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Original article

Bioproducts and NPK fertilization in beans grown on Eutric Nitisols soils

Bioproductos y fertilización NPK en el frijol cultivado sobre suelos Ferralíticos Rojos Lixiviados

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ABSTRACT: Beans are an important food in the Cuban diet and their production is limited by a deficient supply of nutrients, due to the difficult economic situation. Different bioproducts have been developed in the country that provide nutrients and increase the efficiency of nutrient utilization. This work was carried out to establish criteria for effectively managing bioproducts and their relationship with the required fertilizer doses. Five experiments were carried out in experimental areas of the National Institute of Agricultural Sciences, with the cultivar CC-25-9N in Eutric Nitisols soils, from 2013 to 2016. Various combinations of the bioproducts EcoMic®, Fitomas-E®, Azofert-f®, Biobras-16®, Quitomax®, and Pectimorf® were studied in randomized blocks, from the application of two to six and various doses of NPK fertilizer. A beneficial response to the combined application of bioproducts was found, depending on the dose of fertilizer applied and the maximum yield obtained. For yield levels between 1.96 and 2.05 t ha^{-1}, it was necessary to apply doses of 36-39-51 kg ha^{-1} which decreased to 36-26-34 for yields between 1.6 and 1.8 t ha^{-1}. The application of Quitomax® and Biobras-16® together with EcoMic®, Fitomas-E®, and Azofert-f® was effective and increased yields ($p \leq 0.1$) compared to when only one of the two was added. The inclusion of Pectimorf® was not efficient. The combined application of the bioproducts EcoMic®, Fitomas-E®, Azofert-f®, Biobras-16®, and Quitomax® to beans was effective even in the presence of low NPK fertilizer doses.

Key words: mycorrhizal inoculant, chitosan, brassinosteroid analogue, rhizobia.

RESUMEN: El frijol es un alimento importante en la dieta del cubano y su producción se encuentra limitada por un deficiente suministro de nutrientes, derivado de la difícil situación económica. En el país se han desarrollado diferentes bioproductos los cuales aportan nutrientes y/o incrementan la eficiencia en la utilización de estos. Este trabajo se realizó con el objetivo de establecer criterios para el manejo efectivo de bioproductos y su relación con las dosis de fertilizantes requeridas. Se ejecutaron cinco experimentos en áreas experimentales del Instituto Nacional de Ciencias Agrícolas, con el cultivar CC-25-9N en suelos Nitisoles éutricos, durante el periodo 2013-2016. Se estudiaron en bloques al azar diversas combinaciones de los bioproductos EcoMic®, Fitomas-E®, Azofert-f®, Biobras-16®, Quitomax® y Pectimorf®, desde la aplicación de dos hasta los seis y varias dosis de fertilizante NPK. Se encontró una respuesta beneficiosa a la aplicación conjunta de bioproductos, dependiente de la dosis de fertilizante aplicada y del rendimiento máximo obtenido. Para niveles de rendimientos entre 1.96 y 2.05 t ha^{-1} fue necesario aplicar dosis de 36-39-51 kg ha^{-1} que descendieron a 36-26-34 para rendimientos entre 1.6 y 1.8 t ha^{-1}. La aplicación de Quitomax® y Biobras-16® de conjunto con EcoMic®, Fitomas-E® y Azofert-f® resultó efectiva e incrementó los rendimientos ($p \leq 0.1$) en comparación a cuando se adicionó solo uno de los dos. La inclusión del Pectimorf® no fue eficiente. La aplicación conjunta al frijol de los bioproductos EcoMic®, Fitomas-E®, Azofert-f®, Biobras-16® y Quitomax® resultó efectiva aún en presencia de dosis bajas de fertilizantes NPK.

Palabras clave: inoculante micorrízico, quitosano, análogo brasinoesteroide, rizobio.

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INTRODUCTION

The cultivation of beans is important in the diet of a population large part in various regions of the world, especially in developing countries (1). It is one of the fundamental foods in the Cuban diet and it is considered that the annual per capita can reach up to 23 kg (2); however, in 2021 only 70 000 ha were planted, reaching a low average yield of 0.86 t ha-1 (3), far from the yield potentials of commercial cultivars between 2.5 and 3 t ha^{-1} (4) and even those obtained in 2018, prior to the COVID pandemic and the tightening of the blockade, of 147 560 ha planted and yields of 1.09 t ha^{-1} (5).

