

Maize Yield Under Fertilization With Bio-Stimulants, Sulfur, and Zinc in the State of Paraíba, Brazil

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Abstract

Maize (*Zea mays*) is a cereal widely used in human and animal feeding worldwide, with great socioeconomic importance. This study aimed to evaluate the growth, productivity, and absorption of macro and micronutrients of maize under the application of biostimulant, sulfur, and zinc in a Gleisolo Háplico soil in a microclimate region of the semiarid state of Paraíba, Brazil. The experiment was carried out in a complete randomized block design, a 2x2x2 factorial scheme with eight treatments, and four replications was used. The first factor corresponded to treatment with biostimulant (Presence and absence); the second to sulfur (Presence and absence); and the latter to zinc (Presence and absence). Growth, yield, and macro and micronutrient absorption data were evaluated. Data were submitted to analysis of variance and the means compared by the Tukey test at 5% probability. The number of rows per ear and ear diameter responded positively when the biostimulant was used. Maize under foliar fertilization with zinc nitrate did not show yield increase. The application of sulfur promoted an increase in almost all variables, including yield. The combined application of the three products influenced only the number of rows per ear. The application of sulfur separated or together with biostimulant provided an increase in the absorption of some nutrients by the leaf such as copper, N and K. As a result of fertilization with sulfur, which provided an increase in leaf absorption, mainly of N, also promoted a significant increase in all student variables, including grain productivity.

Keywords: biostimulant, maize crop, plant nutrition, sulfur fertilization

1. Introduction

Maize (*Zea mays*) is a cereal widely cultivated and used in human and animal consumption worldwide, with great socio-economic importance, and characterized as a subsistence crop by most producers (Silva & Silva, 2017).

In Brazil, this crop is one of the main economic sources of agribusiness, making it the second most exported grain (Souza et al., 2018). The biggest maize producers are the United States, China, and Brazil, with a potential production of approximately 563 out of 717 million tons/year (Prestes et al., 2019). In Brazil, the higher productions are concentrated in the state of Paraná, responsible for 14.69% of the maize produced, with a production of 34.9 million tons, according to data from the 2017/18 harvest, and the state of Mato Grosso with 32.11% of the total maize produced (Conab, 2019; Embrapa, 2016).

In the state of Paraíba, maize is mainly cultivated by small producers, where yields are very low due to little or no technology employed, in addition to the factors that affect the development of this crop, which depends on the region conditions, such as, precipitation, temperature, and evapotranspiration, that can significantly affect the physiological activities of the crop (Francisco et al., 2017).

According to data from Conab, the state of Paraíba occupies 109 thousand hectares of cultivated area, with an estimated production of 56 thousand tons, which still represents a small production in the grain scenario in Brazil (Conab, 2019).

With the increase in its exploitation by producers, the adoption of new technologies such as the use of biostimulants, stands as a good option to optimize production in different crops (Cunha et al., 2016). The application of biostimulants can morphologically change the plant organs, in which their growth and development are inhibited, in a way that meristematic activity is influenced, resulting in a more uniform formation and harvest of the seeds (Jesus Rodrigues et al., 2020).

The use of sulfur (S) in maize is a great ally in its absorption capacity, it acts positively on the plant metabolism, as component of amino acids, proteins, molecules of the chloroplast, and other diverse metabolic functions (Mendes et al., 2014).

Also, the use of zinc (Zn) stands out as one of the micronutrients with the highest responses of grain yield in maize, and if it is not in optimal concentrations in the soil, the crop responds negatively on its growth and yield due to the important functions played by this micronutrient (Luo et al., 2010).

There are some reports in the literature of the use of mixtures between biostimulants and nutrients, such as the publication by Santana et al. (2017) with mixtures of auxin + gibberellin + cytokinin and combined with N + B + Cu + Mo + Zn and significant effects on broccoli crop. Zeist et al. (2018) evaluated the application of boron, calcium, and plant regulators (separately and together) in pepper and obtained superior values for commercial fruits using the plant regulator Stimulate® when applied in combination with boron + calcium and boron. Santos et al. (2020) worked with biostimulant and a nutrient complex separately and together in soybean seeds and obtained significant effects in radicle length, stem diameter, and dry root mass when used the biostimulant and the nutrient complex together. However, no reports of biostimulants and nutrient combinations in the maize crop are found. For this reason, this work is relevant to obtain information on the positive effects of these mixtures in maize, which will be highlighted in the present research.

