoes stress, but it is also essential for the development of new organs, such as leaves, stems, and roots, in addition to the expansion of the cells that make up these structures (Berglund et al., 2017; Dawood et al., 2019; El-Lethy et al., 2019). This culminates in conditions for new leaves to be issued and for these to develop and accumulate structural components, as observed in the present study (Figures 2C and D).

The highest shoot dry mass, 1.43 g, was obtained at a dose of 30.3 mg L^{-1} of nicotinamide using *A. brasilense*. From this dose, the use of nicotinamide affects the production of dry mass, with the lowest value, 1.08 g, obtained with the dose of 120 mg L^{-1}, which represents a value 22.3% lower than that obtained in the absence of the vitamin application (Figure 3A).

In contrast, the inhibitory effect observed at the highest nicotinamide doses for PH (Figure 2A), SD (Figure 2B), NL (Figure 2C), LA (Figure 2D), and SDM (Figure 3A) was due to high concentrations of nicotinamide, which block the mitotic cycle during the interphase, which can result in a long G2



* and ** - Significant at $p \leq 0.05$ and ≤ 0.01 by the F-test, respectively

Figure 3. Shoot dry mass (SDM) (A), root dry mass (RDM) (B), shoot:root dry mass ratio (RSR) (C), plant height:shoot dry mass ratio (RHS) (D), and Dickson quality index (DQI) (E) of *Coffea arabica* seedlings according to nicotinamide doses and *Azospirillum brasilense*

period, inhibiting DNA synthesis, as verified by Kumar & Shikha (2012). These authors tested different concentrations of nicotinamide (0.5, 1.0, 1.5, and 2.0%), verifying that the active mitotic index (frequency of cell division) of the root meristem progressively decreased with increasing doses of nicotinamide and verified that the relative division rate was negative and directly proportional to the severity of mitotic inhibition.

The use of *A. brasilense* provided the lowest values for RSR, with the application of nicotinamide (Figure 3C). For RHS (Figure 3D), there was no adjustment for the equation using *A. brasilense*. Without the application of bacteria, there was a reduction in the values of this variable with the nicotinamide application. For the DQI (Figure 3E), the highest value was obtained with the dose of 61.8 mg L^{-1} of nicotinamide.

Despite the inhibition caused by the higher doses of nicotinamide on the biometric characteristics of the seedlings, a decrease in the RSR ratio was observed (Figure 3C), indicating that the application of nicotinamide can also be used as a method to increase the rusticity of seedlings. The gain in mass, without the increase in height, is related to the accumulation of matter in cell walls, indicating tissue lignification and, consequently, increased field survival capacity (Massad et al., 2016).

A drop in RSR was also observed as the doses of nicotinamide increased (Figure 3D). Again, this effect is given by the inhibitory action of the vitamin on the development of seedlings, causing stress to the physiological system. In this stressful condition, different plant species, including coffee, tend to invest in root development at the expense of shoot development as a survival strategy (Pastorini et al., 2016). However, as observed for RSR, this effect can increase the survival rate of seedlings after transplanting, as a larger root system results in greater soil exploration capacity and, consequently, greater capacity to absorb nutrients and water (Ferreira et al., 2012).

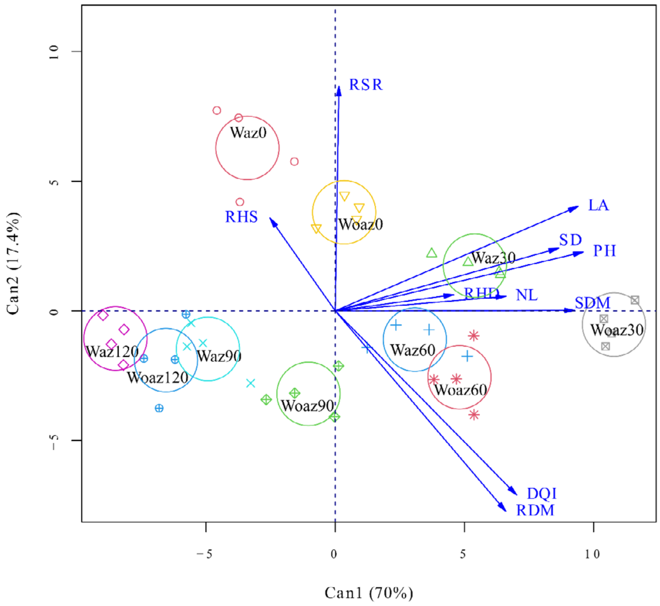
The highest DQI obtained was 0.37 using 61.8 mg L^{-1} of nicotinamide (Figure 3E). According to Gomes & Paiva (2012), the higher the value of this variable, the better the quality standard of seedlings. For Marana et al. (2008), the minimum DQI value for the seedlings to have quality would be 0.21; however, this is variable, according to Trazzi et al. (2010), depending on the species, the management applied, the substrate used, among others.