In Cuba, crop production technologies have commonly been based on guaranteeing nutritional requirements through synthetic fertilizers. The economic situation of the country and the increasing prices of these have decreased the acquisition capacity of these since the end of the last century, limiting crop yields (6), however, beans remained as a priority crop in the period 2011- 2018, receiving a package of 200 to 300 kg ha^{-1} of 9-13-15 and 50 kg ha^{-1} of 46-0-0. Subsequently, these applications could not be maintained and the low availability of fertilizers has been one of the causes of the current drop in yields.

On the other hand, the rethinking of the role of fertilizers in nutrient supply systems (7), including the joint application with beneficial soil microorganisms (8,9), which improve the nutritional status of plants and reduce fertilizer requirements, has boosted the development of bioproducts based on arbuscular mycorrhizal fungi (AMF) and rhizobia (10,11). Other bioproducts have also been developed, such as Quitomax®, Biobras-16®, Fitomas® and Pectimorf® based on compounds with bioactive properties (Department of Soils and Fertilizers (12), which increase yields and efficiency of the inputs applied.

Based on the results obtained with the application of bioproducts and the economic situation of the country, it was decided to implement a Bioproducts Policy in the country (13), which indicated an increase in the production and use of these products. As almost all of these bioproducts have different mechanisms of action (12) that appear to be complementary, the evaluation of the effectiveness of joint applications is based on this. Although there is information on the beneficial effects of mycorrhizal and rhizobial biofertilizer applications on beans (4), the potential of co-management should include other bioproducts with positive results in single crop applications.

Macronutrient extractions in beans are estimated at 102, 10.5 and 73.5 kg t^{-1} of N, P and K, respectively (14). With the exception of inoculants based on rhizobia with which the biological nitrogen fixation (BNF) can reach up to 40 % (15) or perhaps 60 % of the needs of this crop, the rest of the bioproducts referred to do not originate contributions of nutrients to the system and although they promote an absorption or a more efficient use of these by the plants, the sustainable management of bioproducts requires their integration with other sources of nutrients to together with the available elements of the soil to guarantee the nutrients to the crop (16).

A very little studied topic in the country has been the integration of the joint management of different bioproducts with the fertilizer doses necessary to reach the maximum yields of cultivars. The maximum experimental yields achieved under conditions of satisfactory nutrient supply depend not only on the yield potential of the cultivar for a given condition, but also on the specific climatic conditions, the agronomic management used, the water supply and the effectiveness of pest control, among others (17). In such a way that with the same cultivar, even in the same location, different maximum experimental yields can be obtained with different nutrient requirements (4).

Therefore, the objectives of this work were to establish criteria for the joint management of bioproducts for beans, their relationship with the doses of fertilizers required and with the maximum yield levels achieved. This will make it possible to make more efficient use of national production of bioproducts, as well as imports or national production of fertilizers, and thus, by increasing yields, improve the availability of beans for the population.

MATERIALS AND METHODS

Five experiments were carried out during the years 2013-2016, with one of the most widespread commercial bean cultivars in the country (CC-25-9 N). The experiments were developed in the experimental area of the National Institute of Agricultural Sciences (INCA), located in San José de las Lajas municipality, in Mayabeque province, Cuba and located at 22º59'North Latitude and -82º08'West Longitude, at 138 m a.s.l. on leached Red Ferrallitic soils (18) equivalent to eutrophic Nitisols according to the world soil referential WRB (19).

Edaphic conditions

The soils presented neutral pH-H_2O, with adequate Ca and Mg contents of 11.9 and 2.4 cmolc kg^{-1} respectively, and were typical for these soils, with a Ca/Mg ratio of 4.95. The organic matter contents, although average, indicated a good conservation status for this type of soil, subjected to continuous cultivation and located at low altitude. The available phosphorus contents were high and related to previous applications of mineral fertilizers. Potassium contents were low both absolute and relative (< 2 % in relation to exchangeable bases) and indicative of the need to attend to its supply to the crops to guarantee satisfactory yields. The contents of resident AMF spores were low.

Climatic Conditions

The locality is characterized by an average annual precipitation of 1579 mm (historical series 1968-2020). The rainy period extends from May to October with 77.2 % of the precipitation and the rest in the little rainy period. The experimental years were characterized by annual precipitation ranging between 85 and 105 % of the average

Table 1. Chemical characteristics of the soils at the beginning of the experiments and number of resident AMF spores (0-20 cm depth). INCA, San José de las Lajas, period 2013-2016

Soil	pH H_2O	MO (g kg^{-1})	P (mg kg^{-1})	Na^+	K^+	Ca^{2+}	Mg^+	spores in 50 g^{-1}
				(cmol$_c$ kg^{-1})				
Eutric Nitisol	6.7	32	144	0.10	0.24	11.5	2.4	65
CI±	0.12	1.1	5.2	0.02	0.03	0.35	0.25	10