It is important to note that the rates of nutrient extraction by plants can change depending on the cultivar used, the environment and the management of fertilization, however, the quantities exported per ton of grains are moderately similar in various conditions (Resende et al., 2018).

Hence, the use of biostimulants and micronutrients, as an agronomic tool to increase yield in different crops, has grown in recent years. This study aimed to evaluate the growth and yield of maize under the application of biostimulant, sulfur and zinc in a Gleissolo Háplico soil in a microclimate region of the semiarid state of the Paraíba state in Brazil.

2. Method

The experiment was carried out in the municipality of Areia, in an experimental area of the Centro de Ciências Agrárias (CCA) of the Universidade Federal da Paraíba (UFPB), the soil of the experimental area is classified as Gleissolo Háplico, with sandy texture in the 0-20 cm layer, with 820 kg⁻¹ of sand, 96 kg⁻¹ of silt and 84 kg⁻¹ of clay. The soil chemical properties were analyzed at the Laboratório de Química e fertilidade do Solo of the CCA at UFPB and are shown in Table 1.

Table 1. Chemical properties of the soil, in the 0 to 20 cm layer of the experimental area

Chemical properties									
pH	P	S-SO ₄ ⁻²	K ⁺	H ⁺ +Al ⁺³	Al ⁺³	Ca ⁺²	Mg ⁺²	SB	CTC
H ₂ O(1:2,5)	-----mg/dm ³ -----			-----cmol _c /dm ³ -----					
6.2	75.35	2.89	67.03	0.04	0.00	2.00	1.32	3.53	5.71
M.O.	Zn	Fe	Mn	Cu	B				
--g/kg--	-----mg/dm ³ -----								
4.36	0.93	3.42	3.25	0.04	0.50				

P, K, Na: Mehlich 1 Extractor

SB: Sum of exchangeable bases

H + Al: Calcium Acetate Extractor 0,5 M, pH 7,0

CTC: Cation Exchange capacity

Al, Ca, Mg: KCl 1 M Extractor

M.O.: Organic Matter – Walkley-Black

The climate of the region is classified as As' climatic subtype, this corresponds to the subhumid tropical climate (hot humid, with autumn-winter rain) (Kottek et al., 2006). The variation in precipitation, maximum and minimum temperature, insolation, and humidity relative air are shown in Figure 1.

