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ORIGINAL ARTICLE

Biostimulant potential of *Azospirillum brasilense* and nicotinamide for hydroponic pumpkin cultivation[1]

Potencial bioestimulante de *Azospirillum brasilense* e nicotinamida para cultivo hidropônico de abóbora

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HIGHLIGHTS:
Foliar application of nicotinamide, alone or combined with A. brasilense*, increases the number of female flowers.*
Nicotinamide increases gas exchange and plant growth in a hydroponically grown pumpkin.
A. brasilense *applied via foliar is capable of doubling root volume.*

ABSTRACT: The application of biostimulants in agriculture has been studied to increase production while using smaller physical spaces and agricultural inputs to increase sustainability in production systems. Despite the promising results, there is a need to study the effects of joint applications of these products, identifying possible interactions during crop development. Thus, this study aimed to verify the biostimulant potential of the isolated and combined use of nicotinamide and *Azospirillum brasilense* during the initial growth of pumpkin plants in hydroponic cultivation. A completely randomized design with six treatments and four replicates was performed. The treatments were composed by the combination of the application of nicotinamide (foliar) and *A. brasilense* (foliar or applied in the hydroponic solution). Treatments increased the gas exchanges, particularly the assimilation rate and carboxylation efficiency, being superior to the control treatment. However, for growth characteristics, the isolated application of nicotinamide favored the development of the shoot and, when combined with *A. brasilense* via foliar, increased root growth. Thus, the application of nicotinamide and *A. brasilense* alone or together enhances the growth and gas exchanges of pumpkin plants grown in a hydroponic system, especially when using foliar application of *A. brasilense*. Whereby its action can be referred to as a biostimulant effect. Furthermore, the application method affects the efficiency and compatibility of the products applied. Therefore, foliar application is recommended.

Key words: *Cucurbita pepo* L., growth-promoting bacteria, agricultural sustainability, protected environment, B vitamin

RESUMO: A aplicação de bioestimulantes na agricultura tem sido estudada para aumentar a produção, utilizando menores espaços físicos e menos insumos agrícolas, visando aumentar a sustentabilidade nos sistemas de produção. Apesar dos resultados promissores, há necessidade de estudar os efeitos de aplicações conjuntas destes produtos, identificando possíveis interações durante o desenvolvimento da cultura. Assim, este estudo teve como objetivo verificar o potencial bioestimulante do uso isolado e combinado de nicotinamida e *Azospirillum brasilense* durante o crescimento inicial de plantas de abóbora em cultivo hidropônico. Os tratamentos foram compostos pela combinação da aplicação de nicotinamida (foliar) e *A. brasilense* (foliar ou aplicada em solução hidropônica). Foi realizado um delineamento inteiramente casualizado com seis tratamentos e quatro repetições. Os tratamentos aumentaram as trocas gasosas, principalmente a taxa de assimilação e a eficiência de carboxilação, sendo superiores ao tratamento controle. Em complemento, para as características de crescimento, a aplicação isolada de nicotinamida favoreceu o desenvolvimento da parte aérea e, quando combinada com *A. brasilense* via foliar, aumentou o crescimento radicular. Assim, a aplicação de nicotinamida e *A. brasilense* isoladamente ou em conjunto potencializa o crescimento e as trocas gasosas de plantas de abóbora cultivadas em sistema hidropônico, principalmente quando se utiliza aplicação foliar de *A. brasilense*. Desta maneira, sua ação pode ser referida como efeito bioestimulante. Além disso, o método de aplicação afeta a eficiência e compatibilidade dos produtos aplicados, recomendando-se a aplicação foliar.

Palavras-chave: *Cucurbita pepo* L., bactérias promotoras de crescimento, sustentabilidade agrícola, ambiente protegido, vitaminas B

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Introduction

Contemporary agriculture is denoted by the technological development of production processes, encompassing factors that enable greater sustainability in physical space, energy, and water resources (Costa et al., 2021). In addition to these characteristics, there is a demand for products that promote health benefits for consumers, such as bioactive foods rich in antioxidant compounds, capable of helping to prevent different diseases (Fuente et al., 2019; Wimmerova et al., 2022).

In food production, soilless cultivation systems meet the demands imposed on vegetable producers, allowing greater yield and food quality (Majid et al., 2021). Although hydroponic systems are consolidated in plant production, especially vegetables, the insertion of new management methods can contribute to further increasing crop yields.

