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Nicotinamide, *Azospirillum brasilense*, and a mixture of phytohormones as biostimulants in corn

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ABSTRACT

Biostimulants are products capable of improving the performance of the corn crop, resulting in higher grain yields. This study aimed to evaluate the effect of foliar application of nicotinamide, *A. brasilense*, and synthetic phytohormones applied as biostimulants in corn plants. A randomized block design with eight treatments and four repetitions was used. The treatments consisted of the isolated or combined application of nicotinamide at 800 mg L^{-1} water concentration, *Azospirillum brasilense* at a dose of 500 mL ha^{-1}, and a mixture of phytohormones at a dose of 500 mL ha^{-1}. Growth traits, yield components, and grain yield of corn were evaluated. The isolated use of the phytohormone mixture favored corn plants grown, promoting an increase of 24.3, 29.2, and 21.2% in grain mass per ear, 1000-grain weight, and grain yield, respectively. The grain yield reached with this treatment was 8155 kg ha^{-1}. The highest value for leaf chlorophyll index was obtained with the isolated use of *A. brasilense*. Nicotinamide alone and the mixture of biostimulants were unfavorable for the variables evaluated because they did not overcome the control. However, they did not harm the performance of the variables.

Keywords: *Zea mays*, Vitamins, Growth Promoter.

Nicotinamida, *Azospirillum brasilense* e mistura de fitohormônios como bioestimulantes em milho

RESUMO

Os bioestimulantes são produtos capazes de melhorar o desempenho da cultura do milho, resultando em maiores produtividades de grãos. Nesse sentido, o objetivo do trabalho foi avaliar o efeito da aplicação foliar de nicotinamida, *A. brasiliense* e fitohormônios sintéticos aplicados como bioestimulantes na cultura do milho. Foi utilizado o delineamento experimental em blocos casualizados, com oito tratamentos e quatro repetições. Os tratamentos foram constituídos pela aplicação isolada ou combinada de nicotinamida na concentração de 800 mg L^{-1} água, *Azospirillum brasilense* na dose de 500 mL ha^{-1} e mistura de fitohormônios na dose de 500 mL ha^{-1}. Foram avaliadas características de crescimento, componentes de produção e produtividade de grãos do milho. A utilização isolada da mistura de fitohormônios favoreceu a cultura do milho, promovendo aumento de 24,3, 29,2 e 21,2%, na massa de grãos por espiga, massa de mil grãos e produtividade de grãos, respectivamente. A produtividade de grãos atingida com esse tratamento foi de 8155 kg ha^{-1}. O maior valor para índice de clorofila foliar foi obtido com o uso isolado de *A. brasiliense*. A nicotinamida isolada e a mistura dos bioestimulantes não foram favoráveis para as variáveis avaliadas, porque não superaram o controle. De toda forma, não prejudicaram o rendimento das variáveis.

Palavras-chave: *Zea mays*, Vitaminas, Promotor de crescimento.

1. Introduction

The cultivation of corn (*Zea mays* L.) plays a key role in production systems in Brazil and worldwide because it is one of the main cereals produced and consumed. Corn grain is widely used in human and animal food or as a raw material in the industry (Santos et al., 2013a). The highlight scenario occupied by the crop in Brazilian agribusiness is due to the increasing production of the cereal, which can be grown in up to three harvests, with expressive production in the first two harvests, being present in much of the country, resulting in an expected production for the 2022/23 harvest of 125.1 million tons, being the summer crop (first crop) responsible for the production of 26.5 million tons (CONAB, 2023).

Several technologies and management practices are employed to increase corn grain yield, and the use of biostimulants is one of the practices that has gained some attention. Biostimulants can be defined as natural or synthetic substances from the mixture of two or more plant regulators, such as auxins, gibberellins, and cytokinins, in addition to other substances such as amino acids, vitamins, and nutrients (Galindo et al., 2016), and can be applied via seed treatment or directly to the plant (Buchelt et al., 2019). These biostimulants promote hormonal balance in plants, making them express their genetic potential. This occurs through alterations in vital and structural processes that stimulate the development of the root system (Ferreira et al., 2007), providing greater absorption of water and nutrients and favoring the development of the plant, which can result in higher grain yield.

