Original article

Bioestimulants in soybean (*Glycine max* (L.) Merrill) growing and yield

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ABSTRACT

Taking into account the importance of soybeans culture in the world and particularly in Cuba, as well as the need to validate bioproducts which can increase their yields and preserve our environment, this work was developed. The aim was to evaluate the effect of the application of three biostimulants (Azofert-S®, EcoMic® and QuitoMax®) in the growth and yield of soybean cultivation cv. Incasoy-27. The experiment was conducted in experimental areas of the National Institute of Agricultural Sciences, in an agrogenic leachate Red Ferralitic soil. A randomized block design with eight treatments and four replicates was used, which consisted of sowing seeds without the application of biostimulant, as control, with the application of each biostimulant or their combination. A treatment with mineral fertilization was used. The biofertilizers were applied by seed coating and QuitoMax® by its imbibition during 15 min. In flowering phase, growth and symbiosis variables were determined and at harvest time, the crop agricultural performance and its components were also determined. The results showed that the application of Azofert®, EcoMic® and QuitoMax® stimulated nodulation, mycorrhizal colonization, growing and soybean yield.

Key words: mycorrhiza, *Rhizobium*, chitosan, stimulation, leguminous

INTRODUCTION

Agriculture faces the challenge of sustainable production of safe food to supply the world's population. The Food and Agriculture Organization of the United Nations (FAO) estimates a world population growth of 13 % in 2030 and 30 % in 2050, which will require a 70 % increase in production to solve malnutrition problems and guarantee food security [(1)].

Soy represents an important crop in the economy, as it has a high nutritional value, with values of 38–42 % protein and 18–20 % oil. Its consumption increases every day, due to the need to use grain as a raw material in the preparation of concentrated animal feed and for human consumption [(2)]. This crop is among the ten most important in the world, it is planted on more than 90.2 million hectares, whose world production exceeds 345.96 million tons, representing an increase of 10.52 % in world production of recent years [(3)].

Nitrogen (N) and phosphorus (P) are considered two of the elements with the greatest influence on the production of crops and, in particular, soybean [(4)]. However, the use of these nutrients in chemical form is limited, mainly due to their high cost and due to the incompatibility of their excessive use with the conservation of the environment, which is why sustainable alternatives capable of maintaining productive levels and their use are used quality, without damaging agroecosystems [(5)].

Among the agro-ecological alternatives that are proposed today in Cuba and the world, is the application of biostimulants. These products comprise a wide range of compounds, inocula of microorganisms or their derivatives, which when applied to the plant, the seed or the substrate, promote the development and growth of the roots, leaves and stems; in addition to improving the response to abiotic stress [(6)].

Within them, plant growth promoting microorganisms exert beneficial effects on the soil and plants [(7)]. Various studies demonstrate the positive influence of bacteria from the rhizobia group and arbuscular mycorrhizal fungi on soybean yield [(8,9)]. On the other hand, the use of non-microbial biostimulants such as chitosan stimulates the nodulation, growth, protection and performance of different crops [(10-12)].

The objective of this study was to evaluate the effect of the application of Azofert-S®, EcoMic® and QuitoMax® biostimulants, on the growth and yield of soybean cultivar cv. Incasoy-27.

MATERIALS AND METHODS

Characterization of the experimental area

The research was carried out in the experimental areas of the National Institute of Agricultural Sciences (INCA), located in the San José de las Lajas municipality, Mayabeque province, Cuba; in the dry season, from January to April 2018, in an agrogenic leachate Red Ferralitic soil (13), whose chemical characteristics are presented in Table 1.

Table 1. Characteristics of the Agrogenic Leachate Red Ferralitic Soil used in the experiment

pH (H_2O)	MO (%)	P_2O_5 (mg 100 g^{-1})	Changeable cations ($cmol_c$ kg^{-1})			
			Na^+	K^+	Ca^+	Mg^+
6,5	2,8	43,30	Tr	0,44	12,0	1,5

Tr: Trace

pH (H_2O): potentiometric soil-water ratio method (1: 2.5); MO (%): Walkley-Black; Assimilable P (mg. 100 g^{-1}): Oniani (extraction with H_2SO_4, 0.1N); Changeable K and Na ($cmol_c$ kg^{-1}): by extraction with ammonium acetate and flame photometry; Ca and Mg ($cmol_c$ kg^{-1}): Maslova method (1N Ammonium Acetate, pH 7), determination by complexometry

The techniques used for the analysis of the soil are described in the Manual of analytical techniques for the analysis of soil, foliar, organic fertilizers and chemical fertilizers (14).