In the context of the use of new inputs, the use of biostimulants has stood out in the cultivation of grain-producing species, such as corn (Colla et al., 2021), but has also gained prominence for application in the cultivation of horticultural species (Garcia et al., 2023; Gaiotto et al., 2023). Thereby, one of the products that stand out is made up by *Azospirillum brasilense,* which promotes plant growth, improves photosynthetic efficiency, and increases hormonal, nutritional, and pigment content, also modulating the expression of genes that promote growth, yield, and quality of vegetables, including under hydroponic cultivation (Vendruscolo & Lima, 2021; Oliveira et al., 2022; 2023).

Other compounds, such as vitamins from the B complex, have a similar growth-promoting function but are still little explored commercially (Lima et al., 2023). Among these, nicotinamide has a close relationship with the photosynthetic capacity of plants, being a constituent part of NADH (Ferreira et al., 2023). In addition, when applied exogenously, it can result in significant gains in terms of increased gas exchange activity and vegetative growth in lettuce grown in a hydroponic system, for example (Vendruscolo et al., 2023a).

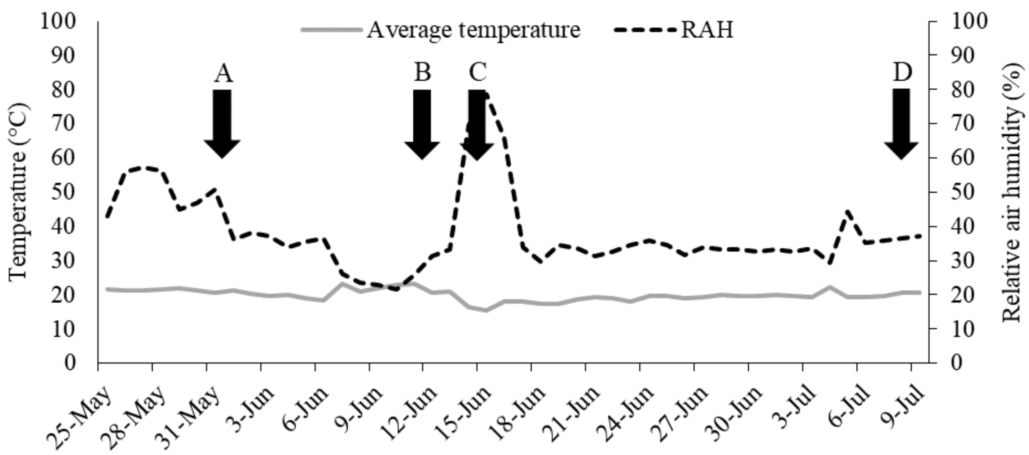
Combined effects, through the interaction of two or more products, can also provide benefits to plant development (Lima et al., 2023); however, they must be studied for different cultivation conditions and species, avoiding the possibility of incompatibility and production and financial losses. In this sense, under the hypothesis that the joint application of products with biostimulant characteristics can result in a synergistic effect, this study aimed to verify the biostimulant potential of the isolated and combined use of nicotinamide and *Azospirillum brasilense* during the initial growth of pumpkin plants in hydroponic cultivation.

Material and Methods

The experiment was performed in a greenhouse, with a dimension of 14.64 m long × 6.40 m wide × 3.5 m height, closed on the roof and side by a 150-micron low-density polyethylene film (LDPE), light, double layer, with 35% shading aluminized thermo-reflective screen (Aluminet®) under the cover film and pad/fan Humil Cool (CELDEX®) climate control system. The environment has six internal metallic benches with dimensions of 1.10 m × 5.0 m and 0.80 m high and concrete floor. The structure belongs to the Experimental Farm of the Mato Grosso do Sul State University (Universidade Estadual de Mato Grosso do Sul - UEMS), in Cassilândia, MS, Brazil (19º 05' 46" S and 51º 48' 50" W, and altitude of 521 m). The daily values of temperature and relative air humidity were recorded by a meteorological station installed inside the greenhouse (Figure 1).