CI±: Confidence interval at P=0.95. Average values of the different experiments. In each experiment 4 composite soil samples were taken. Chemical determinations: pH-H2O potentiometer, OM (organic matter) Walkley Black, P extraction with H2SO4 0.05 M, exchangeable cations by extraction with NH4Ac 1 M solution, pH 7, (20). Quantity of mycorrhizal spores, by the modified wet decantation method (21)

annual precipitation, with only one year below 1400 mm. The temperature regime both the annual average and the period averages were relatively similar in the four years, with values close to the historical annual average of 24.2 °C. In the rainy period, the average monthly temperatures were about 25.8 °C higher by 3.2 °C than in the low rainy period.

Experiments, treatments, and experimental design

Experiment 1. Thirteen treatments (Table 2) were evaluated in a randomized block design with four replications. The response to different combinations of EcoMic® (mycorrhizal inoculant based on INCAM-4/ *Glomus cubense* strain), Azofert-f® (rhizobium-based inoculant) and Biobras-16® (biostimulant based on brassinosteroid analogues) was studied in the presence of three fertilizer doses (36-26-34, 36-39-51 and 36-52-68 kg ha^{-1} of N, P_2O_5 and K_2O, respectively). The treatment with the 100-72-84 dose, recommended by the Technical Instructions (22) and the absolute control (0-0-0) were also evaluated. The bioproduct Fitomas-E® was applied in the background to the fertilized treatments, since it is widely used in the country, with the exception of the one that received the dose of the Technical Instructions. The sowing date was 16/1/2013.

Experiment 2. With objectives, bioproducts, fertilizer doses and experimental design similar to the previous one, but with only 11 treatments (Table 2). In this experiment, the additive effect of the different bioproducts was studied at the 36-26-34 and 36-39-51 kg ha^{-1} doses of N, P_2O_5 and K_2O. Likewise, the combined effect of the three bioproducts was also evaluated in the presence of the 36-52-68 dose. The absolute control treatment was also included. The planting date was 11/29/2013.

Experiment 3. Thirteen treatments were evaluated (Table 3) with similar objectives, fertilizer doses and experimental design to the previous experiments. In this case, the biostimulant Biobras-16® was replaced by Quitomax® (biostimulant based on chitosan hydrolysates). The additive effect of the different bioproducts was studied at doses of 36-26-34 and 36-39-51 kg ha^{-1} of N, P_2O_5 and K_2O, respectively. Also, a treatment that received all the bioproducts in the presence of higher doses of P_2O_5 and K_2O (36-52-68) was included, as well as some other reference treatments with different fertilizer doses and the absolute control. The sowing date was 11/26/2014.

Experiments 4 and 5. Both experiments were conducted with the same nine treatments (Table 4). All treatments received as a common background the dose of 36-26-34 kg ha^{-1} of N, P_2O_5 and K_2O respectively, together with the application of Fitomas-E®. The response to the combined application of EcoMic® and Azofert-f® was studied. In addition, single and combined applications of the bioproducts Biobras-16®, Quitomax® and Pectimorf® (oligogalacturonide-based biostimulant) were studied in addition to the application of EcoMic® and Azofert-f®. The experimental design was a randomized block design with four replications. Experiment 4 was planted on 26/11/2014 and experiment 5 on 26/12/2015.

General issues

The planting frame used in all experiments was 0.7 m between rows and 0.07 m between plants, for a density of 200 000 plants ha^{-1} and 14 plants per linear meter. The experimental plots were composed of 7 rows of 5 meters long. The calculation area was 14 m^2, based on evaluating the 5 central furrows and using only the plants corresponding to 4 m, not including 0.5 m at the beginning and end of each furrow.

Table 2. Treatments studied in experiments 1 and 2. Eutric nitisol Soil. San José de las Lajas, Mayabeque. Years 2013-2014

Doses of N, P_2O_5, and K_2O kg ha^{-1}	Experiment 1				Experiment 2			
	Without addition	Eco	Eco+Az	Eco+Az+Bb	Without addition	Eco	Eco+Az	Eco+Az+Bb
0-0-0	1				1			
36-26-34+ Ft	2			3	2	3	4	5
36-39-51+ Ft	4	5	6	7	6	7	8	9
36-52-68+ Ft	8	9	10	11	10			11
59-52-68+Ft	12							
100-72-84	13							

Each treatment evaluated and identified by a number, resulted from the combination of the row (set dose) with the column (application of bioproducts) in question. The column with no addition means that the treatments did not receive bioproducts and corresponds to the application alone of the fertilizer doses in each row. Ft: Fitomas®; Eco: EcoMic®; Az: Azofert-f®; Bb: Biobras-16®