The synergistic potential of the use of *A. brasilense* and nicotinamide can be verified with the gains obtained by the variables PH, NL (Figures 2A and C), and SDM (Figure 3A).

In this work, the accumulated variance in the first two canonical variables was 87.4% (Figure 4). A clustering pattern is observed between treatments, where the nicotinamide doses showed similar behaviors, regardless of the presence or absence of *A. brasilense*.

The SDM and RSR vectors were the variables with the greatest influence on the canonical variables, with the SDM having a strong influence on component 1 and RSR having a great influence on the second canonical variable.

The vectors LA, SD, PH, RDH, NL, and SDM, had similar behavior, suggesting that they generate the same variability between doses of nicotinamide and inoculation. The Woaz30 treatment (absence of *A. brasilense* +30 mL L^{-1} of nicotinamide)



Woaz - Absence of *A. brasilense*; Waz - Presence of *A. brasilense*; PH - Plant height; SD - Stem diameter; NL - Number of leaves; LA - Leaf area; RDM - Root dry mass; SDM - Shoot dry mass; RHD – Plant height:stem diameter ratio; RHS – Plant height:shoot dry mass ratio; RSR – Shoot:root dry mass ratio; DQI - Dickson quality index; 0, 30, 60, 90, and 120 are nicotinamide doses; Can1 and Can2 - Canonical variables

Figure 4. Canonical analysis for growth and quality variables of *Coffea arabica* seedlings according to the nicotinamide application and *Azospirillum brasilense*

was highlighted in the SDM vector, as it is in the same order of distance from the origin in the canonical variables 1, indicating that this treatment was effective in increasing the shoot dry mass. The Waz30 treatment was highlighted in the vectors LA, SD, PH, RHD, and NL, indicating that the dose of 30 mL L^{-1} of nicotinamide in the presence of *A. brasilense* was effective in increasing the total height, stem diameter, number of leaves, leaf area, and plant height:stem diameter ratio, with the RHD and NL vectors having less influence on the canonical variables.

The Woaz60 and Waz60 treatments were similar. The variables that contributed to this were DQI and RDM, indicating that the highest values for these variables can be obtained independently of the use of *A. brasilense*.

Thus, it is possible to verify that the effects of nicotinamide as a growth regulator (El-Lethy et al., 2019; Mohamed et al., 2020) were probably decisive for the gains observed in the variables SDM, DQI, and RDM.

The treatments in the absence and presence of *A. brasilense* at doses of 90 and 120 mL L^{-1} of nicotinamide (Woaz90; Waz90; Woaz120; Waz120) did not stand out specifically in terms of any variable analyzed.

The canonical variables indicate the synergistic effect of *A. brasilense* with nicotinamide when observing the responses of the variables LA, SD, PH, RHD, and NL at the dose of 30 mL L^{-1} of nicotinamide and the presence of bacteria (Figure 4). Although the isolated effects of the vitamin (El-Lethy et al., 2019; Mohamed et al., 2020) and *A. brasilense* (Galeano et al., 2019; Vega et al., 2020) have already been observed in several plants, this experiment indicated that the synergism between these two compounds has application potential. It is possible that the ability of nicotinamide and *A. brasilense* to promote the formation of growth regulators was maximized when they are associated, benefiting the growth of coffee seedlings.

Conclusions

1. A synergistic effect of the use of *A. brasilense* and nicotinamide was verified for *Coffea arabica* seedlings growth and quality, being that doses up to 33.5 mg L^{-1} increase the biometric characteristics and accumulation of dry mass.

2. Nicotinamide doses between 30 and 61.8 mg L^{-1} increase the quality of *Coffea arabica* seedlings.

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Literature Cited

Abdelhamid, M. T.; Sadak, M. S. H.; Schmidhalter, U. R. S.; El-Saady, A. K. M. Interactive effects of salinity stress and nicotinamide on physiological and biochemical parameters of faba bean plant. Acta Biológica Colombiana, v.18, p.499-510, 2013.