Pumpkin seedlings were obtained by sowing in conical containers with a volume of 290 cm^3, filled with commercial peat substrate (CarolinaSoil®). After 12 days, the containers containing seedlings with two leaves were transferred to a hydroponic system with a static solution aerated by a set of air compressors and hoses (Figure 2). Each pot was filled with 4 L of nutrient solution for hydroponic crops (18% N, 8% P, 30% K, 15% Ca, 3% S, 3% Mg, 0.14% Fe, 0.04% B, 0.04% Mn, 0.03% Cu, 0.019% Mo, 0.006% Ni, and 0.002% Co), which was replaced weekly. The pH and electrical conductivity of the solution were regularly adjusted so that they remained at values of 6.0 ± 0.5 and 1.5 ± 0.3 mS cm^{-1}, respectively.

A completely randomized design with six treatments and four replicates was adopted. The treatments were: T1 - Control; T2 - 300 mg L^{-1} of nicotinamide applied via foliar spray; T3 - 2 mL L^{-1} of *A. brasilense* (Azototal®) applied via foliar spray (AZF); T4 - 300 mg L^{-1} of nicotinamide combined to 2 mL L^{-1} of *A. brasilense* applied via foliar spray (AZFN); T5 - 2 mL L^{-1}



A - Sowing date; B - Date of transfer of seedlings to the hydroponic system; C - Date of treatment application; D - Date of harvest and measurement

Figure 1. Average temperature and relative air humidity (RAH) inside the greenhouse during the experiment

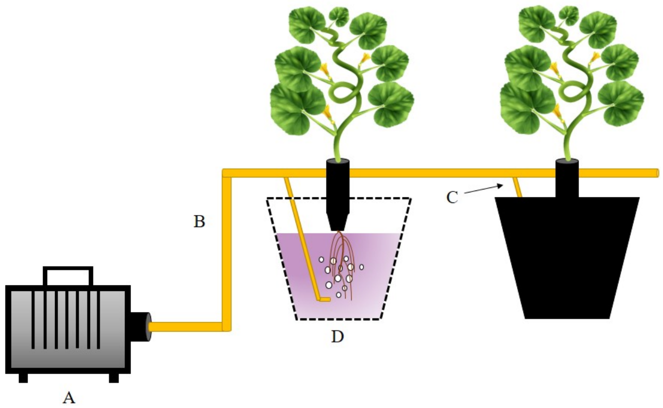


Figure 2. Aerated static solution hydroponic system, consisting of an air compressor (A), main air distribution hose (B), distribution hose per production unit (C), and containers with 4 L of nutrient solution for hydroponics (D)

of *A. brasilense* applied to the hydroponic solution (AZS); T6 - 300 mg L^{-1} of nicotinamide applied via foliar spray combined to 2 mL L^{-1} of *A. brasilense* applied to the hydroponic solution (AZSN). The foliar spray applications were conducted with a manual sprayer (2 mL $plant^{-1}$) or adding the *A. brasilense* directly to the hydroponic solution. The concentrations used were based on the literature on the application of nicotinamide (Vendruscolo et al., 2017) and *A. brasilense* (Vendruscolo & Lima, 2021) in horticultural species.

Treatments were applied two days after the seedlings transfer (DAS) to the hydroponic system. Each experimental plot consisted of a pot containing a pumpkin seedling of the cultivar Mini Jack (Isla®).

