http://dx.doi.org/10.22161/ijeab/4.1.36 ISSN: 2456-1878

Effect of biostimulants on the nutrition of maize and soybean plants

Ana Carolina Feitosa de Vasconcelos

Post-doc researcher. Federal University of Campina Grande, Brazil.
E-mail: ana3carol@yahoo.com.br

Abstract— *The presence of humic substances in biostimulants composition affect positively plantgrowth by the improvement of the cation exchange capacity of the soils (CEC) and also by theformation of water soluble complexes with ions which can be uptaken by roots. Biostimulantsimprove plant growth due to the cytokinin and humic acids present in their composition. Over theyears the use of these products has been increasing and it is necessary to conduct more studies toevaluate their efficiency in promoting plant growth. The aim of this research was to evaluate theeffect of biostimulants (Brotax Solo®, Naturvital®, PT4-O® and Brotax-5®), applied with andwithout mineral fertilization, on nutrient uptake by maize and soybean plants. A greenhouseexperiment was conducted in Piracicaba, State of São Paulo, Brazil. Three-liter pots were filledwith 0-20 cm depth samples of Quartzipsamment soil. Base saturation was increased to 60% byapplying lime in the samples following incubation for 20 days at 80% the water retentioncapacity. After this period, mineral fertilizers were added to pots of specific treatments. Theapplied doses in L ha-1 were:Brotax Solo®: 0; 150 and 300; Brotax-5®: 0; 8 and 16; Naturvital®:0, 25 and 50; PT4-O®: 0; 0.5 and 1; referring to control, recommended dose by manufacturer and100% higher than this one, respectively. The products were diluted in water and they wereapplied in the pots 21 days after planting. Two months after planting, plant tops were collected,dried, weighted and nutrient contents in plants were determined. Data were subjected to analysisof variance (ANOVA) and means were compared by the LSD test (α = 0.05). Nutrient content inplants was higher in fertilized than in non-fertilized pots, for all products and crops. Ca and B;Zn; K; Ca and Mg contents were higher in maize plants treated with Brotax Solo®, Naturvital25®, PT4-O® and Brotax-5®, respectively. In soybean plants only the product PT4-O® showedincrement in Fe content. The amount of nutrients and humic acids in the studied products was notsufficient to increase significantly the amount of nutrients in the maize and soybean plants.*

Keywords— nutrients, biostimulants, yield.

I. INTRODUCTION

Biostimulants are defined by Russo and Berlyn (1990) as products that, when applied to plants, reduce the need for fertilizers and increase their productivity and resistance to water and climatic stress. Casillas et al. (1986) and Zhang and Schmidt (2000) state that these substances are efficient when applied in small concentrations, favoring the good performance of the plant's vital processes and allowing higher yields and higher quality products.Recently, Patrick du Jardin (2015) stated that biostimulantscorrespond 'to any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content'.

The major plant biostimulants are humic and fulvic acids, protein hydrolysates, seaweed extracts, silicon, chitosan, inorganic compounds, beneficial fungi (i.e., arbuscularmycorrhizal fungi; AMF and *Trichoderma*spp.) and plant growth-promoting bacteria (Canellas et al., 2015; Colla et al., 2015a; Rouphael et al., 2015b; Ruzzi and Aroca, 2015).

Biostimulants can increase plant growth and development, stimulating cell division and cell differentiation and stretching; these effects depend on the concentration, the nature and the proportion of the substances present in the products. Biostimulants may also increase the uptake and utilization of water and nutrients by plants (Vieira, 2001). In addition, Colla et al. (2015) citedmany studies conducted on greenhouse and open-field vegetables suggesting that applications of biostimulant substances can promote nutrient uptake and assimilation.

Some factor can be attributed to the increase in plant uptake, such as an increase in soil enzymatic and microbial activities, modifications in root architecture as well as an enhancement in micronutrient mobility and solubility (Ertani et al., 2009; Colla et al., 2013, 2014; Lucini et al., 2015).

Thus, the objective of this work was to study the effects of biostimulants on dry matter production and nutrient uptake by maize and soybean plants submitted to the presence and absence of mineral fertilization.

http://dx.doi.org/10.22161/ijeab/4.1.36 ISSN: 2456-1878

II. MATERIAL AND METHODS

In a greenhouse of the Department of Soil Science of the University of São Paulo, in Piracicaba (22°42'30" S 47°38'30" W), Brazil, two experiments were installed using maize and soybean crops for this study.