Berglund, T.; Wallstrom, A.; Nguyen, T. V.; Laurell, C.; Ohlsson, A. B. Nicotinamide; antioxidative and DNA hypomethylation effects in plant cells. Plant Physiology Biochemistry, v.118, p.551-560, 2017. https://doi.org/10.1016/j.plaphy.2017.07.023

Bhering, L. L. Rbio: A tool for biometric and statistical analysis using the R platform. Crop Breeding and Applied Biotechnology, v.17, p.187-190, 2017. https://doi.org/10.1590/1984-70332017v17n2s29

Coelho, V. P. de M.; Rosa, K. M.; Paiva, P. E. B.; Moreira, É. F. A.; Carvalho, M. Fertigation and growth regulator on coffee seedling production in tubes. Pesquisa Agropecuária Tropical, v.48, p.350-357, 2018. https://doi.org/10.1590/1983-40632018v4852148

Colla, R. L. da; Lima, S. F. de; Vendruscolo, E. P.; Secco, V. A.; Piati, G. L.; Santos, O. F. dos; Abreu, M. S. Does foliar nicotinamide application affect second-crop corn (*Zea mays*)? Revista de la Facultad de Ciencias Agrarias – UNCuyo, v.53, p.64-70, 2021. https://doi.org/10.48162/rev.39.040

CONAB – Companhia Nacional de Abastecimento. Acompanhamento da safra brasileira de café, Brasília, v.9, safra 2022, n. 3, setembro, 2022.

Costa, C. E. de M.; Rezende, T. T.; Silva, A. B. da. Efeito de bioestimulantes no crescimento de cafeeiros esqueletados. Research, Society and Development, v.11, p. e12811527871, 2022. https://doi.org/10.33448/rsd-v11i5.27871

Dawood, M. G.; Abdel-Baky, Y. R.; El-Awadi, M. E. S.; Bakhoum, G. S. Enhancement quality and quantity of faba bean plants grown under sandy soil conditions by nicotinamide and/or humic acid application. Bulletin of the National Research Centre, v.43, p.1-8. 2019. https://doi.org/10.1186/s42269-019-0067-0

Dickson, A.; Leaf, A. L.; Hosner, J. F. Quality appraisal of white spruce and white pine seedling stock in nurseries. Forest Chronicle, v.36, p.10-13, 1960. https://doi.org/10.5558/tfc36010-1

El-Lethy, S. R.; Talaat, I. M.; Tarraf, S.; El Moursi, A. Impacts of some biostimulants on guar (*Cyamopsis tetragonoloba* L.) plants. Bulletin of the National Research Centre, v.43, p.1-7, 2019. https://doi.org/10.1186/s42269-019-0196-5

Ferreira, B. C.; Lima, S. F. de; Simon, C. A.; Andrade, M. G. de O.; Ávila, J. de; Alvarez, R. de C. F. Effect of biostimulant and micronutrient on emergence, growth and quality of arabica coffee seedlings. Coffee Science, v.13, p.324-332, 2018. https://doi.org/10.25186/cs.v13i3.1450

Ferreira, D. F. Sisvar: a computer analysis system to fixed effects Split plot type designs: Sisvar. Brazilian Journal of Biometrics, v.37, p.529-535, 2019. https://doi.org/10.28951/rbb.v37i4.450

Ferreira, W. N.; Zandavalli, R. B.; Bezerra, A. M. E.; Medeiros Filho, S. Crescimento inicial de *Piptadenia stipulacea* (Benth.) Ducke (Mimosaceae) e *Anadenanthera colubrina* (Vell.) Brenan var. cebil (Griseb.) Altshul (Mimosaceae) sob diferentes níveis de sombreamento. Acta Botanica Brasilica, v.26, p.408-414, 2012. https://doi.org/10.1590/S0102-33062012000200016

Fukami, J.; Ollero, F. J.; La Osa, C.; Valderrama-Fernández, R.; Nogueira, M. A.; Megías, M.; Hungria, M. Antioxidant activity and induction of mechanisms of resistance to stresses related to the inoculation with *Azospirillum brasilense*. Archives of microbiology, v.200, p.1191-1203, 2018. https://doi.org/10.1007/s00203-018-1535-x

Galeano, R. M. S.; Campelo, A. P. S.; Mackert, A.; Brasil, M. S. Initial development and quantification of corn proteins inoculated with new strains of *Azospirillum brasilense*. Revista Agricultura Neotropical, v. 6, p. 95-99, 2019. https://doi.org/10.32404/rean.v6i2.2613

Galindo, F. S.; Teixeira Filho, M. C. M.; Silva, E. C. da; Buzetti, S.; Fernandes, G. C.; Rodrigues, W. L. Technical and economic viability of cowpea co-inoculated with *Azospirillum brasilense* and *Bradyrhizobium* spp. and nitrogen doses. Revista Brasileira de Engenharia Agrícola e Ambiental, v.24, p.304-311, 2020. https://doi.org/10.1590/1807-1929/agriambi.v24n5p304-311

Gomes, J. M.; Paiva, H. N. de. Viveiros florestais: propagação sexuada. Viçosa: UFV, 2012. 116p.