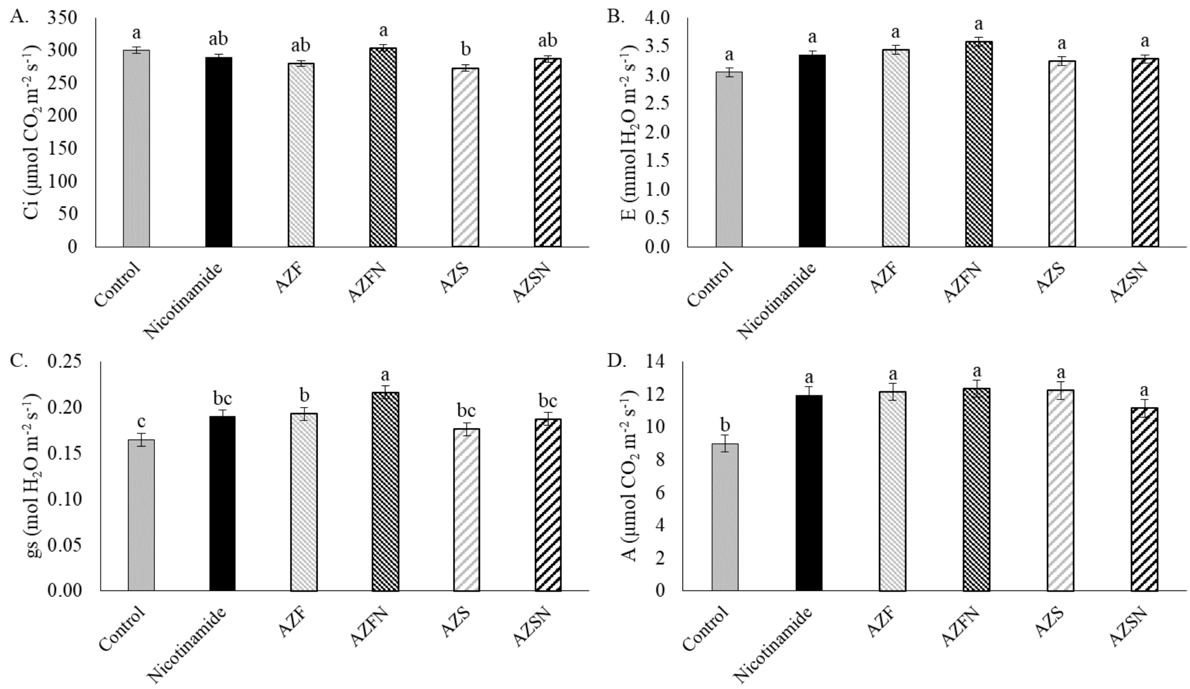
All plants were evaluated 26 days after sowing (DAS) for CO_2 assimilation rate (A), stomatal conductance (gs), intracellular CO_2 concentration (Ci), and transpiration (E). The measurements were performed between 8:00 and 10:00 a.m., when the plants were in full gas exchange activity, on a leaf from the middle portion of the plant. A portable infrared gas-analyzer (LCi, ADC Bioscientific, Hertfordshire, United Kingdom) was used for the measurements, and the parameters of the narrow-leaf cuvette (6.25 cm^2) were a flow rate of 200 µmol s^{-1}, CO_2 concentration of 440 ppm, cuvette temperature of 32 °C (ambient), and no light supplementation. In addition, water use efficiency (WUE) was calculated as the ratio between net photosynthesis and transpiration (A/E), and the intrinsic carboxylation efficiency (iCE) as the ratio between net photosynthesis and intracellular CO_2 concentration (A/Ci).

The number of leaves, leaf area, number of female flowers, plant length, stem diameter, root volume, and shoot dry weight were also evaluated. Leaf area was obtained with the Easy Leaf Area software (Easlon & Bloon, 2014). The length was measured with a graduated ruler, and the stem diameter was measured with a digital caliper in the median part of the first internode. The dry weight was obtained by drying the plants in an air-forced circulation oven at 65 °C for 72 hours and subsequently weighing them on a digital scale.

The data were subjected to preliminary tests of normality and homoscedasticity and then submitted for analysis of variance. The means were compared by the Tukey test ($p \leq 0.05$). The Sisvar software (Ferreira, 2019) was used for the data analysis. Pearson's linear correlation was performed using Excel software (2013 version).

Results and Discussion

The treatment consisting of *A. brasilense* applied to the hydroponic solution (AZS) reduced the intracellular CO_2 concentration (Ci) (Figure 3A), while for transpiration,



Bars with the same lowercase letter do not differ by the Tukey test ($p \leq 0.05$). AZF - 2 mL L^{-1} of *A. brasilense* applied via foliar spray; AZFN - 300 mg L^{-1} of nicotinamide combined with 2 mL L^{-1} of *A. brasilense* applied via foliar spray; AZS - 2 mL L^{-1} of *A. brasilense* applied to the hydroponic solution; AZSN - 300 mg L^{-1} of nicotinamide applied via foliar spray combined to 2 mL L^{-1} of *A. brasilense* applied to the hydroponic solution

Figure 3. Intracellular CO_2 concentration (Ci- A), transpiration (E- B), stomatal conductance (gs- C), and CO_2 assimilation rate (A- D) of pumpkin plants under *A. brasilense* and nicotinamide management

no difference was found among treatments (Figure 3B). In addition, the application of combined foliar *A. brasilense* and nicotinamide (AZFN) and foliar *A. brasilense* (AZF) increased stomatal conductance (gs) (Figure 3C). In contrast, all treatments composed of nicotinamide and/or *A. brasilense*, regardless of the application way, positively increased the CO_2 assimilation rate (A) (Figure 3D).