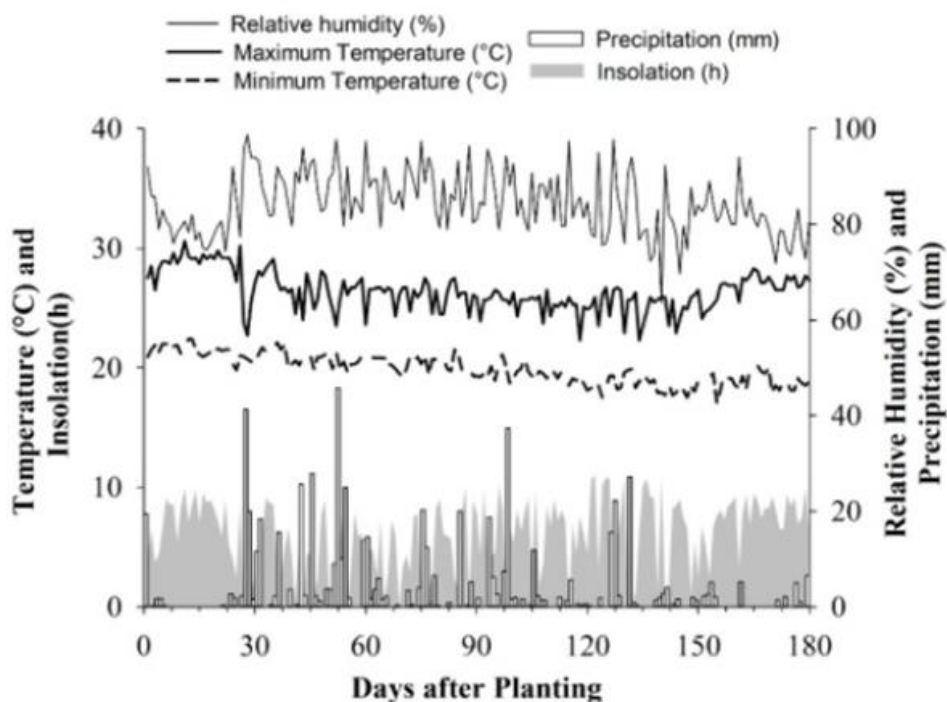


Figure 1. Precipitation, maximum and minimum temperature, insolation, and relative humidity in the municipality of Areia, PB during the experimental period with maize under field conditions (Inmet, 2020)

A randomized block design experimental design was used, in a 2x2x2 factorial scheme with 8 treatments, and four replications (Table 2). The first factor corresponded to the biostimulant (Presence and absence); the second to sulfur (Presence and absence); and the latter to zinc (Presence and absence).

Table 2. Treatments composition used in the experiment

Treatment	Biostimulant	Sulfur	Zinc
T1	1500 mL / 100 kg		
T2			400 g
T3		20 kg	
T4		20 kg	400 g
T5	1500 mL / 100 kg		400 g
T6	1500 mL / 100 kg	20 kg	
T7	1500 mL / 100 kg	20 kg	400 g
T8			

Source: Author data.

The plots were composed of 4 lines of 5 meters in length, spaced 0.50 m (10 m² per plot) apart. The two central lines were used in the evaluations, the first three plants on the plot edges were not considered in the evaluations (total useful area of 6 m²).

The sowing occurred manually, the spacing of 0.50 m between rows and 0.33 m between lines was used, and two seeds were sown at 3 to 4 cm depth (3 seeds per linear meter). Twenty days after planting, thinning was carried out, and one plant per hole was left (population of 60,600 plants per hectare).

The maize hybrid DKB 310-PRO2 (Dekalb) was used, a transgenic cultivar (simple hybrid) with 870 degrees days and planting recommendation of 60 to 65 plants per square meter, resistant to Bt pests and glyphosate, semi dent kernels, good resistance to lodging, average ear insertion of 1.25 m, plant height of 2.20 m.

Basal potassium fertilization was performed (approximately 10 cm deep), along with 30% of the nitrogen. The remaining nitrogen was applied at 30 and 45 days after sowing in a side-dressing way, without incorporation.

Urea was applied and adjusted in the plots that received zinc nitrate at the dose of 371 kg ha⁻¹; potassium chloride at the dose of 192 kg ha⁻¹; and the source of phosphorus was the residue present in the area according to soil analysis. Foliar fertilization was performed at the doses of 2 L ha⁻¹ of zinc nitrate (400 g ha⁻¹ of Zn) and 25 kg ha⁻¹ of fungicide with 80% S in its composition (no registered for the crop) at the V5 stage.

The biostimulant contains kinetin - 0.09 g / L, gibberellic acid GA3 - 0.05 g/L, and 4-indole-3-butyl acid - 0.05 g/L and was applied via seeds in the dose of 1500 mL for 100 kg of seeds.

Measurements were performed at 50 days after sowing (DAS) to monitor plant growth on the following variables:

Plant height (PH) - measured with the aid of a 5-meter measuring tape, measured from the ground to the upper end of the plant;

Stem diameter (SD) - measured with the aid of a 150 mm Stainless Hardened digital caliper, measured at the second internode;

Number of leaves (NL) - done manually in the useful plot area in 5 plants and calculated the arithmetic mean between them;

Leaf area (LA) - Measured in the leaves from the middle third height of the plant in a non-destructive way using the formula $CF \times LF \times \text{factor}$ (0.7458), where CF is the leaf length and LF is the leaf width;

Flag leaf area (FLA) - Made similarly to the Leaf Area using the same formula and measuring the length of the flag leaf (LFL) and the width of the flag leaf (WFL) at 106 days after sowing.

The harvest occurred at 134 days after sowing, and the ears of the plants marked at 30 DAP

were harvested manually. Evaluations of ear length (EL), ear diameter (ED), number of rows per ear (NRE), number of kernels per row (NKR), and grain yield (YLD) (kg ha^{-1}) were calculated.