Table 3. Treatments studied in experiment 3. Eutric nitisol Soil. San José de las Lajas, Mayabeque. Years 2014-2015

Fertilizer doses N, P_2O_5, and K_2O kg ha^{-1}	Experiment 3			
	Without adding	Eco	Eco+Az	Eco+Az+Qx
0-0-0	1			
36-26-34	2			
36-26-34 + Ft	3	4	5	6
36-39-51 + Ft	7	8	9	10
36-52-68 + Ft	11			12
100-72-84	13			

Each treatment evaluated and identified by a number, resulted from the combination of the row (states the dose) with the column in question. The column without addition means that the treatments correspond to the information presented in the row. Ft: Fitomas®; Eco: EcoMic®; Az: Azofert-f®; Qx: Quitomax®

Table 4. Treatments studied in experiments 4 and 5. Eutric nitisol soil. San José de las Lajas, Mayabeque. Years 2014-2016

Treatments
36-26-34 + Ft
36-26-34 + Ft + Eco+Az
36-26-34 + Ft + Eco+Az+Bb
36-26-34 + Ft + Eco+Az+Pc
36-26-34 + Ft + Eco+Az+Qx
36-26-34 + Ft + Eco + Az+ Bb+Pc
36-26-34 + Ft + Eco+Az+Bb+Qx
36-26-34 + Ft + Eco+Az+Pc+Qx
36-26-34 + Ft + Eco+Az+Bb+Pc+Qx

36-26-34: fertilizer doses kg ha^{-1} of N, P_2O_5 and K_2O respectively. Ft: Fitomas®; Eco: EcoMic®; Az: Azofert-f®, Bb: Biobras-16®; Pc: Pectimorf®; Qx: Quitomax®

Bioproducts and forms of application

Mycorrhizal inoculant. The commercial inoculant EcoMic® based on *Glomus cubense* (Y. Rodr. & Dalpé) / INCAM-4, DAOM241198/, with a minimum of 20 spores g^{-1} and ain CF1/Rhizobium leguminosarum with a titer of 108 CFU in quantities of 240 ml per 50g of seed (12). It was applied together with EcoMic® via seed coating, so that part of the seed wetting was carried out with this inoculant (10). Peundetermined amounts of rootlets and mycelium, was used. It was applied via seed coating, previously moistened with water, in quantities of 4 kg of EcoMic® per 50 kg of seed (10). This inoculant is effective for crops grown on soils with a pH-H^2O range between 5.8 and 7.2 (16).

Rhizobium-based inoculant. Azofert-f® inoculant was used, formulated liquid based on strctimorf® biostimulant. In liquid formulation. It was applied by imbibing seeds in a solution of 10 mg L^{-1} for 30 minutes (12), before coating them with EcoMic® and Azofert-f®.

Fitomas® Biostimulant. It was applied via foliar spraying at two times. In each one with a dose of 1 L ha^{-1} sprayed at 25 and 45 days after planting (das) in high volume applications, equivalent to 200 L ha^{-1} (12).

Biobras-16® biostimulant. Based on brassinosteroid analogues in liquid formulation. It was applied by foliar spraying at two times, 25 and 45 days after planting the beans (das). At each moment, the equivalent of 20 mg ha^{-1} (12) was applied, dissolved in water for high volume applications.

Quitomax® biostimulant. Based on chitosans in liquid formulation. It was applied via foliar spraying (Qxf), in two moments at 25 and 45 days, with doses of 100 mg (12) dissolved in 200 L of water and applied to one hectare (high volume).

Pectimorf® biostimulant. In liquid formulation. It was applied before sowing via seed imbibition in a solution of 10 mg L-1 for 30 minutes. (15). It was done before seed coating with other bioproducts.

In the treatments with foliar applications of several biostimulants, these were applied together in the same high volume application equivalent to 200 L ha^{-1}.

Fertilizers and cultural attentions

Different doses of fertilizers were formed from applying phosphorus and potassium by using the formula (9-13-17) and the amounts of nitrogen supplemented with urea (46-0-0). The amounts of complete formula were applied at the bottom of the furrow prior to planting and the urea was applied at 30 days to the soil around the plants and incorporated with it. In the bulk of the treatments, similar amounts of nitrogen were maintained in the order of 36 kg ha-1 of N, taking into account the application of rhizobium-based inoculants, so that the amounts of phosphorus and potassium received by the beans varied. The application 100-72-84 that responds to the recommendation of NPK fertilizers for beans in the absence of inoculation with rhizobia, as well as the cultural attentions including irrigation were carried out according to the Technical Instructions of the crop (12).