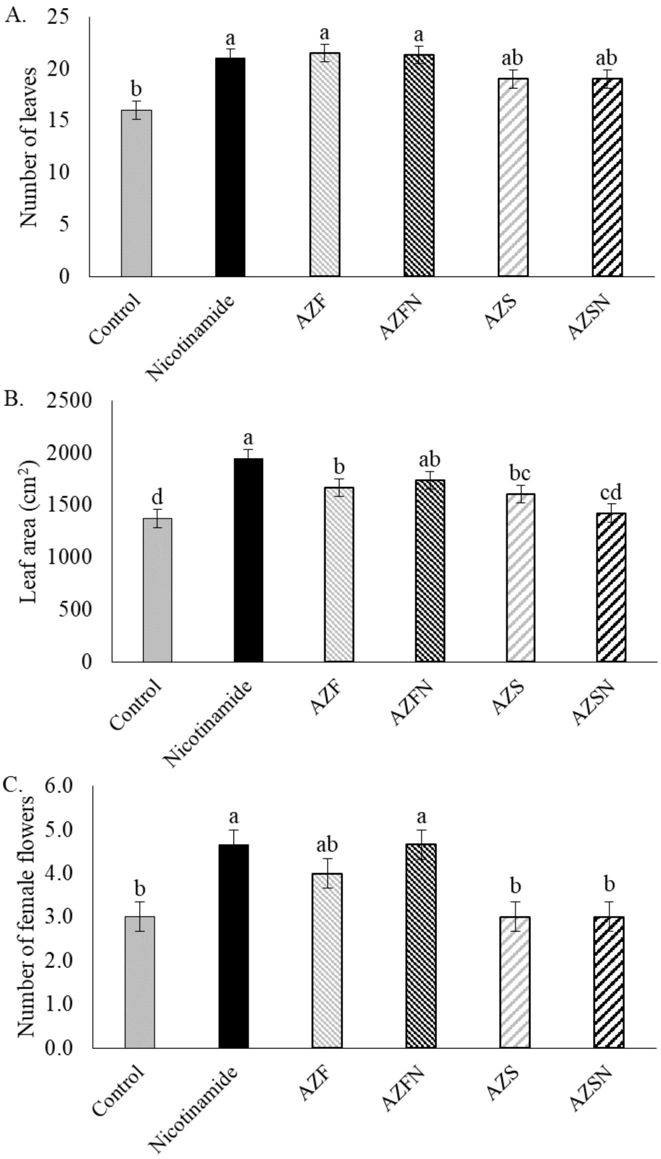
The treatments did not affect WUE (Figure 4A); however, there was a positive effect of treatments consisting of the isolated or joint application of nicotinamide and *A. brasilense*, foliar or via nutrient solution, on iCE, increasing it concerning the control treatment (Figure 4B).

The number of leaves (NL) was increased by the application of nicotinamide and *A. brasilense* via foliar, alone or combined (Figure 5A). In contrast, the leaf area (LA) was positively affected by the application of nicotinamide, followed by the AZFN, AZF, and AZS application, which were also superior to the control treatment (Figure 5B). Also, the application of nicotinamide and AZFN resulted in gains in the number of female flowers (NFF) (Figure 5C).

The application of nicotinamide alone also provided a significant increase in plant length (PL) and stem diameter (SD), not differing from the AZFN, AZS, and AZSN treatments for PL (Figure 6A) and for AZF, AZFN, AZS, and AZSN treatments for SD (Figure 6B). Root volume (RV) was also positively affected by the application of treatments. AZFN stood out from the others, followed by the treatments composed of nicotinamide, AZF, and AZS, which also differed from the control treatment (Figure 6C). The greatest increase in shoot dry weight (SDW) was provided by the application of nicotinamide alone, which did not differ significantly from the AZF, AZFN, and AZS treatments (Figure 6D).