The ears were harvested within the useful plot area and left to dry under direct sunlight for 72 hours, after this period the weight of individual ears was obtained. The ear length was measured with a ruler and the ear width with a caliper. The row number and the number of kernels per ear were obtained through manual counting. The yield was made based on the grain weight per ear and multiplied by 60,000 ears.

The leaf samples were dried in an oven and ground in a Wiley mill. The samples were packed in properly identified paper bags for the determination of macro and micronutrient contents.

Analysis of variance was performed by the F test for the variables of growth, yield, and leaf analysis, the means were compared by the Tukey test at the 0.05 level of significance. The statistical software R 4.02 (R Core Team, 2020) was used.

3. Results and Discussion

No significant differences were observed in the analysis of variance in the triple interaction (Biostimulant x Zinc x Sulfur) at 5% probability for Plant Height (PH), Stem Diameter (SD), Leaf Area (LA), Flag Leaf Area (FLA), Ear Height (EH), Number of Leaves (NFO), Ear Length (CME), Ear Diameter (DE), Number of kernels per Row (NKR) and yield (YLD), except for Number of Rows per Ear (NFE) (Table 3). Also, there no significant differences for the double interaction (Zinc and Sulfur) were observed in all variables, except for the Number of kernels per Row (NKR).

Table 3. Summary of the analysis of variance of plant height (PH), stem diameter (SD), leaf area (LA), flag leaf area (FLA), ear height (EH), number of leaves (NOL), ear length (EL), ear diameter (ED), number of rows per ear (NRE), Number of kernels per row (NKR) and yield (YLD) under doses (presence and absence) of Biostimulant (Bio), zinc (Zn) and sulfur (S)

VS	DF	Medium squares					
		PH	SD	LA	FLA	EH	NOL
Block	3	78,20 ^{NS}	0,0317 ^{NS}	16971,6 ^{NS}	290,8 ^{NS}	73,00 ^{NS}	0,19125 ^{NS}
Biostimulant (Bio)	1	24,40 ^{NS}	0,0210 ^{NS}	3818,0 ^{NS}	11,3 ^{NS}	77,50 ^{NS}	0,10125 ^{NS}
Zinc (Zn)	1	11,59 ^{NS}	0,1128 ^{NS}	4230,7 ^{NS}	334,6 ^{NS}	172,05*	0,03125 ^{NS}
sulfur (S)	1	519,87*	0,0300 ^{NS}	2809,7 ^{NS}	5639,0**	778,15**	1,71125**
Bio x Zn	1	143,74 ^{NS}	0,0120 ^{NS}	10025,5 ^{NS}	848,1 ^{NS}	3,78 ^{NS}	0,28125 ^{NS}

Bio x S	1	61,99 ^{NS}	0,0006 ^{NS}	1240,7 ^{NS}	388,9 ^{NS}	45,60 ^{NS}	0,15125 ^{NS}
Zn x S	1	178,89 ^{NS}	0,0105 ^{NS}	942,7 ^{NS}	205,8 ^{NS}	11,28 ^{NS}	0,55125 ^{NS}
Bio x Zn x S	1	234,69 ^{NS}	0,0325 ^{NS}	4219,9 ^{NS}	22,5 ^{NS}	37,41 ^{NS}	0,15125 ^{NS}
Resíduo	21	91,67	0,0334	3662,5	327,2	35,83	0,20649
CV %		12,74	8,4	20,9	13	9,2	4,8

VS	DF	Medium squares				
		EL	ED	NRE	NKR	YLD
Block	3	3,127 ^{NS}	0,13508 ^{NS}	3,5760 ^{NS}	10,302 ^{NS}	2291390 ^{NS}
Biostimulant (Bio)	1	7,078 ^{NS}	0,64695 ^{**}	11,2812 ^{**}	24,500 ^{NS}	4533913 ^{NS}
Zinc (Zn)	1	1,182 ^{NS}	0,01320 ^{NS}	1,1250 ^{NS}	2,000 ^{NS}	825383 ^{NS}
sulfur (S)	1	76,416 ^{**}	0,39383 [*]	0,0000 ^{NS}	288,000 ^{**}	17079072 [*]
Bio x Zn	1	0,272 ^{NS}	0,00195 ^{NS}	0,5000 ^{NS}	2,531 ^{NS}	187494 ^{NS}
Bio x S	1	5,080 ^{NS}	0,13133 ^{NS}	1,1250 ^{NS}	16,531 ^{NS}	567985 ^{NS}
Zn x S	1	2,284 ^{NS}	0,05695 ^{NS}	0,2813 ^{NS}	42,781 [*]	3458825 ^{NS}
Bio x Zn x S	1	0,463 ^{NS}	0,00195 ^{NS}	7,0313 [*]	32,000 ^{NS}	43824 ^{NS}
Resíduo	21	2,319	0,06032	1,0283	9,510	2275845
CV %		9,5	4,4	5,8	9,5	18,5