Evaluations

Soil analysis. At the beginning of each experiment and in each replicate, two composite samples were taken, each of 10 subsamples taken randomly at a depth of 0-20 cm. The determinations listed in Table 1 were made on each sample.

Harvesting. In all experiments, harvesting was carried out between 105 and 110 das, in the calculation area of each plot. The total pods were processed and the yield was expressed in t ha^{-1} of grains at 14 % moisture.

Statistical analysis. In each experiment, the normality of the data and homogeneity of variance were checked. Subsequently, the corresponding ANOVAs were performed and yields were denoted according to Duncan's test at $p \leq 0.1$

RESULTS

Experiment 1

In the presence of the common application of Fitomas-E® a positive response ($p \leq 0.1$) to mineral fertilization was presented (Figure 1) and the highest yields in the order of 2.0 t ha^{-1} were found with the application of the 59-52-68 kg ha^{-1} dose, which did not differ significantly ($p \leq 0.1$) with the 2.15 t ha^{-1} achieved when applying the higher Instructive dose (100-72-94). It was also observed that in the presence of similar amounts of nitrogen fertilizer, the use of phosphorus and potassium fertilizer amounts of 39 and 51 kg ha^{-1} of P_2O_5 and K_2O respectively, originated higher yields than those receiving 26 and 34 kg ha^{-1}; higher amounts of P_2O_5 and K_2O did not cause significant yield increases.

The response to the combined application of the four bioproducts was positive ($p \leq 0.1$) although dependent on the dose of fertilizer applied. The application in the presence of the lowest dose of fertilizer studied (36-26-34) originated lower yields and with the application of the dose of 36-39-51 already higher yields ($p \leq 0.1$) of the order of 2 t ha^{-1} were reached, which did not differ from the highest obtained, either with the combined application of the bioproducts in the presence of the higher dose of 36-52-68 or with the single application of fertilizers at the dose of 100-72-94, recommended by the Technical Instructions in the absence of biofertilizers.

In the two doses in which the effect of increasing the amount of bioproducts applied was studied (36-39-51 and 36-52-68), higher yields were achieved in such a way that the addition of EcoMic® was always superior ($p \leq 01$) to Fitomas-E® application. The additional application of Azofert-f® while achieving higher yields, did not differ significantly from the yield when applying EcoMic® + Fitomas-E®. The additional application of Biobras-16® differed significantly from the yields obtained when using EcoMic® + Fitomas-E® and although it did not significantly exceed ($p \leq 0.1$ %) the application of the three bioproducts, it was with the only combination that yields similar to the maximum yield achieved were achieved. No differences were found between these fertilizer doses.

Experiment 2

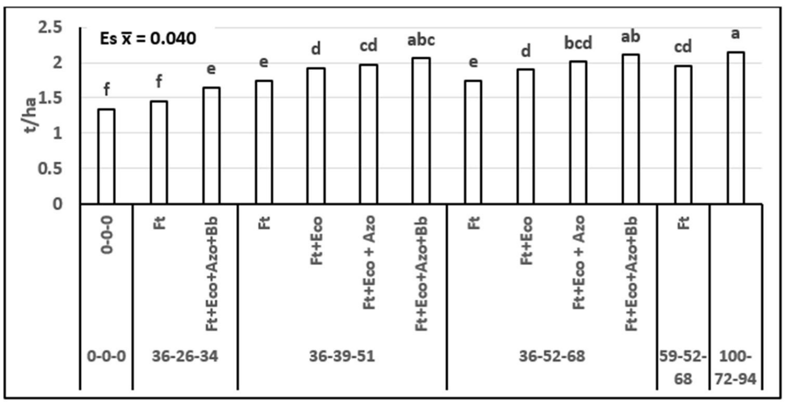
In this experiment the maximum yields reached of 1.6 t ha^{-1} were lower than those obtained in experiment 1 (Figure 2). A positive response ($p \leq 0.1$) to increasing fertilizer applications in the presence of Fitomas-E® application was also found, however, the higher yields obtained with the highest fertilizer dose studied were lower than those obtained with the combined application of the bioproducts in the presence of the lowest dose studied (36-26-34).

A positive response ($p \leq 0.1$) to EcoMic® application was found at each fertilizer dose, reaching maximum yields in the order of 1.6 t ha^{-1} already in the presence of the lowest fertilizer dose (36-26-34). Subsequent additions of the other bioproducts and fertilizer doses did not increase yields and in all cases remained in the order of 1.6 t ha^{-1}.