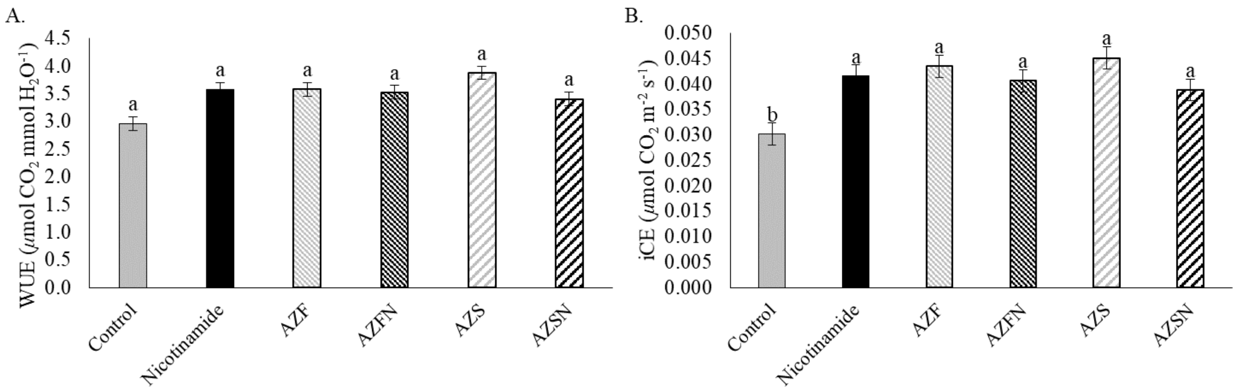
The Pearson linear correlation revealed a strong positive correlation between biometric characteristics and the CO_2 assimilation rate (A). Also, root volume (RV) was positively correlated to most characteristics, gas exchange, and growth. The variable NFF had a strong positive correlation with the NL, LA, RV, and SDW. The only negative correlations were between WUE and Ci, WUE and E, and iCE and Ci (Table 1).

Despite being widely studied for use as a sustainable technology in crops produced on a large scale, such as corn (Hungria et al., 2022), and had satisfactory results for vegetable



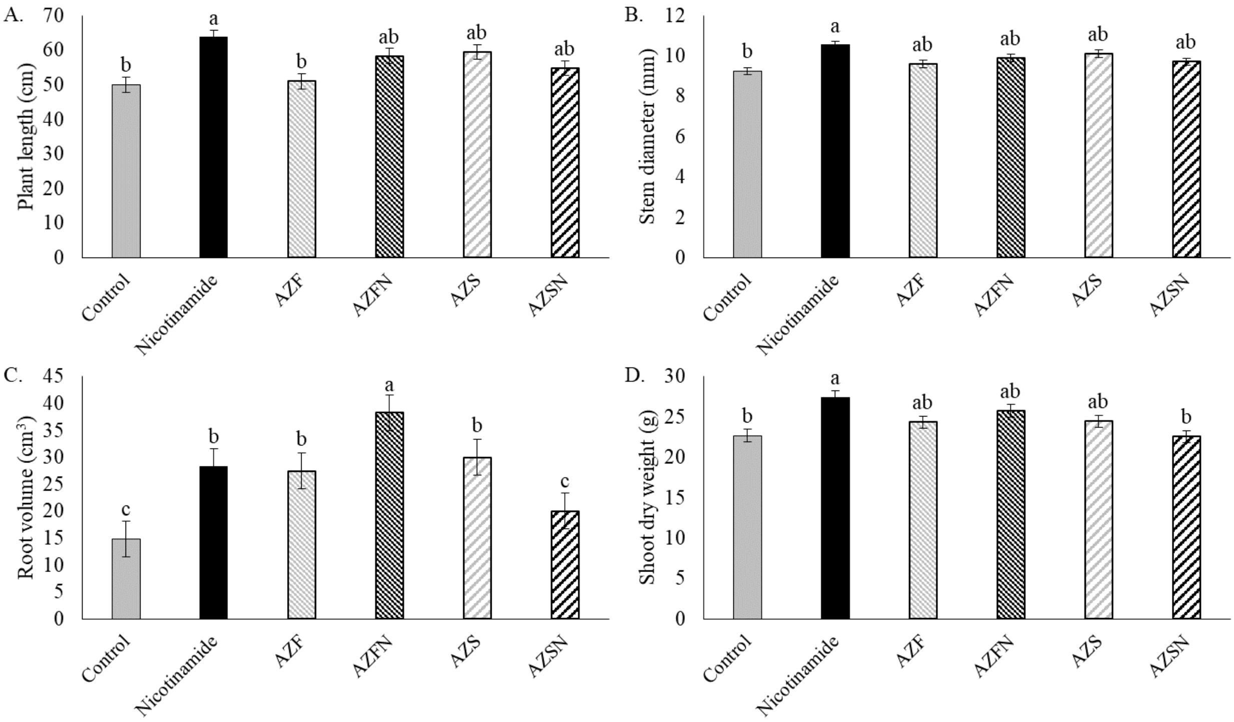
Bars with the same lowercase letter do not differ from each other by the Tukey test ($p \leq 0.05$); AZF - 2 mL L^{-1} of *A. brasilense* applied via foliar spray; AZFN - 300 mg L^{-1} of nicotinamide combined with 2 mL L^{-1} of *A. brasilense* applied via foliar spray; AZS - 2 mL L^{-1} of *A. brasilense* applied to the hydroponic solution; AZSN - 300 mg L^{-1} of nicotinamide applied via foliar spray combined to 2 mL L^{-1} of *A. brasilense* applied to the hydroponic solution