^{**}Significant at 1% probability; ^{*} significant at 5% probability and ^{NS} by the Snedecor F test.

For the double factorial interaction (Biostimulant x Zinc and Biostimulant x Sulfur), no significant effect was observed for the studied variables. For the isolated biostimulant factor, a significant difference was observed only for the variables Ear diameter (ED) and Number of rows per ear (NRE). For Zinc (Zn), a significant effect was observed only for Ear Height (EH). For Sulfur (S), a significant effect for the Flag Leaf Area (LFA), Ear Height (EH), Number of Leaves (NOL), Ear Length (EL), and Number of Kernels per Row (NKR) were observed at 1%

probability. Significant effects were also observed on Plant Height (PH), Ear Diameter (ED), and Yield (YLD) at 5% probability.

For the triple interaction (Biostimulant x Sulfur x Zinc) (Table 4), Zn within S and Bio, a significant difference was observed only for 20 Kg of Sulfur + 1500 mL of biostimulant treatment in the dose of 0g of zinc. Thus, when applying zinc, under these conditions, it can be inferred that it caused a reduction in the number of rows per ear. This was probably due to the fact that the biostimulant used in the experiment contains indole butyric acid (auxin) 0.005%, kinetin (cytokinin) 0.009%, and gibberellic acid (gibberellin) 0.005%. The adequate hormonal balance of auxin and cytokinin are very important for the best development of plants (Taiz et al., 2017), however, when Zn was applied, a hormonal imbalance may have occurred, affecting the synthesis of tryptophan, a precursor amino acid for the biosynthesis of this hormone. Zn immobilization may also have occurred due to this imbalance since cytokinin is responsible for mobilization, a well-known function of this hormone (Taiz et al., 2017). Similar results were found by Lima et al. (2020), where they found an increase in ear length with the use of biostimulant, but not for the number of rows per ear.

Table 4. Number of rows per ear in the Bio x S x Zn triple interaction

		Bio (mL)	Zn (g)	
			0	400
S (Kg)	0	0	13.9 a A α^*	13.0 a B α
	0	1500	14.8 a A β	15.2 a A α
	20	0	13.5 a A β	14.1 a A α
	20	1500	15.5 a A α	13.8 b A α

* Means followed by the same letter are not statistically different, the lower case letter between columns compares Zn within S and Bio; uppercase letter between lines compares S with Bio and Zn; and Greek letters, between columns, compares Bio with Zn and S.

In addition, it can be observed that for Sulfur within Bio and Zn interaction, all treatments had higher values than those observed on treatments under the dose of 0 kg of S and 0 mL of biostimulant for the dose of 400 g of Zn. This may have occurred due to the interaction between these nutrients and the biostimulant. Thus, these results can be explained based on the influence of these nutrients on the plant, essential for its development, as an alternative to support the crop in overcoming abiotic stresses, acting as a hormonal and nutritional amplifier (Oliveira et al., 2016).

For Biostimulant within the zinc and sulfur interaction, it can be observed that when the dose of 400 (g) of zinc was applied, a significant effect was observed, with higher values than those

obtained for 0 Kg of Sulfur + 1500 mL of Biostimulant, with an increase of 2.7% and an increase of 4.4% with 20 Kg of Sulfur + 0 mL of Biostimulant. This effect was probably because the two nutrients used are very important for maize crop. Zinc in optimal concentrations can improve the functioning of the plant metabolism promote plant growth and still inhibit the increase of reactive oxygen species (Luo et al., 2010) while the use of sulfur is important to increase the content of methionine in cereal proteins, interact with nitrogen, phosphorus, and magnesium, an important enzymatic activator. The latter interaction is a key point for the photosynthesis process, respiration, synthesis of organic compounds, ionic absorption, and mechanical work such as root expansion (Marschner, 2012).