Kumar, G.; Shikha, D. Mitodepressive and chromotoxic impact of nicotinamide on root meristems of *Cyamopsis tetragonoloba* L. Chromosome Botany, v.7, p.105-110, 2012. https://doi.org/10.3199/iscb.7.105

Marana, J. P.; Miglioranza, E.; Fonseca, E. P.; Kainuma, R. H. Índices de qualidade e crescimento de mudas de café produzidas em tubetes. Ciência Rural, v.38, p.39-45, 2008. https://doi.org/10.1590/S0103-84782008000100007

Massad, M. D.; Dutra, T. R.; Cardoso, R. L.; Santos, T. B.; Sarmento, M. F. Q. Produção de mudas de *Anadenanthera peregrina* em resposta a substratos alternativos com bagaço de cana. Revista Ecologia e Nutrição Florestal – ENFLO, v.4, p.45-53, 2016. https://doi.org/10.5902/2316980X24308

Mehnaz, S. *Azospirillum*: A biofertilizer for every crop. In: Arora, N. K. (ed.). Plant microbes symbiosis: Applied facets. New Delhi: Springer, 2015. p.2297-314.

Mohamed, M. H.; Badr, E. A.; Sadak, M. S.; Khedr, H. H. Effect of garlic extract, ascorbic acid and nicotinamide on growth, some biochemical aspects, yield and its components of three faba bean (*Vicia faba* L.) cultivars under sandy soil conditions. Bulletin of the National Research Centre, v.44, p.1-8, 2020. https://doi.org/10.1186/s42269-020-00359-z

Pastorini, L. H.; Romagnolo, M. B.; Barbeiro, C.; Guerreiro, R. G. de O.; Costa, P. M.; Sert, M. A.; Souza, L. A. Germinação e crescimento inicial de *Machaerium brasiliense* Vogel (Fabaceae) em casa de vegetação. Floresta, v.46, p.83-92, 2016. http:/dx.doi.org/10.5380/rf.v46i1.39625

Prieto, C. A.; Alvarez, J. W. R.; Figueredo, J. C.; Trinidade, S. A. Bioestimulante, biofertilizante e inoculação de sementes no crescimento e produtividade da soja. Revista de Agricultura Neotropical, v.4, p.1-8, 2017. https://doi.org/10.32404/rean.v4i2.1167

Silva, L. C. da; Barbosa, C. K. R.; Franco Junior, K. S. Evaluation of the effect of *Azospirillum brasilense* inoculation on arabic coffee seedlings. Coffee Science, v.15, p.e151678, 2020. https://doi.org/10.25186/cs.v15i.1678

Trazzi, P. A.; Caldeira, M. V. W.; Colombi, R. Avaliação de mudas de Tecoma stans utilizando biossólido e resíduo orgânico. Revista de Agricultura, v.85, p.218–226, 2010. https://doi.org/10.37856/bja.v85i3.2853

Vega, M. E. G.; Rodriguez, A. H.; Diaz, Y. H.; Espino, J. A. L.; Viva, M. F.; Ardisana, E. F. H.; Pérez-Álvarez, S. Characterization and plant growth promoting potential of microbial groups associated with a *Coffea* sp. Collection. Emirates Journal of Food and Agriculture, v. 32, p. 358-367, 2020. https://doi.org/10.9755/ejfa.2020.v32.i5.2105

Vendruscolo, E. P.; Lima, S. F. de. The *Azospirillum genus* and the cultivation of vegetables. A review. Biotechnology, Agronomy and Society and Environment, v.24, p.236-246, 2021. https://doi.org/10.25518/1780-4507.19175

Vendruscolo, E. P.; Rodrigues, A. H. A.; Correa, S. R.; Seleguini, A.; Lima, S. F. de. Different soaking times and niacine concentrations affect yield of upland rice under water deficit conditions. Agronomia Colombiana, v.37, p.166-172, 2019. https://doi.org/10.15446/agron.colomb.v37n2.72765