Experiment 3

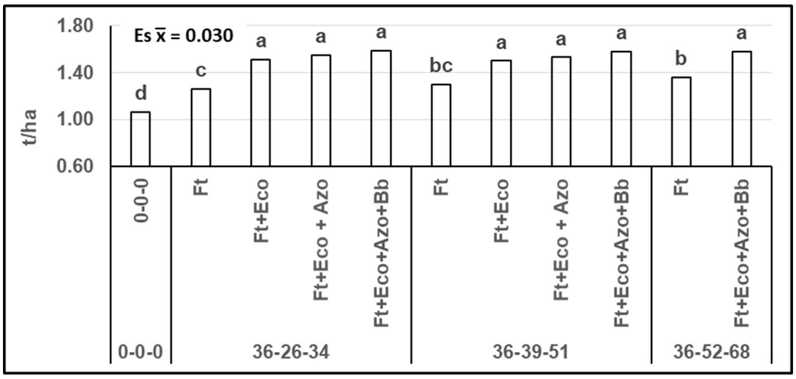
In the absence of the application of bioproducts, a positive response to increasing fertilizer application was also found (Figure 3) and maximum yields in the order of 1.95 t ha^{-1} were reached with the application of the highest fertilizer dose used (91-65-85). This maximum yield was slightly lower than that obtained in experiment 1, but higher than that obtained in experiment 2.

In this experiment, Biobras-16® was replaced by Quitomax®, both biostimulants. A significant response ($p \leq 0.1$) to bioproduct applications was found, with the highest yields with the application of the four bioproducts, although dependent on the fertilizer dose. Yields obtained in the presence of the 36-39-51 dose were similar ($p \leq 0.1$) to the experimental maximums achieved with the higher dose of fertilizer and without bioproducts. In the presence of the 36-39-51 dose, sharper differences were established between successive additions of the bioproducts, than in the presence of the 36-26-34 dose; moreover, the application of the four bioproducts in the presence of 36-26-34 originated yields lower than the maximum obtained. Applications of the bioproducts with doses higher than 36-39-51 did not significantly increase yields.



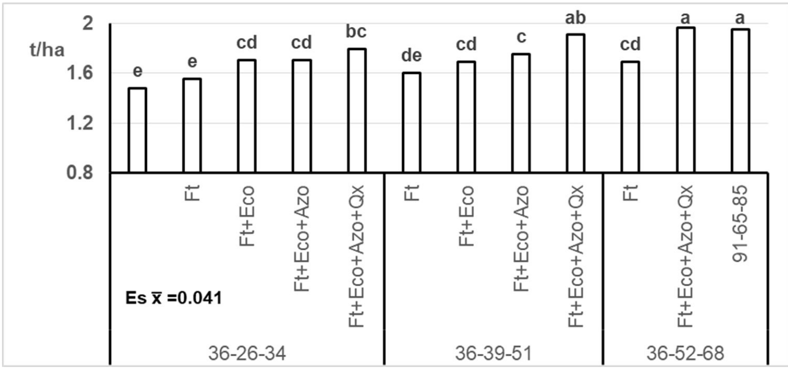
Planting date 10/12/2012. Ft: Fitomas-E®; Eco: EcoMic®; Az: Azofert-f®; Bb: Biobras-16®. Different letters correspond to significant differences at $p \leq 0.1$ by Duncan's test

Figure 1. Effect of bioproduct applications and fertilizer doses (NPK) on bean, "CC25-9N". Nutric Nitisol soil, San José de las Lajas, Mayabeque



Planting date 12/2013. Ft: Fitomas-E®; Eco: EcoMic®; Az: Azofert-f®; Bb: Biobras-16®. Different letters correspond to significant differences at p ≤ 0.1 by Duncan's Test

Figure 2. Experiment 2. Effect of bioproduct applications and fertilizer doses (NPK) on bean, "CC25-9N". Nutrient eutric Nitisol soil, San Jose de las Lajas, Mayabeque



Date of sowing 12/2014. Ft: Fitomas-E®; Eco: EcoMic®; Az: Azofert-f®; Qx: Quitomax®. Different letters correspond to significant differences at p≤0.1 by Duncan's Test

Figure 3. Experiment 3. Effect of bioproduct applications and fertilization doses (NPK) on bean, "CC25-9N". Nutric Nitisol soil, San José de las Lajas, Mayabeque

Experiments 4 and 5

The results in both experiments were similar (Figure 4 A and B). In the presence of the 36-26-39 dose, the combined application of EcoMic® + Azofert-f® + Fitomas-E® increased yields (p≤0.1) compared to the application of Fitomas-E® The single and combined additions of Biobras-16®, Quitomax® and Pectimorf® on the pool of the previous three bioproducts, presented a differentiated behavior. The greatest effects were associated with the application of Biobras-16® and Quitomax® bioproducts and especially with their combination, reaching higher yields (p≤0.1) than the rest of the treatments, which were found to be between 1.75 and 1.82 t ha^{-1}. The additional application of Pectimorf® was inferior, not increasing yields in any of the combinations studied.