For the number of kernels per row, no significant effect for zinc doses was observed. However, a significant difference was observed when 20 kg of sulfur was applied (Table 5). This shows the importance of sulfur as an essential macronutrient for this crop, which actively collaborates to the protein composition and other constituents essential for the plant development (Fiorini et al., 2017). On the other hand, the number of kernels per row is closely linked to grain yield, in this sense, Fiorini et al. (2016), when studied the application of different sources of sulfur, observed that they affected the agronomic characteristics of the maize crop in a similar way regardless of the micronutrient supply and when compared to the control (without application of S), a significant yield increase was observed.

Table 5. Number of kernels per row in the Zn x S interaction

Products	Doses	Means	
		Sulfur (S)	
		0 kg	20 kg
Zinc (Zn)	0 g	22.3 a B*	30.6 a A
	400 g	24.1 a B	27.8 a A

* Means followed by the same lower case letter in the lines compares Zn (zinc) and upper case letter in the column for S (sulfur), do not statistically differ by the Tukey test at 5%.

For the Biostimulant, a significant effect on ear diameter (ED) and the number of rows per ear (NRE) was observed with an increase of 6.5% and 8.8% in the 1500 mL dose, respectively, when compared to the dose of 0 mL (Table 6). This increase may have occurred because the composition of the biostimulant have concentrations of auxin, cytokinin, and gibberellin, these hormones are active in the physiological and morphological processes, known to improve the plant development (Martins et al., 2016). Similar results were found by Dourado Neto et al. (2014), in which they verified that the use of the Biostimulant bioregulator provided satisfactory results when compared to the control in the maize crop.

Table 6. Plant height (PH), flag leaf area (FLA), ear height (EH), number of leaves (NOL), ear length (EL), ear diameter (ED), number of rows per ear (NRE), number of kernels per row (NKR) and yield (YLD) for isolate factors Biostimulant, Zinc and Sulfur

Products	Dose	Means								
		PH (cm)	FLA (cm ²)	EH (cm)	NOL (unid)	EL (cm)	ED (cm)	NRE (u)	NKR (u)	YLD Kg ha ⁻¹
Bio (mL)	0	76.0 a	112 a	54.5 a	7.67 a	12.5 a	4.42 b	13.6 b	25.3 a	6247 a
	1500	74.3 a	113 a	51.4 a	7.79 a	13.4 a	4.71 a	14.8 a	27.1 a	7000 a
Zn (g)	0	75.8 a	116 a	50.6 b	7.70 a	13.1 a	4.55 a	14.4 a	26.5 a	6784 a
	400	74.6 a	109 a	55.3 a	7.76 a	12.8 a	4.59 a	14.0 a	26.0 a	6463 a
S (Kg)	0	71.1 b	99,5 b	48.0 b	7.50 b	11.4 b	4.46 b	14.2 a	23.2 b	5893 b
	20	79.2 a	126 a	57.9 a	7.96 a	14.5 a	4.68 a	14.2 a	29.2 a	7354 a

*Means followed by the same lower-case letter in the columns do not differ by the Tukey test at 5%.

For the zinc factor, significant effects were only observed for ear height (EH), at the dose of 400 g, with an increase of 9.2% when compared to the dose of 0 g of Zn. This occurred due to the lengthening of internodes and a sufficient increase in auxin (phytohormone) (Taiz et al., 2017). In maize cultivation, Zinc is required for the tryptophan biosynthesis, which is one of the precursors of natural auxin, indole-3-acetic acid (AIA) (Peixoto, 2020). Thus, applying zinc to the plant, improves plant growth, since it is linked to metabolic functions that trigger several changes in the synthesis of carbohydrates, proteins, and auxins, thus influencing ear height (Ohse et al., 2012).