Experimental design

A randomized block experimental design was used, consisting of eight treatments and four replicates, separated at 1 m to avoid mixing between the different treatments. The treatments imposed were: T1 (Control), T2 (NPK), T3 (Azofert-S®), T4 (Azofert-S®+EcoMic®), T5 (EcoMic®), T6 (Azofert-S®+QuitoMax®), T7 (QuitoMax®) and T8 (Azofert-S®+EcoMic®+QuitoMax®). Mineral fertilizer (complete formula 9-13-17), at a rate of 120 kg ha^{-1}, was applied thoroughly before sowing, in all treatments. The experiment was carried out in two consecutive campaigns.

Plant material and biostimulants

As plant material, certified soybeans were used, from the commercial cultivar Incasoy-27, from the Germplasm Bank of the National Institute of Agricultural Sciences (INCA).

Biostimulants used were Azofert-S®, EcoMic® and QuitoMax®, which are at the National Institute of Agricultural Sciences produced and marketed.

Azofert-S®, with Registry No. RCF 005/13, at a concentration of 2.9 x 109 CFU mL^{-1} (Colony forming units), was applied at a dose of 200 mL for every 50 kg of seed. EcoMic®, with Registry No. RCF 004/15, and a fungal richness of 20 spores per gram of inoculant, was applied at a dose of 4 kg for every 50 kg of seed. These biofertilizers were applied to the seeds at planting time using coating technology (15).

In the case of QuitoMax®, with Registration No. RCF 010/17, at a concentration of 4 g L^{-1}, it was applied by imbibing the seed for 15 min, at the dose of 0.5 g L^{-1} per every 50 kg of seed.

Variables evaluated

The experiments were sown in an experimental area of 0.15 ha, with 32 plots of 15 m^2 each, with four furrows (5 m long x 3 m wide). The distance between rows was 0.75 m x 0.05 m between plants, for a total of 20 plants per linear meter.

At 35 days after planting (DAS), ten plants were randomly taken from each plot (replica) in the central rows. The number of total and main root nodules, mycorrhizal colonization and visual density (%) were determined by root staining [(16)]; the height of the plants (cm), the length of the root (cm), the number of leaves per plant, the dry mass of the aerial part and the root (g) and the content of N, P, K (%) foliar [(14)]. At harvest time, 96 days after planting (DAS), the yield variables and their components were determined. For this, the two central rows of each replica were used, from which 1 linear meter per plot was harvested, and the following indicators were evaluated: number of pods per plant, mass of 100 grains (g) and agricultural yield (kg ha^{-1}, at 13 % humidity).