Among the available biostimulants, nicotinamide is a water-soluble vitamin belonging to the group of B-complex vitamins known as vitamin B or niacin (Abdelhamid et al., 2013). This vitamin is a constituent of coenzymes and acts as a precursor of bioactive molecules such as nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), acting as cofactors in redox reactions important for plant metabolism (Berglund et al., 2017). Due to its participation in the pentose phosphate oxidative pathway, mitochondrial metabolism, and the photosynthetic process, nicotinamide becomes essential for plant development (Abreu et al., 2020), in addition to indirectly contributing to plant growth by performing energy transport in the plant cell (Dong et al., 2015).

In this sense, nicotinamide is considered a biostimulant when applied in small amounts, as it can cause physiological changes favorable to plant development (Dawood et al., 2019). El-Bassiouny et al. (2014) point out that nicotinamide is associated with stress signaling, promoting the regulation of secondary metabolic accumulation and/or the manifestation of plant defenses. Berglund et al. (2016) report that with the foliar application, nicotinamide is efficient for the traits that make up the yield. Another biostimulant with potential application in agriculture is bacteria from the genus *Azospirillum*. These bacteria, classified as diazotrophs, act directly in biological nitrogen fixation and, when associated with the plant rhizosphere, can contribute to nitrogen nutrition (Martins et al., 2012, Moreira et al., 2020, Barbosa et al., 2023). Among the bacteria belonging to this genus, the one with the greatest potential is *Azospirillum brasilense*, which is present in several inoculants marketed in the country (Mumbach et al., 2017).

Azospirillum can generate several stimuli, promoting plant growth through various mechanisms such as biosynthesis and release of amino acids, and production of phytohormones that favor root system growth, thus enabling greater nutrient and water uptake by the plant (Zeffa et al., 2019). *A. brasilense* releases indoleacetic acid (IAA), gibberellins, and cytokinins that promote root growth (Galindo et al., 2016) and are considered biostimulants for corn crops. The biostimulants that contain auxin, cytokinin, and gibberellin in their formulation, when applied directly to the plants, cause physiological changes because their mode of action is similar to that of plant hormones, although they are synthetic substances and are applied exogenously, each phytohormone has a function in the plant.

The auxin is responsible for promoting the formation of lateral and adventitious roots, cell elongation, and accentuating the permeability of cell membranes (Bontempo et al., 2016). The action of cytokinins is related to cell division, elongation, differentiation, and establishing drains, promoting the greater distribution of photoassimilates (Santos et al., 2013b). Gibberellins act mainly on the development of the shoot of the plants (Ferreira et al., 2007). Thus, the hypothesis was established that using different biostimulants in corn plants can improve its yield components and grain yield. The objective of the study was to evaluate the effect of foliar application of nicotinamide, *A. brasilense,* and synthetic phytohormones as biostimulants in corn plants.

2. Material and Methods

The experiment was conducted in the experimental area of the Federal University of Mato Grosso do Sul, Campus of Chapadão do Sul - MS, located at an altitude of 820 meters, with a latitude of 18°48'459" S and longitude of 52°36'003" West. The soil of this area was classified as Latossolo Vermelho distrófico (Santos et al., 2018). The climate of the region is characterized by a rainy season in summer, a dry season in winter, and an annual precipitation of 1,800 mm, with an average annual temperature between 13ºC and 28ºC (Cunha et al., 2013). The randomized block design was used, with eight treatments and four repetitions, totaling 32 plots. The treatments are on the Table 1.

Table 1. Description of the treatments.

ID	Treatment	Dose	Stage of application
1	Control	-	-
2	Stimulate® (S)	500 mL ha^{-1}	V5
3	Nicotinamide (N)	800 mg L $água^{-1}$	V5
4	*Azospirillum brasilense* (A)	500 mL ha^{-1}	V5
5	S + N	500 + 800	V5 + V5
6	S + A	500 + 500	V5 + V5
7	N + A	800 + 500	V5 + V5
8	S + N + A	500 + 800 + 500	V5 + V5 +V5

They were constituted by the isolated or combined application of nicotinamide at a concentration of 800 mg L^{-1} water, the commercial product AzoTotal max® containing *Azospirillum brasilense* 2.0 x 108 CFU mL^{-1} strain AbV5 and AbV6, at a dose of 500 mL ha^{-1}, and Stimulate® (kinetin 0.09 g L^{-1} + gibberellic acid 0.05 g L^{-1} + 4-indol-3ilbutyric acid 0.05g L^{-1}) at a dose of 500 mL ha^{-1}. The treatments were applied using a 16-liter backpack sprayer with constant pressure and a 400 L ha^{-1} spray volume.