For the sulfur factor, significant effects were observed for almost all variables, except for the number of rows per ear (NRE), with increments of 11.39% for plant height, 26.63% for flag leaf area, 20.62% for ear height, 6.13% for the number of leaves per plant, 27.2% for ear length, 4.9% for ear diameter, 25.56% for the number of kernels per row, and 24.79% for grain yield. These increments demonstrate that this macronutrient is of significant importance for maize plants. It is a nutrient found in the composition of amino acids (cystine, cysteine, and methionine) (Taiz et al., 2017), and it is also a component of several coenzymes that are essential to plant metabolism (Fiorini et al., 2016).

For a good development of maize plants, specific quantities of each macro and micronutrient are needed for its establishment. With the crop well-nourished, the plant has greater resistance

to pathogens. On the other hand, the adequate fertilization with macro and micronutrient allow greater uptake of nutrients, improving the nutritional aspect, with a larger size, better reproductive structures. Therefore, studies on the requirements of each nutrient for each specific crop are very important (Schoninger et al., 2015).

The Analysis of Variance did not show significant differences for the triple factorial interaction (Bio x Z x S), or for the double factorial Z x S and Bio x Z (Table 7). However, there was a significant effect for the double factorial Bio x S, in the variable Sulfur (S) and Copper (Cu) at 5% probability. Also, there was a significant effect for the separate factor Sulfur (S) at 5%, in the variable Nitrogen (N) at 1% for the variables Potassium (K) and Magnesium (Mg). For the separate factor Zinc (Z), there was only a significant effect for the variable Z.

Table 7. Summary of analysis of variance for the variables Nitrogen (N), Phosphorus (P), Potassium (K), Sulfur (S), Calcium (Ca), Magnesium (Mg), Boron (B), Iron (Fe), Zinc (Zn), Copper (Cu) and Manganese (Mn), under the doses (presence and absence) of Biostimulant (Bio), zinc (Zn) and sulfur (S)

SV	DF	Mean square				
		N	P	K	S	Ca
Block	3	16.42	31.11	10.38	6.16	6.30
Bioest (Bio)	1	72.33	86.92	0.68	0.77	3.84
Zn (Z)	1	15.92	19.16	4.03	0.20	0.34
S (S)	1	184.49*	56.92	35.87**	2.67	1.14
Bio x Z	1	6.27	30.30	9.61	1.70	0.02
Bio x S	1	55.57	21.88	14.44	6.61*	2.26
Z x S	1	0.00	94.39	0.90	1.66	3.56
Bio x Zn x S	1	9.78	60.33	13.89	2.66	2.24
Erro	21	34.66	54.56	4.30	1.42	4.85
CV (%)		24.56	12.75	9.55	26.72	18.20

SV	GL	Mean square				
		Mg	B	Fe	Zn	Cu

Block	3	0.25	371.31	339131.46	173.76	3.24	303.66
Bioest (Bio)	1	0.37	9.19	4243.28	34.01	2.15	0.00
Zn (Z)	1	0.02	4.52	82047.94	4874.05**	4.66	71.97
S (S)	1	14.43**	8.29	83942.41	444.09	3.02	0.05
Bio x Z	1	1.67	97.33	379536.10	242.93	2.20	176.39
Bio x S	1	0.43	77.53	2529.56	145.39	10.20*	19.33
Z x S	1	0.17	3.32	92534.94	7.61	2.71	53.74
Bio x Zn x S	1	0.06	0.02	160692.06	221.50	1.51	233.33
Error	21	0.82	37.28	133929.48	342.01	2.37	109.65
CV (%)		14.99	32.92	21.53	30.83	24.86	30.99

**Significant at 1% probability; *significant at 5% probability and NS by the Snedecor F test.

The unfolding of means was performed when a significant effect was observed, to observe nutrients absorption behavior by the plants and their interaction with the products applied during the experiment.

As observed in Table 8, no significant effect was observed for S in the application of the biostimulant in any dose of the Sulfur (S). However, a significant effect for Cu was observed, when 1500 mL of the biostimulant was applied, with an increase of 31.36% when compared to the dose of 0 mL.

Table 8. Mean of Sulfur (S) and Copper (Cu) for the double interaction Biostimulant (Bio) x Sulfur (S)

Products		Sulfur (S)			
		0 kg	20 kg	0 kg	20 kg
		S		Cu	
Biostimulant	0 mL	2.56 aA*	2.23 aA	6.19 aA	5.68 bA
	1500 mL	1.96 aB	3.45 aA	5.58 aB	7.33 aA

* Means followed by the same lower-case letter in the column and upper case in the line do not differ by the Tukey test at 5%.