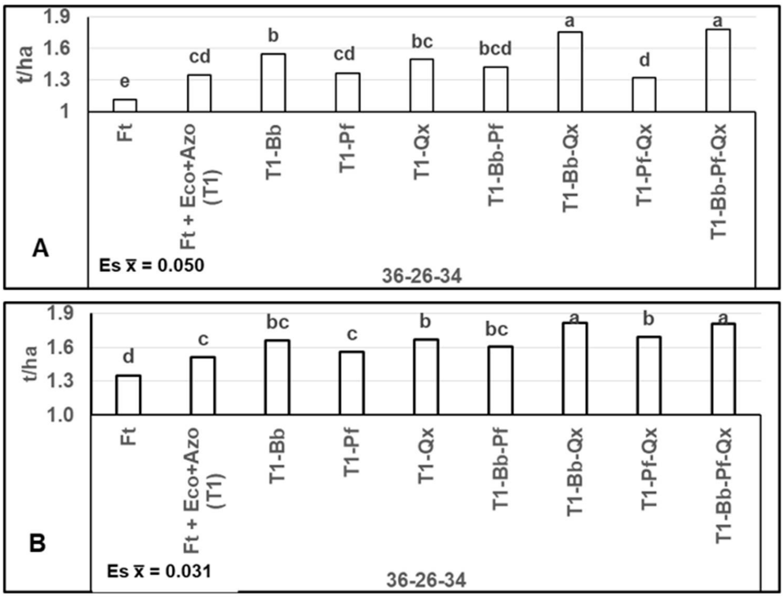
RESULTS

The results in the different experiments were quite reproducible. In two of the three experiments in which fertilizer doses and the application of up to four bioproducts (Fitomas®-EcoMic®-Azofert®-Biobras16® or Quitomax®) were studied, the maximum yields ranged between 1.95 and 2.15 t ha^{-1} and the effective utilization of the four bioproducts required the dose of 36-39-51. In the remaining experiment when the maximum yield achieved was lower (1.6 t ha^{-1}) the dose required decreased to 36-26-34, as well as only the combined application of EcoMic® + Fitomas-E® was sufficient.

In the two experiments in which up to six bioproducts were applied, the combined application of Fitomas-E® - EcoMic®-Azofert®-Biobras16®-Quitomax® was the most effective and allowed higher yields. Pectimorf® use was not effective in the combinations studied. The information seems to suggest that the yield of 1.6 t ha^{-1} achieved consistently with the 36-26-34 dose and four bioproducts (Fitomas-E®-EcoMic®-Azofert®-Biobras16® or Quitomax®), can be increased to 1.75 - 1.82 t ha^{-1} when these five bioproducts are applied together.

DISCUSSION

Beans responded positively to the application of mycorrhizal inoculants and their combination with inoculants based on rhizobia, which had been obtained previously by other researches in Cuba, in beans (4) as well as in other leguminous plants (23-25), corroborating the general statements about the effectiveness of the tripartite symbiosis rhizobia and mycorrhizae in leguminous plants (26,27).



4 A: Experiment 4, 4 B: Experiment 5. Date of sowing 12/2014 y 12/2015 respectively. Ft: Fitomas-E®; Eco: EcoMic®; Az: Azofert-f®; Bb: Biobras-16®; Qx: Quitomax®; Pf: Pectimorf®. Different letters correspond to significant differences at p≤0.1 by Duncan's Test

Figure 4. Effect of different combinations of bioproducts in the presence of the fertilization dose 36-26-24 on the bean "CC25-9N". Nitisol eutrophic soils, San José de las Lajas, Mayabeque

The addition of different biostimulants to the application of EcoMic® and rhizobia was positive, demonstrating the hypothesis that the combined management of these bioproducts with complementary mechanisms of action should show satisfactory results. However, the effectiveness of the combined application of several bioproducts will depend on the maximum experimental yield obtained, the nutrient supply and of course the bioproducts applied.