The experimental plots consisted of five rows five meters long and spaced 0.45 m apart. The three central rows were used in the evaluations. The hybrid used was NK 505 VIP3.

The experiment was conducted in a no-tillage system from December 17, 2020, to April 20, 2021. Before the installation of the experiment, soil sampling was performed in the 0.0-0.20 m layer, and the results of chemical analysis were: pH ($CaCl_2$) = 5.3; P ($Mehlich^{-1}$) = 10.8; K = 62; S = 4.2; B = 0.16; Cu = 1.4; Fe = 50; Mn = 15.2, and Zn = 5.7 (all in mg dm^{-3}); Ca = 2.20; Mg = 0.50; H+Al = 5.4, and CEC = 8.3 (all in $cmol_c$ dm^{-3}); Base saturation = 34.6% and Organic matter = 29 g dm^{-3}. The particle size analysis evidenced the following results: clay = 52.5%, silt = 5%, and sand = 42.5%. About 30 days before sowing, 3.3 t ha^{-1} of dolomitic limestone (ECCE=90%) was applied on the surface, without incorporation, to correct soil acidity, aiming to raise the base saturation to 70%.

Sowing was performed on December 17, 2020, with a tractor-driven seven-row seed drill. Along with the sowing, triple superphosphate fertilizer at 222 kg ha^{-1} (100 kg ha^{-1} of P_2O_5) was applied in the sowing furrow. Topdressing fertilization was performed when the corn plants were at the V5 stage, using 80 kg ha^{-1} of K_2O and 180 kg ha^{-1} of N. Potassium chloride and urea were used as sources of potassium and nitrogen, respectively.

During the development of the crop, treatments to control weeds, pests, and diseases were performed. At 15 days after sowing (DAS) was applied 1 L e.a. ha^{-1} of glyphosate potassium, and 1 L a.i. ha^{-1} of Atrazine for weed control. Pest control was done by applying Thiamethoxan (35.3 mL a.i. ha^{-1}) + Lambda-cyhalothrin (26.5 mL a.i. ha^{-1}) at 7, 15, and 24 DAS. Disease control was carried out three times: at V7 (seven expanded leaves) with an application of Azoxystrobin (70 mL a.i. ha^{-1}) + Cyproconazole (28 mL a.i. ha^{-1}); at R1 (one or more silks extends outside of husk leaves), with an application of Difenoconazole (75 mL a.i. ha^{-1}) + Cyproconazole (45 mL a.i. ha^{-1}) and Azoxystrobin (90 mL a.i. ha^{-1}) + Benzovindiflupir (45 mL a.i. ha^{-1}) and 15 days after R1, with an application of Azoxystrobin (70 mL a.i. ha^{-1}) + Cyproconazole (28 mL a.i. ha^{-1}) and Propiconazole (87.5 mL a.i. ha^{-1}) + Difenoconazole (87.5 5 mL a.i. ha^{-1}).

At the stage of corn tasseling, plant height (PHG), first ear insertion height (FEIH), and stem diameter (SD) were evaluated. The height was determined from the base of the plant to the end of the stalk for PHG and from the base to the insertion of the first ear for FEIH with the aid of a graduated ruler. The diameter of the stem was measured with a digital caliper positioned above the adventitious roots from the base of the plant. PHG, FEIH, and SD data were obtained from the evaluation of ten plants in each plot.

The relative chlorophyll index (RCI), determined with a digital chlorophyll meter CFL 1030 (Falker, Porto Alegre, RS), was also measured during the corn tasseling. The measurements were taken on the insertion leaves of the last ear, on five leaves per plot from five different plants, and the evaluation was taken at the median portion of the leaf.