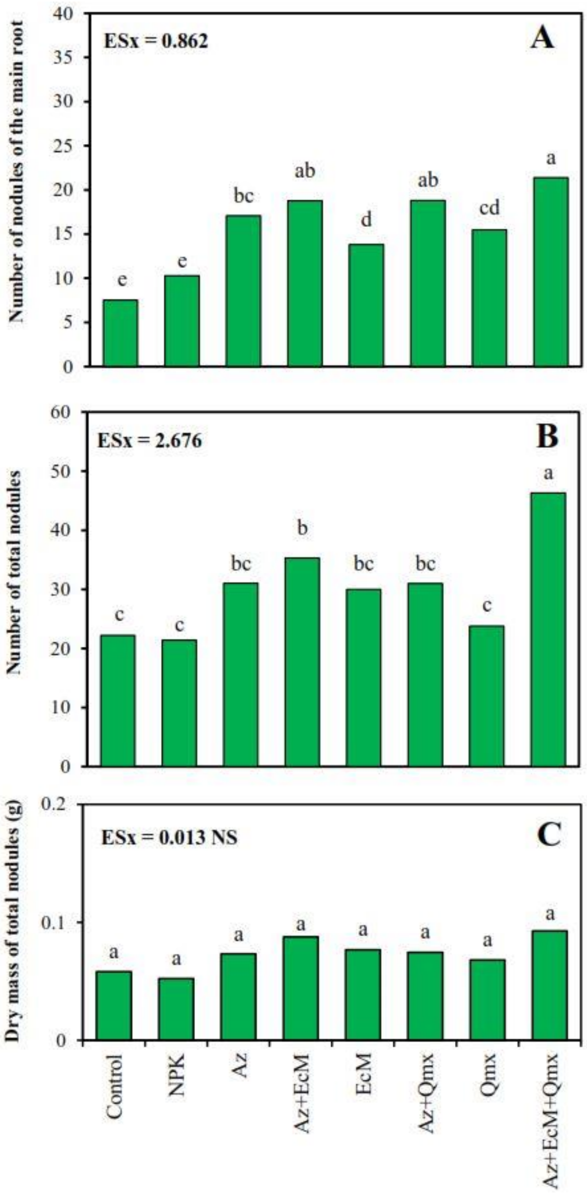
Statistical analysis

After checking the theoretical assumptions of normality and homogeneity of variance, a double classification analysis of variance (ANOVA) was applied to each variable and when there were significant differences, these were verified by Tukey's multiple range test $p<0.05$. For the analysis of the data, the statistical package SPSS version 19 on Windows was used and the results were processed in Excel 2013.

RESULTS AND DISCUSSION

Effect of biostimulants on nodulation

Inoculation with Azofert-S®, EcoMic® and the application of QuitoMax® in soybean plants, caused an increase in the number of nodules in the main root 35 days after emergence. (Figure 1 A). In the total number of nodules, only the combination of Azofert-S®+EcoMic® and the three biostimulants outperformed the control (Figure 1B).



Az (Azofert-S®), EcM (EcoMic®), Qmx (QuitoMax®), DAS (Days after planting)

Figure 1. Number of nodules in the main root (A), number of total nodules (B) and dry mass of total nodules (C), in Incasoy-27 soybean plants at 35 DAS cultivated under field conditions. Different letters differ significantly ($p < 0.05$) according to Tukey's Multiple Range Test

In the case of the dry mass of the total nodules (Figure 1 C), no significant differences were found between the imposed treatments; however, with the application of Azofert-S® in bean cultivation, other authors found increases in dry nodular mass, compared to the control treatment [17].

The three variables related to nodulation showed a similar trend, with superior results where Azofert-S® was applied in combination with EcoMic® and the combination of the three biostimulants. Another aspect to highlight in this experiment is the presence of nodules in the control treatment, where Azofert-S® was not inoculated, which indicates that the soil used contained rhizobia strains compatible with soy.

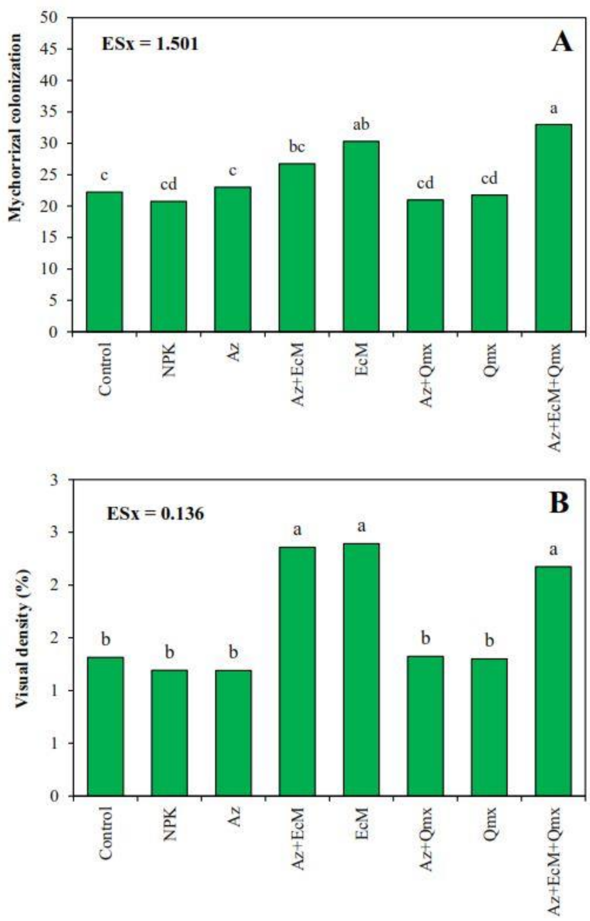
These results indicate that the action of inoculating a PGPR using the Azofert-S® biostimulant, increases the concentrations of these bacteria in the soil. Soy has the ability to establish symbiosis with bacteria of the *Bradyrhizobium* genus residing in soils, however, inoculation provides a greater number of bacteria and in better physiological conditions, which guarantees greater effectiveness in infection [(18)].