The biostimulants used in the experiments present the sugarcane residues and the leonardite as raw material (Table 1).

Table.1: Characterization of the biostimulantsused in the greenhouse study

Parameter	Biostimulant			
	Brotax Solo®	Brotax-5®	PT4-O®	Naturvital 25®
pH	4.7	5.6	1.5	13.0
TotalN (g L^{-1})	98.0	138.2	14.0	4.6
P_2O_5(g L^{-1})	19.4	2.0	233.8	0.06
K_2O (g L^{-1})	22.0	23.0	5.6	63.0
Ca (g L^{-1})	0.40	0.32	0.41	4.9
Mg (g L^{-1})	1.1	0.33	0.7	1.9
S (g L^{-1})	52.1	26.8	1.1	10.1
Cu (mg dm^{-3})	47.0	15.0	3.0	2.0
Fe (mg dm^{-3})	360.0	261.0	162.0	1890.0
Mn (mg dm^{-3})	235.0	12.0	7.0	18.0
Zn (mg dm^{-3})	98.0	1100.0	6000.0	6.0
Organic Matter (g L^{-1})	384.6	343.2	145.5	102.5
Total C (g L^{-1})	213.7	190.6	80.8	56.9
Humic acid (g L^{-1})	71.3	58.6	N.A.*	163.6
Fulvic acid (g L^{-1})	120.3	114.7	185.4	84.3
C/N ratio	2/1	1/1	6/1	13/1
Density (g L^{-1})	1.25	1.25	1.24	1.16

* Notavailable

The experimental design was completely randomized using a 3x2 factorial scheme with three replicates: three doses of the product in the absence or presence of mineral fertilization, totaling 18 experimental plots for each product and crop. The doses of the biostimulants used are shown in Table 2. Treatments 1 and 2 correspond to the dose recommended by the manufacturer and a dose of 100% higher, respectively.

Table.2: Doses of the biostimulants used in the experiments

Bioestimulant	Doses						
	Control	----- Treatament 1 ----			---- Treatament 2 ----		
			maize	soybean		maize	soybean
		L ha^{-1}	--- mL/pot ---		L ha^{-1}	--- mL/pot ---	
Brotax Solo®	0	150	5.0	1.2	300	10.0	2.4
Brotax-5®	0	8	0.3	0.06	16	0.6	0.12
Naturvital 25®	0	25	0.8	0.2	50	1.6	0.4
PT4-O®	0	0.5	0.02	0.004	1	0.04	0.008

For field application, field doses were calculated based on the number of plants of each crop per hectare and then related to two plants per pot.The experiments were carried out in three-liter pots where samples of the 0-20 cm layer of a QuartzarenicNeosol of the city of Piracicaba, whose chemical characterization, according to Raij et al. (2001), is shown in Table 3.

Table.3: Chemical and physical characterization of the soil used in the experiments

Parameter	Value
pH $CaCl_2$	4.1
P (mg dm^{-3})	2.8
K ($mmol_c$ dm^{-3})	1.3
Ca ($mmol_c$ dm^{-3})	2.0
Mg ($mmol_c$ dm^{-3})	1.5
Al ($mmol_c$ dm^{-3})	6.0
H+Al ($mmol_c$ dm^{-3})	28.0
S. B. ($mmol_c$ dm^{-3})	4.8
C.T.C ($mmol_c$ dm^{-3})	32.8

http://dx.doi.org/10.22161/ijeab/4.1.36 ISSN: 2456-1878

V (%)	14.6
M.O. (g dm^{-3})	1.4
Cu (mg dm^{-3})	0.3
Zn (mg dm^{-3})	0.5
Mn (mg dm^{-3})	7.1
Fe (mg dm^{-3})	22.3
B (mg dm^{-3})	0.2
S (mg dm^{-3})	17.7
Areia (g kg^{-1})	840
Silte (g kg^{-1})	40
Argila (g kg^{-1})	120

As the soil showed high acidity and low base saturation, the samples were treated with 0.6 mg dm^{-3} of high purity calcium carbonate; and 0.2 mg dm^{-3} of high purity magnesium carbonate, the doses of which were calculated to raise the base saturation to 60%. After incorporation of the carbonates, the samples were incubated for 20 days with humidity referring to 70% of the water retention capacity.