At full maturity, the length (LE) and diameter (DE) of the ear were evaluated with a digital caliper. The number of grain rows per ear (NRE), the number of grains per row (NGR), and the grain mass per ear (GME) were evaluated in three ears. The 1000-grain weight (1000W) was obtained from counting and weighing one thousand grains per plot. The grain yield (YIEL) was obtained from the harvest and threshing of all plants in the useful area of each plot, with the correction of grain humidity to 13%.

The data obtained were subjected to analysis of variance, and the means were grouped by the Scott-Knott test at the 5% probability level. The Sisvar software (Ferreira, 2019) was used for the data analysis.

3. Results and Discussion

The analysis of variance (Table 2) shows that only the variables plant height, grain mass per ear, 1000-grain weight, relative chlorophyll index, and grain yield were influenced by the application of treatments. Regardless of which biostimulant and its combinations were used, the first ear insertion height was not different from the control treatment, except for the combination Stimulate® + nicotinamide + *A. brasilense*, where this variable was lower (Figure 1).

One should pay attention to this variable because plants with very high ear insertion heights are more prone to breakage or toppling, besides needing the harvesting platform to be adjusted higher. It can be seen in the experiment that the S+N+A treatment resulted in an average reduction of 9.0 cm compared to all other treatments and the control. This phytohormone, because it has the same mode of action as plant hormones (Bontempo et al., 2016), usually results in increased plant growth (Fancelli, 2013) and, consequently, results in increased first ear insertion height. However, in the present experiment, this gain was not observed for plant height (Table 2) nor the first ear insertion height (Figure 2). The use of Stimulate® alone provided an average gain of 24.3% in grain mass per ear compared to the other treatments and control (Figure 2). The synthetic phytohormones present in this product have the same function as plant phytohormones (Bontempo et al., 2016), which can lead to beneficial physiological changes in the plant and, as a result, the increase of some yield components and grain yield (Fancelli, 2013).

Table 2. Summary of analysis of variance for plant height (PHG), first ear insertion height (FEIH), stem diameter (SD), number of grain rows per ear (NRE), number of grains per row (NGR), grain mass per ear (GME), 1000-grain weight (1000W), ear length (LE), relative chlorophyll index (RCI), and grain yield (YIEL).

Source of Variation	GL	Mean Square of the Residue				
		PHG	FEIH	SD	NRE	NGR
Block	3	0.0022	0.0093	0.0002	0.1981	0.5916
Treatments	7	0.0029^{ns}	0.0059^{*}	0.0001^{ns}	0.7925^{ns}	2.0219^{ns}
Error	21	0.0023	0.0024	0.0001	0.6305	3.0264
CV (%)		2.00	4.60	5.28	5.01	5.26
		GME	1000W	LE	RCI	YIEL
Block	3	0.0007	0.0004	0.3289	0.5307	$1.46x10^{4}$
Treatments	7	0.0009^{**}	0.0049^{**}	0.7789^{ns}	5.0430^{**}	$1.7351x10^{6**}$
Error	21	0.0002	0.0002	0.3476	0.7152	$1.0111x10^{5}$
CV (%)		8.91	4.05	3.52	1.44	4.59

* and ** Significant at 5% and 1% probability level, respectively, by Skott Knott test.