The non-significant effect for S may have occurred due to several factors such as soil fertility, genotype unresponsive to biostimulant use, or even low dose. Martins et al. (2016) also did not observe statistical differences in leaf nutrient content in maize plants when applied biostimulants and liquid fertilizers, the authors related this to adequate soil conditions and nutrients already available at the beginning of the cultivation year.

Another factor that must be considered for the lack of significant difference between the doses of the biostimulant, is the nutritional balance of the seed used for planting, as some elements may have been present in small amounts (Bontempo et al., 2016).

The tested concentrations of the biostimulants were probably not enough to cause a statistical difference for the variable mentioned.

It was also observed a significant effect for the Sulfur (S) only for the dose of 1500 mL of biostimulant, both for S and for Cu, with an increase of 76.02 and 31.36%, respectively.

This increment can be explained by the addition of the biostimulant in the seeds since they act similarly to the groups of known plant hormones. This product may have favored the genetic potential of plants due to changes in their vital and structural processes, generating a hormonal balance, and stimulating the root system development (Silva et al., 2008). Also, with better absorption of Cu, which has important functions in primary metabolism (photosynthesis and respiration), as well as metabolic routes related to pathogens resistance (Guo et al., 2010) therefore, improving the performance of the plants.

As observed in Table 9, a significant effect was only observed for the isolate factor Zinc (Z), when the dose of 400 g was applied an increase of 74.90% was observed when compared to the dose of 0 g. This was already expected since an amount of 0.93 mg dm⁻³ (Table 1) was already present in the soil, when the dose of 400g was applied, it enabled a greater absorption of this micronutrient.

Table 5. Nitrogen (N), Potassium (K), Magnesium (Mg) e Zinc (Zn) for the isolated factor Zinc (Zn) and Sulfur (S)

Products	Doses	N	K	Mg	Zn
		g kg ⁻¹	g kg ⁻¹	g kg ⁻¹	mg kg ⁻¹
Zinc (Zn)	0 g	23.27 a*	21.37 a	6.04 a	32.95 b
	400 g	24.68 a	22.08 a	6.00 a	57.63 a
Sulfur (S)	0 kg	21.57 b	20.66 b	6.69 a	49.01 a
	20 kg	26.37 a	22.78 a	5.35 b	41.56 a

* Means followed by the same lower-case letter between lines do not differ by the Tukey test at 5%.

For the isolate factor Sulfur (S), an increase in N and K of 22.25 and 10.26%, respectively, was observed, when compared to the application and absence of it, showing that when 20 Kg of Sulfur (S) was applied, an increase of other nutrients was observed. This may have occurred because sulfur is an essential macronutrient and a common constituent of proteins, and other cellular compounds that act in the vegetative development and fructification of maize, such as the synthesis of amino acids, which are responsible for 90% of S in plants (Fiorini et al., 2016). It also participates in the biological nitrogen fixation through the activation of nitrogenase (Hungria et al., 2015), which justifies the increase in the N accumulation in the leaves, as observed in the present study.

It can also be linked to the high diversity of secondary compounds that contain N and K in the sulfur structure, with an important role in the defense against pests and diseases (Stipp & Casarin, 2010).

For Mg, an opposite effect was observed, when Sulfur (S) was applied a decrease of 20% was observed. This can be explained due to the irregular release of this nutrient, which influences the competition in the absorption among the other elements, where one nutrient can impair the absorption of other, leading to nutrient deficiency in the plant, in addition to an accumulation of the nutrient in the soil, as reported by Fangueiro et al. (2015).

4. Conclusion

The application of sulfur alone or in combination with the biostimulant provided an increase in nutrients uptake by the leaf, such as copper and sulfur as observed in the double factorial Bio x Sulfur and N, and K for the separate factor sulfur.

Due to the sulfur fertilization, which caused an increment in leaf absorption, mainly of N, a significant increase in all variables, including grain yield was observed.

There are few studies in the literature using mixtures of biostimulants and macros and micronutrients. Therefore, more studies regarding the combination of biostimulants and other essential nutrients in the development of plants are recommended.

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