After this period, the sampleswere submitted to mineral fertilization in those plots where it was intended to evaluate the effects of the interaction between the mineral fertilization and the doses of the conditioners. 150 mg dm^{-3} of N as ammonium sulfate (with 20% N), 100 mg dm^{-3} of K (KCl with 60% K_2O) and 300 mg dm^{-3} of P (single superphosphate with 18% of P_2O_5), following recommendations of Raij et al. (2001) to raise these nutrients from low to medium-high in the soil. The micronutrients, as a solution, were applied together with the irrigation water, namely: 0.5 mg dm^{-3} of boron (H_3BO_3 with 17.7% of B); 0.8 mg dm^{-3} Cu ($CuSO_4 .5H_2O$ with 25.6% Cu), and Zn ($ZnSO_4 7H_2O$ with 22.6% Zn).

After the mineral fertilization, sowing was done using five seeds per pot, leaving two plants per pot after thinning.

Biostimulantswere diluted in water at the established doses and applied to the soil twenty-one days after sowing. As the doses had low volumes to be applied per pot, the following application criterion was adopted: each pot received 100 mL of the dilution made with the dose of each product, in order to allow the soil to receive the doses in a more homogeneous way.

Two months after sowing, the aerial part of the plants was harvested, oven dried at 65oC, weighed and submitted to determination of macro and micronutrients contents according to Malavolta (1997).

Statistical analyzes were performed using the statistical software SAS version 8.2 (SAS INST., 2002). The effect of the treatments was evaluated by analysis of variance (ANOVA) and the means compared by the minimum significant difference at the 5% level of probability by the Tukey test.

III. RESULTS AND DISCUSSION

The biostimulant Naturvital 25® showed a significant effect among the doses applied only to the zinc content of maize plants, not causing a significant effect on the other nutrients. The dose recommended by the manufacturer of this product was the one that favored the highest zinc content in these plants.

The biostimulant PT4-O showed a significant difference between the doses only for the potassium content of maize plants. The dose recommended by the manufacturer of the biostimulant PT4-O was the only one that significantly increased the potassium content in the plant, showing no significant effect for the other nutrients.

For the biostimulantBrotax 5®, there were statistically significant differences for the calcium and manganese averages of maize plants, but the variations were not very significant in both cases. For calcium, doses 1 and 2 of the biostimulantBrotax 5® were effective, whereas for manganese, only dose 1 exceeded the control.

For the Brotax Solo®, Naturvital 25®, PT4-O® and Brotax-5® products, the average between the fertilized plots exceeded the average of the non-fertilized plots at the 5% probability level in the soybean plants.

In the comparison between the doses applied in the experiment for soybean, none of the products showed significant effect, except for the biostimulant PT4-O that showed significant effect only for the iron content. However, this effect was not consistent, since none of the doses was superior to the control.

Chen et al. (2004) and Marschner (1995) state that the plants respond to the application of humic acid products by increasing the availability of soil micronutrients to plants, especially iron and zinc, by complexing or complexing these metals with humic substances.

In general, the results obtained in this study showed that the application of four commercial biostimulants to the soil resulted in little or no significant increase in the content of the nutrients in the plants. These values were below the range of values suitable for maize and soybean crops (Malavolta, 1997). In some cases, the results were similar to those found by Csizinszky (1990) who observed higher nutrient content in control plants than in those submitted to biostimulant applications. Canellas et al. (2015) did not obtain effects of humic substances on the growth parameters of lettuce plants. Dry matter production in the presence of mineral fertilization was higher than that obtained in the absence of this fertilization at the 5% probability level.