The combination of an efficient *Bradyrhizobium* inoculum with AMF increased nodulation. Coinoculation of rhizobia and AMF has been reported to be an example of beneficial interaction, as colonization of roots by AMF stimulates carbohydrate flow from foliage to root, and these carbohydrates are essential sources of carbon for growth and functioning of said bacteria [(19)].

The increase in the number of nodules in the main root with the treatment where Azofert-S®+QuitoMax® was combined may be due to the structural similarity of chitin and the nodulation factors produced by these bacteria, which basically contain a composite skeleton by chitin oligosaccharides and are responsible for initiating the nodule formation process in the roots of legumes [(20)].

Effect of biostimulants on mycorrhizal infection

The behavior of the fungal indicators in the plants at 35 days DAS, show significant differences before the different treatments (Figure 2). In the case of the frequency of mycorrhizal colonization (Figure 2 A), it was found that the treatments with EcoMic® alone and the combination of the three bioproducts, showed the highest values, with 30.3 and 33 % colonization respectively, although the first of them it did not differ from the treatment inoculated with Azofert-S® + EcoMic®, which reached values of 26.75 % colonization.



Az (Azofert-S®), EcM (EcoMic®), Qmx (QuitoMax®), DAS (Days after planting)

Figure 2. Mycorrhizal colonization (A) and visual density (B), in Incasoy-27 soybean plants at 35 DAS cultivated under field conditions. Different letters differ significantly for ($p < 0.05$) according to Tukey's Multiple Range Test

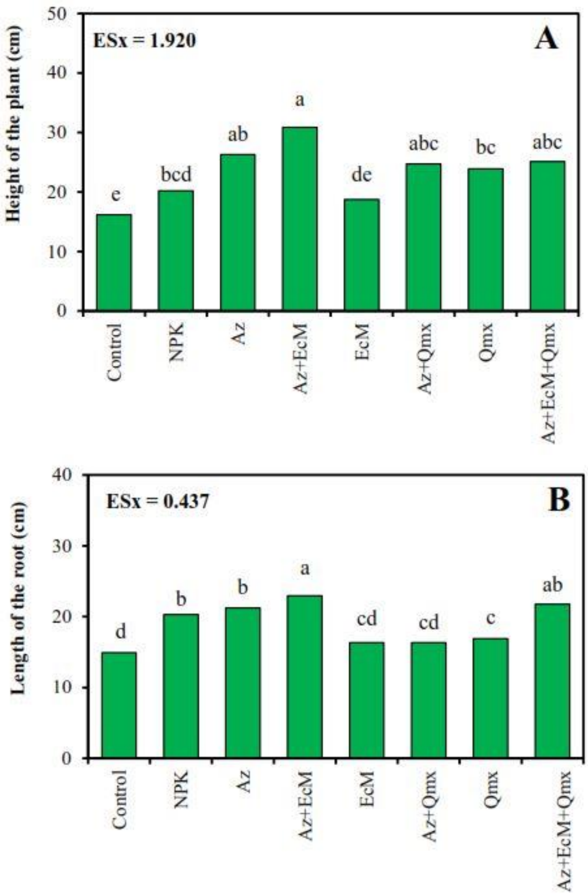
The visual density of mycorrhizal colonization (Figure 12 B) showed two groups of values: a first group where the treatments where Azofert-S®+EcoMic® was applied were highlighted; EcoMic® and the combination of the three Azofert-S®+EcoMic®+QuitoMax® biostimulants and a second group formed by the rest of the treatments, without significant differences between them.

Similar to what was found in the nodulation for Azofert-S®, the treatments where EcoMic® was not applied showed lower values in these fungal variables, but indicate the presence of these fungi in the soil, with the ability to colonize the roots of this legume. In this regard, it is reported that the AMF inoculation efficiency to promote plant growth depends on its ability to compete with native AMF. This is related to the infectivity of the strain, its capacity to produce external hyphae, the speed of the hypha to colonize the roots and its ability to maintain colonization levels under competitive conditions [21].

Effect of biostimulants on soybean growth and development

In Figure 3 it can be seen how the height of the plants and the length of the root fluctuated before the different imposed treatments. All the treatments with the product, except the one that contained EcoMic® alone, increased the height of the plants (Figure 3 A) and the combination of Azofert-S®+EcoMic® stood out with the highest values.