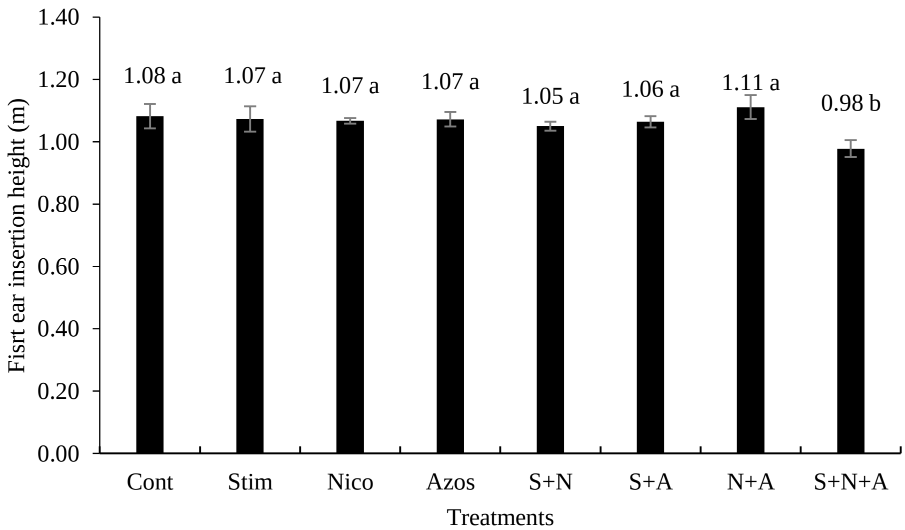

Figure 1. First ear insertion height (FEIH) of corn plants according to the biostimulant treatments and their combinations. Cont = control; Stim = Stimulate®; Nico = nicotinamide; Azos = *Azospirillum brasilense*; A= *Azospirillum brasilense*; N = nicotinamide; S = Stimulate®. Bars indicate the standard deviation.

The use of Stimulate® alone was also responsible for a 12.5% increase in 1000-grain weight compared to the Azos, S+A, and N+A treatments. Compared to the control, this gain was 29.2% (Figure 3). The gains observed for 1000-grain weight are probably justified by the same reasons observed for the increase in grain mass per ear. These results disagree with those observed by Dourado Neto et al. (2014), who did not obtain an increase in the 1000-grain weight using this biostimulant.

The use of *A. brasilense* provided a 6.5% increase in the relative chlorophyll index in corn plants compared to the control (Figure 4). According to Galindo et al. (2016), *Azospirillum* inoculation causes improvement in leaf photosynthetic parameters, including the chlorophyll index in the leaf, probably due to the ability that this bacterium has in biological nitrogen fixation. Kappes et al. (2013) observed that inoculation of *A. brasilense* in corn promoted a higher relative leaf chlorophyll index which can be attributed to the biostimulant action, possibly increasing the ability of plants to absorb nutrients and fix nitrogen.

The use of Stimulate®, Stimulate®+ *Azospirillum*, and Nicotinamide + *Azospirillum* was also able to improve the RCI, on average 4.0% higher than the value obtained in the control treatment (Figure 4).

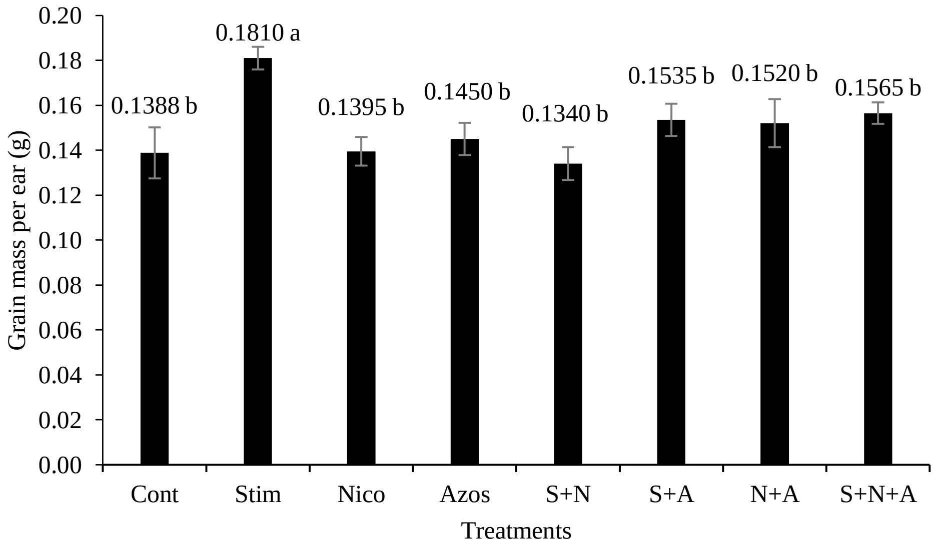

Figure 2. Grain mass per ear (GME) of corn plants according to the biostimulant treatments and their combinations. Cont = control; Stim = Stimulate®; Nico = nicotinamide; Azos = *Azospirillum brasilense*; A= *Azospirillum brasilense*; N = nicotinamide; S = Stimulate®. Bars indicate the standard deviation.