Figure 5. Number of leaves (A), leaf area (B), and number of female flowers (C) of pumpkin plants under *A. brasilense* and nicotinamide management



Bars with the same lowercase letter do not differ from each other by the Tukey test ($p \leq 0.05$); AZF - 2 mL L^{-1} of *A. brasilense* applied via foliar spray; AZFN - 300 mg L^{-1} of nicotinamide combined with 2 mL L^{-1} of *A. brasilense* applied via foliar spray; AZS - 2 mL L^{-1} of *A. brasilense* applied to the hydroponic solution; AZSN - 300 mg L^{-1} of nicotinamide applied via foliar spray combined to 2 mL L^{-1} of *A. brasilense* applied to the hydroponic solution

Figure 4. Water use efficiency - WUE (A) and carboxylation efficiency - iCE (B) of pumpkin plants under *A. brasilense* and nicotinamide management



Bars with the same lowercase letter do not differ from each other by the Tukey test ($p \leq 0.05$); AZF - 2 mL L^{-1} of *A. brasilense* applied via foliar spray; AZFN - 300 mg L^{-1} of nicotinamide combined with 2 mL L^{-1} of *A. brasilense* applied via foliar spray; AZS - 2 mL L^{-1} of *A. brasilense* applied to the hydroponic solution; AZSN - 300 mg L^{-1} of nicotinamide applied via foliar spray combined to 2 mL L^{-1} of *A. brasilense* applied to the hydroponic solution

Figure 6. Plant length (A), stem diameter (B), root volume (C), and shoot dry weight (D) of pumpkin plants under *A. brasilense* and nicotinamide management

Table 1. Estimate of Pearson's linear correlation coefficient among characteristics of pumpkin plants under *A. brasilense* and nicotinamide management

	Ci	E	gs	A	WUE	iCE	NL	NF	LA	PL	SD	RV	SDW
Ci		0.370	0.42	-0.34	-0.63	-0.66	-0.07	0.01	-0.07	0.08	-0.15	-0.02	-0.02
E	ns		0.88	0.34	-0.44	0.12	0.49	0.30	0.33	0.12	0.18	0.43	0.24
gs	*	**		0.46	-0.24	0.19	0.61	0.48	0.37	0.17	0.17	0.64	0.25
A	ns	ns	*		0.69	0.93	0.69	0.45	0.66	0.54	0.45	0.78	0.50
WUE	**	*	ns	**		0.80	0.25	0.16	0.35	0.40	0.33	0.42	0.27
EiCi	**	ns	ns	**	**		0.58	0.34	0.57	0.42	0.44	0.64	0.43
NL	ns	*	**	**	ns	**		0.65	0.71	0.45	0.28	0.71	0.66
NF	ns	ns	*	*	ns	ns	**		0.68	0.24	0.07	0.58	0.70
LA	ns	ns	ns	**	ns	**	**	**		0.71	0.63	0.67	0.88
PL	ns	ns	ns	**	ns	*	*	ns	**		0.59	0.52	0.64
SD	ns	ns	ns	*	ns	*	ns	ns	**	**		0.37	0.47
RV	ns	*	**	**	*	**	**	**	**	**	ns		0.50
DM	ns	ns	ns	*	ns	*	**	**	**	**	*	*	
					$p \leq 0.01$	$p \leq 0.05$	$p \leq 0.05$	≤ 0.01					