Regarding the radical length (Figure 3B), the highest values also corresponded to the Azofert-S®+EcoMic® treatment, followed by Azofert-S®+EcoMic®+QuitoMax®, although the use of the three biostimulants together it did not differ from the Azofert-S® treatments alone or from the one where the full NPK formula was applied. The Azofert-S®+QuitoMax® treatment did not differ from the control.



Az (Azofert-S®), EcM (EcoMic®), Qmx (QuitoMax®), DAS (Days after planting)

Figure 3. Height (A) and root length (B), in Incasoy-27 soybean plants at 35 DDS cultivated under field conditions. Different letters differ significantly ($p < 0.05$) according to Tukey's Multiple Range Test

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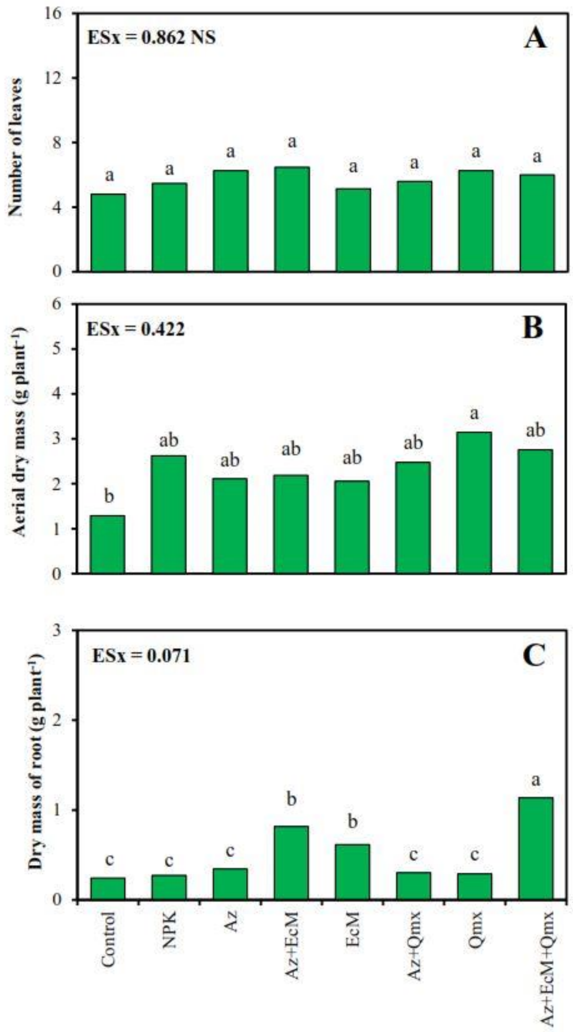
Regarding the indicator number of total leaves per plant, no significant differences were observed between the treatments used (Figure 4 A). However, the dry mass of the aerial part, as well as the dry mass of the root of soybean plants at 35 DAS, found a differential behavior (Figure 4 B and C).



Az (Azofert-S®), EcM (EcoMic®), Qmx (QuitoMax®), DAS (Days after planting)

Figure 4. Number of leaves (A), aerial dry mass (B) and dry mass of the root (C), in Incasoy-27 soybean plants at 35 DAS cultivated in field conditions. Different letters differ significantly ($p < 0.05$) according to Tukey's Multiple Range Test

As for the aerial dry mass, the highest values were found in the treatment where the seeds were embedded with QuitoMax®, with differences with the control treatment, but without differences with the rest of the treatments (Figure 4B). However, the highest values of radical dry mass accumulation were found in the treatment where the three bioproducts were combined, followed by the treatments where Azofert-S®+EcoMic® and EcoMic® only were applied. The rest of the treatments did not show significant differences between them (Figure 4C).

Other authors found similar results, where combinations of AMH and rhizobia increased plant growth (22), while the combination of rhizobia and chitosans or brassinosteroids stimulated the content of chlorophylls, proteins, and the process of photosynthesis (17).

For its part, the network of hyphae that creates the mycorrhizal infection allows exploring a larger area of soil (22) and thus accessing a greater content of nutrients, which allow for greater growth.

Results with chitosan explain the stimulation of the physiological processes in the plant and the increase in the size of the phloem parenchymal cells, which makes the nutrients more assimilable by the plant and increases their growth and development (12). The combined application of this compound with biofertilizers (Azofert-S® and EcoMic®) benefits the nutrient supply processes (10).