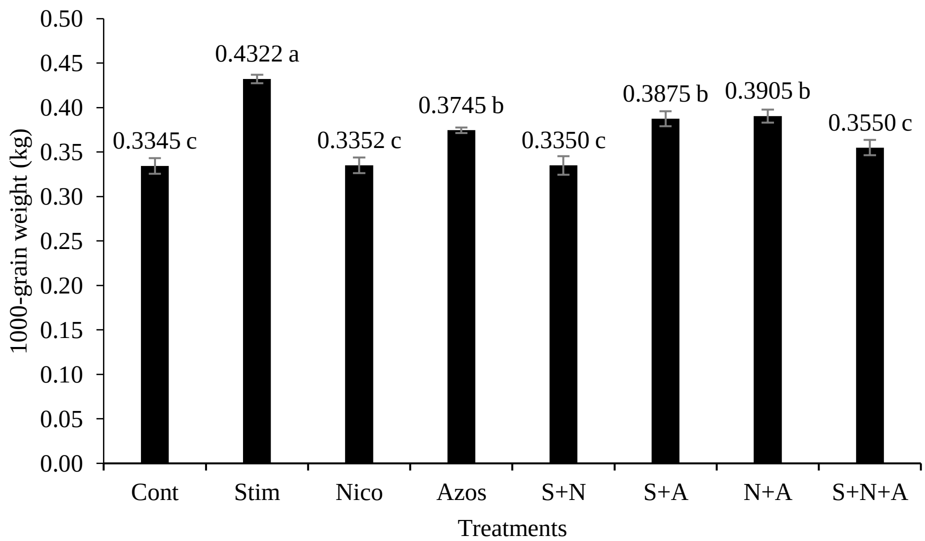

Figure 3. 1000-grain weight (1000W) of corn plants according to the biostimulant treatments and their combinations. Cont = control; Stim = Stimulate®; Nico = nicotinamide; Azos = *Azospirillum brasilense*; A= *Azospirillum brasilense*; N = nicotinamide; S = Stimulate®. Bars indicate the standard deviation.

The treatment with Stimulate® promoted an increase in grain yield of 6.3% compared to the second-best treatment, *Azospirillum*, and 21.2% compared to the control (Figure 5). This is probably justified by the fact that the phytohormone promotes an increase in plant metabolism, intensifying photosynthesis and increasing the uptake of nutrients present in the soil (Yakhin et al., 2017). It is possible to verify the potential use of *A. brasilense* that obtained a grain yield 14.0% higher than the control. The use of nicotinamide and the mixtures of biostimulants were not beneficial for improving corn grain yield. However, it is important to consider that the other biostimulants or their mixtures can contribute to the corn crop when applied in other dosages or forms of use, indicating potential use.

For example, Colla et al. (2021) obtained higher corn grain yields when they applied nicotinamide at a concentration of 96.65 mg L^{-1}. No studies with these biostimulant mixtures are yet found in the literature to justify this study. Regarding the isolated use of nicotinamide in corn, the literature has always shown a positive effect on grain yield, so it is possible that the dose was a determinant factor in the study, indicating that further studies need to be done with the use of vitamins considering different doses, plant species, environments, and form of use.