Table 4 shows the dry matter yield of maize and soybean plants subjected to the application of the doses of the products

Table.4: Dry matter yield of aerial part of maize and soybean plants in response to the application of biostimulants in association or not with mineral fertilization

Treatment	Dose	Maize			Soybean		
		Mineral Fertilization			Mineral Fertilization		
		Presence	Absence	Average+	Presence	Absence	Average+
	L ha^{-1}	g					
		Brotax Solo®					
Control	0	49.4	2.2	25.8 ab	7.8	1.4	4.6 a
1	150	54.0	3.2	28.6 a	9.7	1.5	5.6 a
2	300	47.6	2.6	25.1 b	7.2	1.4	4.3 a
Average+		50.4 x	2.7 y		8.2 x	1.4 y	
		Naturvital®					
Control	0	50.5	1.9	26.2 a	9.6	1.4	5.5 a
1	25	49.2	2.4	25.8 a	10.6	1.4	6.0 a
2	50	51.3	2.9	27.1 a	9.2	1.4	5.3 a
Average+		50.3 x	2.4 y		9.8 x	1.4 y	
		PT4-O®					
Control	0	50.7	2.1	26.4 a	10.6	1.4	6.0 a
1	0.5	51.1	2.3	26.7 a	9.3	1.5	5.4 a
2	1	49.2	2.4	25.8 a	10.9	1.2	6.1 a
Average+		50.3 x	2.3y		10.3 x	1.4 y	
		Brotax-5®					
Control	0	52.5	1.9	27.2 a	9.1	1.4	5.2 a
1	8	48.4	2.2	25.3 a	6.8	0.9	3.9 a
2	16	48.6	3.0	25.8 a	6.9	1.2	4.0 a
Average+		49.8 x	1.8 y		7.6 x	1.2 y	

+ Averages followed by the same letter (a or b in the column and x or y in the row) do not differ significantly from each other at the 5% level by the t test

Among the average doses of biostimulantsapplied there was no statistically significant difference for both maize and soybean plants, except for the Brotax Solo® product applied to maize. However, the differences were small and none of the doses differed from the control.

Delfine et al. (2005) did not find positive results in the yield of wheat submitted to the application of humic acids. In addition, the authors state that the application of products with the presence of humic acids in their composition does not increase the nutrient content in wheat plants and, therefore, its application is not necessary in order to improve the mineral nutrition of the plants.

The amounts of nutrients present in these doses supplied to the plants were very low, as shown in Table 5. These quantities are not sufficient to reflect a significant increase of nutrients in the plant tissue when compared with the necessary amounts of fertilizers recommended by Raij et al. (1997) for maize and soybean.

Table.5: Amounts of nutrients supplied to the soil with the application of the doses of the products

Nutrient	Bioestimulants							
	Brotax Solo®		Naturvital 25®		PT4-O®		Brotax-5®	
	dose 1	dose 2	dose 1	dose 2	dose 1	dose 2	dose 1	dose 2
N (kg ha^{-1})	14.7	29.4	0.11	0.23	0.00	0.01	1.11	2.21
P (kg ha^{-1})	1.27	2.53	0.00	0.00	0.05	0.10	0.01	0.01
K (kg ha^{-1})	2.74	5.48	1.31	2.61	0.00	0.00	0.15	0.31
Ca (kg ha^{-1})	0.06	0.12	0.12	0.25	0.00	0.00	0.00	0.01
Mg (kg ha^{-1})	0.16	0.32	0.05	0.09	0.00	0.00	0.00	0.01
S (kg ha^{-1})	7.82	15.6	0.25	0.51	0.00	0.00	0.21	0.43
Cu (g ha^{-1})	7.05	14.1	0.05	0.10	0.00	0.00	0.12	0.24
Fe (g ha^{-1})	54.0	108.0	47.2	94.5	0.08	0.16	2.09	4.18
Mn (g ha^{-1})	35.2	70.5	0.45	0.90	0.00	0.00	0.09	0.19
Zn (g ha^{-1})	14.7	29.4	0.15	0.30	3.0	6.0	8.8	17.6

The application of higher doses of these products to the soil could be a way to provide greater increases of nutrients in the plants. In addition, the products could present higher concentrations of nutrients in their composition in order to allow greater supply to the plants at the recommended doses.

According to the conditions of this experiment, the application of the products used does not present advantages to increase the yield of maize and soybean, representing another cost to the producer.

IV. CONCLUSIONS

The use of the biostimulants selected for this study did not increase the nutrient content in maize and soybean plants at the applied doses.

The weight of dry matter of aerial part of the plants was not increased with the application of the doses of the biostimulants.

The concentrations of nutrients present in the biostimulants and the recommended doses result in nutrient amounts much lower than those required to obtain satisfactory yields.

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