Effect of biostimulants on the nutritional status of plants

When analyzing the content of N, P and K in the plants (Table 2), a superior positive effect was found with the application of the three joint products. In the N contribution, the other treatments where biostimulants were applied were also highlighted, except for the QuitoMax® only, which did not differ from the control.

For the phosphorus (P) and potassium (K) content, in addition to the three linked products, the treatments where NPK mineral fertilization and Azofert-S®+EcoMic® were applied were highlighted.

Table 2. N, P and K content in the aerial biomass of soybean plants cv Incasoy-27, at 35 DAS, grown under field conditions

Treatments	N (%)	P (%)	K (%)
Control	3.20 c	0.43 c	1.42 c
NPK	4.93 ab	0.54 a	1.81 ab
Az	4.71 ab	0.47 bc	1.68 bc
Az + EcM	5.03 ab	0.52 ab	1.80 ab
EcM	4.93 ab	0.46 bc	1.66 bc
Az + Qmx	4.79 ab	0.49 abc	1.73 abc
Qmx	3.88 bc	0.45 c	1.62 bc
Az + EcM + Qmx	5.67 a	0.54 a	1.89 a
ESx	0.130	0.015	0.070

Different letters differ significantly for p <0.05, according to Tukey's Multiple Range Test.

Az (Azofert-S®), EcM (EcoMic®), Qmx (QuitoMax®)

The NPK values accumulated in the aerial part of the soybean plants in the treatments where the biostimulants were applied, without differences with the treatment with mineral fertilization (Complete formula); corroborate the positive effect of the use of these products in nutrition [23, 24, 25] and in the stimulation of mechanisms to promote plant growth and development [12].

Effect of biostimulants on agricultural performance and its components

The analysis of the behavior of agricultural performance and some of its components is shown in Table 3.

Table 3. Effect of biostimulants on the yield of soybean cv. Incasoy-27, at 96 DAS in field conditions

Treatments	NVP	M100 seeds (g)	Yield (kg ha^{-1})
Control	46.76 c	10.53	1 348.30 d
NPK	54.31 a	12.76	1 950.75 b
Az	51.10 ab	11.99	1 702.56 c
Az + EcM	52.31 ab	12.22	2 122.65 ab
EcM	51.03 ab	11.86	1 679.12 c
Az + Qmx	51.91 ab	12.66	2 043.61 b
Qmx	48.78 bc	10.72	1 554.85 c
Az + EcM + Qmx	54.76 a	12.84	2 233.72 a
ESx	4.280	0.85 NS	93.42

Different letters differ significantly for ($p < 0.05$) according to Tukey's Multiple Range Test

There were no differences between the treatments for the variable mass of 100 seeds. In the NVP there was a positive effect of all the products and their combinations, except for the QuitoMax® only that it did not differ from the control.

In the performance, the Azofert-S®+EcoMic®+QuitoMax® and Azofert-S®+EcoMic® treatments stood out, with 2 233 and 2 122 kg ha^{-1}, respectively. These results exceeded the fertilized treatment by 14 and 9 % and the control by 66 and 57 %, respectively. The fertilized treatment followed in positive results, which did not differ from Azofert-S® + EcoMic®, nor from Azofert-S®+QuitoMax®, but the latter exceeded the control by 36 and 34 %, respectively. Treatments where Azofert-S®, EcoMic® and QuitoMax® alone were applied also outperformed the control in 21, 20 and 13 % of the yield, respectively.

The performance results correspond, in general, with the results obtained in the nodulation, mycorrhizal colonization, and growth and nutrient content variables of the plants to the 35 DDS. Similarly, other authors found that the application of Azofert-S® in combination with QuitoMax®, stimulated the number of pods per plant and the agricultural yield of soybean cv. INCAsoy-27 [26], as well as that in bean cultivar cv. CC-25-9N [17].

CONCLUSIONS

- The application of Azofert-S®, EcoMic® and QuitoMax® biostimulants to soybean cv. Incasoy-27, increases mycorrhizal colonization and culture growth.
- The joint application of the bioproducts increased the NPK concentration of the plants with respect to the absolute control, particularly when they were applied in combination, and equalized the levels of these macroelements in the NPK-fertilized control. The triple combination of the bioproducts significantly increased the performance of all the treatments, except for the combination of Azofert-S® + EcoMic®, with an increase of 66 and 14 % of the yield.

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