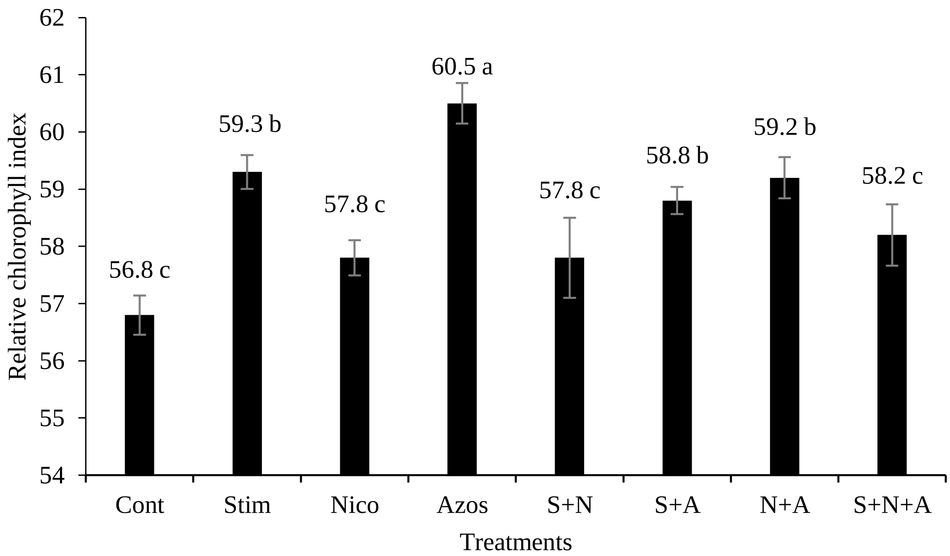

Figure 4. Relative chlorophyll index (RCI) of corn plants according to the biostimulant treatments and their combinations. Cont = control; Stim = Stimulate®; Nico = nicotinamide; Azos = *Azospirillum brasilense*; A= *Azospirillum brasilense*; N = nicotinamide; S = Stimulate®. Bars indicate the standard deviation.

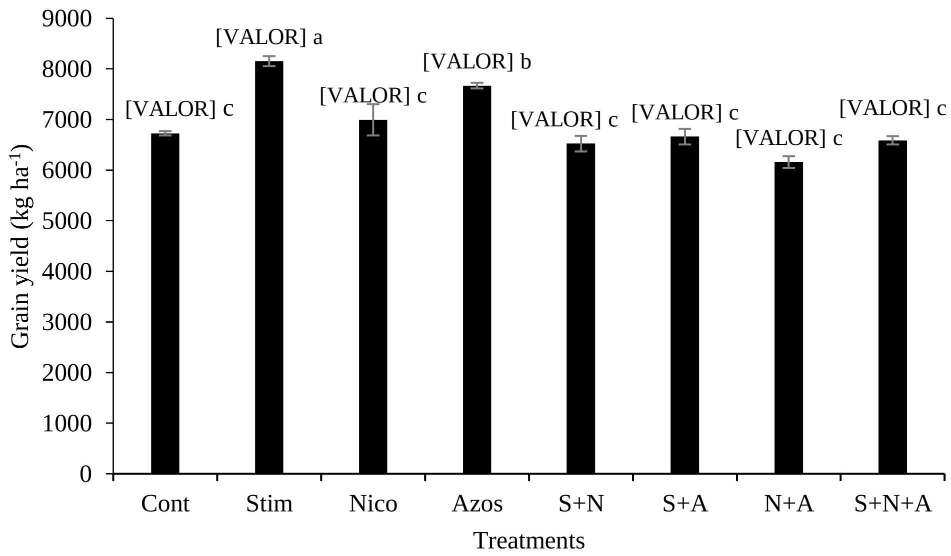

Figure 5: Corn grain yield according to the biostimulant treatments and their combinations. Cont = control; Stim = Stimulate®; Nico = nicotinamide; Azos = Azospirillum brasilense; A= Azospirillum brasilense; N = nicotinamide; S = Stimulate®. Bars indicate the standard deviation.

4. Conclusions

The isolated use of Stimulate® favored corn plants grown, promoting increased grain mass per ear, 1000-grain weight, and grain yield. The highest value for the relative chlorophyll index was obtained with the isolated use of *A. brasilense*. Nicotinamide alone and the mixture of biostimulants were unfavorable for the variables evaluated because they did not overcome the control. In any case, they did not harm the performance of the variables.

Authors' Contribution

Augusto Henrique Pires Gaiotto and Sebastião Ferreira de Lima contributed to the organization of the research, execution of the experiment, data collection, statistical analysis of the data, interpretation of the results, writing of the manuscript, and final correction of the manuscript. Eduarda Samara dos Santos, Lincoln Lara Ferreira, Guilherme Slaviero, and Eduardo Pedroso Ferreira also contributed to organizing the experiment, interpreting the results, and final correction of the manuscript.

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