If the application of nutrients is less than necessary, the effectiveness of the combined application of several bioproducts (more than two) will be limited, since only the benefits obtained with the application in this case of two bioproducts (EcoMic® and Fitomas®) will guarantee these requirements. A similar result is obtained if in the presence of an adequate supply of nutrients the experimental conditions limit the obtaining of high yields, then in addition to requiring lower amounts of nutrients, the benefits of the combined application of all the bioproducts will not be necessary either.

If the experimental conditions allow the expression of higher yields, the effectiveness of the bioproducts is increased, a greater number of bioproducts can be applied, and although greater amounts of fertilizers may be required, these amounts will always be less than those needed to achieve those yields in the absence of the application of the bioproducts. A similar dependence has been found for the effectiveness of mycorrhizal inoculants related in this case to the yield potentials and planting time of different sweet potato cultivars (28), so that the differences between the yields caused by the different inoculants are more clearly established at the time when the highest yields occur.

The combined application of different bioproducts with mycorrhizal inoculants and fertilizer doses has been satisfactorily evaluated in different crops such as coffee (29) and cocoa (30), soybean (31), corn (32), and forage grasses (33), and in all cases has led to guarantee high yields with lower fertilizer doses, although in these studies generally only two bioproducts were combined. In none of these studies was the relationship between the maximum experimental yield levels and the effectiveness of the bioproducts evaluated.

Although the presence of mycorrhizal inoculants in the bioproducts applied is the basis for the increase in the efficiency of nutrient uptake by the crops (16), a direct relationship was also established between the maximum yield levels achieved and the doses of phosphoric-potassium fertilizers, which can be explained by the higher nutrient requirements as yields increase. The higher nitrogen requirements associated with higher yields seem to be guaranteed by the biological nitrogen fixation (BNF) associated with rhizobia and the synergistic effect on BNF of the joint management of rhizobia and mycorrhizal inoculants (26,27).

Interestingly, the combined application of the biostimulants Quitomax® and Biobras-16® together with EcoMic®, Azofert® and Fitomas-E®, up to five bioproducts, significantly and consistently increases the yields achieved with the application of the 36-26-34 kg ha^{-1} dose and four bioproducts (EcoMic®, Azofert®, Azofert®, Fitomas-E® and Biobras-16®), Fitomas-E® and Biobras-16® or Quitomax®) which seems to indicate that these two biostimulants not only present complementary mechanisms but also the satisfactory effectiveness of their joint application, with increases in yields and therefore in the agronomic efficiency of the fertilizer applied, improving the benefits and the yield ceiling reached with that dose even when four of these bioproducts were present.

As a result of the experimental schemes followed, it was not possible to evaluate the effect of the application of these five bioproducts in the presence of the higher dose of 36-39-51 kg ha-1 and to know if under these conditions it was also effective and surpassed the results and yields achieved with the application of the four bioproducts (Fitomas® -EcoMic®-Azofert®-Biobras16® or Quitomax®). This should be evaluated in subsequent experiments.

However, the results are also indicative of the feasibility of applying these five bioproducts for bean production, even in the presence of the low fertilizer doses that farmers have been commonly applying (36-26-34 kg ha^{-1}). This result has a high practical impact because it supports the joint management of these bioproducts in bean production and supports that at least these five bioproducts present complementary mechanisms, whose effects are clearly expressed, contributing to better exploit the potential yield of the cultivars.

The inclusion of the biostimulant Pectimorf® did not show positive effects when it was applied in combination in the bioproduct package; however, other authors (34,35) have reported favorable effects when applied alone or combined with rhizobia (Azofert-f®) in the bean crop. In other words, it is not that Pectimorf® is not an effective product, but it seems that its effects on beans have already been achieved previously with the combination EcoMic®+Azofert® + Fitomas-E® and therefore its application does not bring additional benefits. This situation is different from that already discussed when applying Quitomax® and Biobras-16®.

CONCLUSIONS

The joint application to beans of different bioproducts such as EcoMic®, Fitomas-E®, Azofert-f®, Biobras-16® and Quitomax® is beneficial, increases yields and always requires lower amounts of fertilizers (NPK) than when the bioproducts are not applied. In the presence of the application of four bioproducts (EcoMic®-Fitomas-E®-Azofert-f® and Biobras-16® or Quitomax®), the maximum yield levels of 2 to 2.15 t ha^{-1} demand a greater amount of fertilizers than when maximum experimental yields of 1.6 t ha^{-1} are reached, for which the application of the 36-26-34 dose is sufficient. The joint application of Quitomax® and Biobras-16® for a total of five bioproducts is effective and improves the yield obtained with the application of four bioproducts in the presence of the lower dose of 36-26-34. The inclusion of Pectimorf® in the bioproduct package is not effective.

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