Ci - Intracellular CO_2 concentration; E - Transpiration; gs - Stomatal conductance; A - CO_2 assimilation rate; WUE - Water use efficiency; iCE - Carboxylation efficiency; NL - Number of leaves; NF - Number of female flowers; LA - Leaf area; PL - Plant length; SD - Stem diameter; RV - Root volume; SDW - Shoot dry weight; **, *, ns – Significant at $p \leq 0.01$ and $p \leq 0.05$, and not significant by t-test, respectively. Blue and orange colors indicate positive and negative correlations, respectively

species grown in the field (Vendruscolo & Lima, 2021), the use of bacteria in hydroponic crops requires further development in terms of management and identification of applicability in the production sector. In this sense, these findings point to the biostimulant action of *A. brasilense* applied to pumpkin plants and the optimization of the effect when this bacterium is combined with nicotinamide in foliar application.

The foliar application of *A. brasilense* resulted in a series of positive changes regarding the gas exchange capacity (Figures 3 and 4) and, consequently, the growth of shoot and roots (Figures 5 and 6) of pumpkin plants since there is a high correlation, mainly between the CO_2 assimilation rate and biometric characteristics (Table 1). These gains are driven by the action of the bacteria in association with the plant.

Bacteria of the *Azospirillum* genus are considered plant growth-promoting rhizobacteria, and their action on plants encompasses a wide range of changes, such as increasing endogenous IAA levels and nutrient absorption. These responses will positively affect growth and the number of female reproductive organs in the case of species from the Cucurbitaceae botanical family (Vendruscolo & Lima, 2021), as verified in the present study (Figures 5 and 6). This can increase yield, as seen for melon cultivation (Vendruscolo et al., 2023b).

For other species of fruit-producing vegetables, such as tomatoes, it was also found that exposure of plants to *A. brasilense* resulted in greater photosynthetic capacity, in addition to significant growth of the shoot and roots of the plants, when compared to the control treatment, which is closely related to the greater production of proteins mainly involved in the processes of photosynthesis, respiration, and organization of chloroplasts (Lade et al., 2018). In addition, for lettuce and arugula crops, under hydroponic system conditions, this bacteria significantly increased leaf nutrient content, boosting plant growth and root development (Oliveira et al., 2022; 2023).

These effects are even more noticeable when the bacteria are applied combined with nicotinamide via foliar spray, identifying a synergistic effect between the two products when applied in this way. The effect of the combined application of *A. brasilense* and nicotinamide was also verified for the coffee crop, for which there was a significant increase in growth and dry matter accumulation (Lima et al., 2023).

When the products are applied together, the positive effects of the application of *A. brasilense* are added to the biostimulant action of the vitamin, which includes an increase in photosynthetic activity (Vendruscolo et al., 2023b), stomatal functionality (Ramos et al., 2023) and leaf pigment content (Elsayed et al., 2022), resulting in higher yield of species such as corn (Colla et al., 2021) and lettuce (Vendruscolo et al., 2023a). Also, the presence of nicotinamide stimulates root development (Figure 6C) since its application favors the expression of genes related to auxin production and, consequently, root development (Ahmad et al., 2021; Laurell et al., 2022).

Nicotinamide plays a prominent role in the energy transport activities in the photosystem, participating in the constitution of $NADP^+$/NADPH, acting as an electron donor in anabolic reactions and as an electron acceptor in catabolic reactions (Ferreira et al., 2023). Its participation in the constitution of NADPH also affects the assimilation of nitrogen by plants since this coenzyme actively participates in the reduction of nitrate into ammonia (Waskell & Kim, 2015). This vitamin also influences the photosynthetic capacity of plants (Figure 3D) since many of the responses related to the transformation of light energy into chemical energy by the plant are dependent on the oxidized form of NADPH, NADP, which acts as an electron acceptor enzyme (Waskell & Kim, 2015).

Conclusions

1. The use of nicotinamide and *A. brasilense* alone or combined increases the growth and gas exchange of pumpkin plants grown in a hydroponic system, especially when using foliar application of *A. brasilense*.

2. The positive effect of nicotinamide can be described as a biostimulant.

3. The application method implies changes in the efficiency and compatibility of the products applied. Therefore, foliar application is recommended.

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