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Efficient Microorganisms as Biostimulators to Enhance Yields of *Phaseolus vulgaris L.* Cultivar Delicia Rojo 364

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ABSTRACT

The aim of this paper was to evaluate the effect of efficient microorganisms (EM) on beans cv. Delicias Rojo 364. The study took place on La Rosa Farm, 3 km northeast of Esmeralda municipality, on a typical fersialitic brown reddish soil, between January and March, 2014. A randomized block design was made with four treatments (inoculation of the seed with foliar application every 7 days before planting; foliar application after germination; inoculation of the seeds before planting without foliar application of efficient microorganisms (EM); and a control), and four replications. The indicators evaluated were plant height, stem thickness, number of internodes, amount of pods per plant, seeds per pod, and crop yields. The best results were observed after the inoculation of the product to the seed and foliar application, with the best yields (1.82 t/ha), much better than the control (0.7 t/ha).

KEYWORDS:/ efficient microorganisms, biostimulators, beans, Phaseolus vulgaris L.

INTRODUCCION

Beans (*Phaseolus vulgaris L*.) play an important role in world agriculture in terms of cultivated area and consumption and as an indispensable element in the diet, mostly in Central and South America, the Far East, and Africa. Beans are the most important crop cultivated in tropical and subtropical regions for human consumption, as the cheapest source of protein and an indispensable component of the diet. On top of that, beans are an important source of income for small farmers (Martínez, *et al*., 2004. Cited by Calero, Y. Pérez & Pérez 2016).
In recent years, it became the main income-generating crop on Cuban farms (Alonso, 2011). Although it is a commonly cultivated crop among small Cuban farmers, large bean-growing areas do not exist in the country, especially due to the low yields, in spite of the efforts made to reduce imports and hard currency payments.

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The 166th item of the Guidelines of the Social and Economic Policy of the Party and the Revolution approved during the 7th Congress of the Communist Party of Cuba, and passed by the National Assembly of the People's Power (2016), calls for the need to support bean production programs that favor yield improvements, reduction of imports, and increased consumption, in a scenario where bean yields is a major issue to address. p. 37.

Bean production is affected by several agronomic factors, like soil fertility, pests and diseases, soils with poor physical conditions, deficient seed quality and preservation, and adverse climatic conditions (Ministry of Agriculture, MINAG, 2012). In Cuba, the drop observed in yields is mainly originated by plant nutritional deficit. This crop does not tolerate humidity, though it needs adequate water distribution. Hence, irrigation should be based on the type of soil and the sowing season. Furthermore, rain excess may destroy plants by asphyxiation, cause root decay, and increase the outbreak of diseases. High temperatures, can seriously limit the production of this legume.

Today, bean production in the municipality of Esmeralda is facing various problems related to quality reduction and low yields, which are attributed to different factors, such as, inappropriate nutrition management practices, perhaps due to the lack of information about growth biostimulators. The utilization of biostimulators has become a useful and cost-effective practice for small and mid-sized farmers, with improvements in soil fertility and preservation.

Many natural products, such as biopesticides, biofertilizers, and biostimulants, have been used to improve ecological management of agroecosystems, and increase productions (Peña, Rodríguez & Santana, 2015).

The application of plant growth stimulants to increase crop quality and yields is a basic and relevant aspect of agricultural research due to the social and economic implications they bring.

Mostly agricultural, Cuba must ensure increased food production levels to prevent imports and raise yields. It should be accomplished by reducing synthetic fertilizer and pesticide use, the two with the highest negative impact, which also cause pollution of the ecosystems.

The natural microorganisms present in the soil favor the physical and chemical conditions of the land. Various compounds transformed by these microorganisms in the soil can contribute to favorable biostimulation processes in plants. Soils contain beneficial organisms from three main groups: phototrophic bacteria, lactic acid bacteria, and yeasts (Rodríguez, 2006; Toalombo, 2012). When interacting with organic matter, the efficient microorganisms secrete beneficial substances, like vitamins, organic acids, chelated minerals, and antioxidants that stimulate the growth of plants (Méndez, Chang & Salgado, 2011; Hernández, 2013)**.**

The utilization of efficient microorganisms to propagate plants is intended to stimulate germination, rooting, and growth, through the action of hormones, amino acids, and antioxidant substances. It is also meant to establish beneficial microorganisms that can compete with pathogenic microorganisms in the root system, (Gil *et al*., 2005. Cited by Calero *et al*., 2016).

Accordingly, the use of efficient microorganisms is an easy and economical alternative to improve bean development and yields. Therefore, the aim of this research was to determine the influence of efficient microorganisms on bean (*Phaseolus vulgaris L.)* cultivar Delicia Rojo 364 growth and development, on fersialitic brown reddish soil, typically found in Esmeralda.

MATERIALS AND METHODS

This study took place on La Rosa Farm, 3 km northeast of the municipality of Esmeralda, as part of the suburban agriculture project, in the province of Camagüey, located on the 21º 50´ 5´´ north latitude, and 78º 06´ 15´´ west longitude, 27.5 m above sea level (El Carmen Cartographic Chart 4680-ll, scale 1-25000).

The experiment was conducted between January 12 and March 28, 2014, on typical brown reddish fersialitic soil, with a mean fertility pH 7.1, and medium calcium and organic matter contents. The assimilable phosphorus and potassium are in the high and mid ranges, respectively. The soil is slightly saline, poorly eroded, with an effective depth of 30.0 cm, and clay loam.

The climatic variables observed in the area during the experiment were relative humidity (%), precipitations (mm), and mean temperature (°C), based on the data collected by the provincial meteorological station in Camagüey.

Table 1: Climatic variables observed

Month	Mean temp.	Relative humidity	Precipitations
January	29.4ºC	82%	34.0 mm
February	29.6ºC	80%	22.7 mm
March	30.0ºC	73%	53.8 mm

A randomized block design with 4 treatments and 4 repetitions was used, for a total of 16 plots (6 m wide x 10 m long) in a 60 m^2 surface. Soil preparation was made according to the standards of the Bean Technical Institute (MINAG, 2009).

The procedure used consisted in mixing 16 L of water with 2L of molasses from sugar cane. Then, 7 lb. of solid microorganisms from the Indio Hatuey Experimental Station of Pastures and Forages (Matanzas province) were added. The mix was dissolved by shaking thoroughly; then it was poured into a plastic container with a screw top, and the content was kept air-tight. Following 12 days of rest, the liquid was sieved and put into another similar container. When the efficient microorganism (EM) was obtained, the seed was prepared for sowing. Accordingly, 1.5 ml was dissolved in a liter of water in order to inoculate the seed for 2 h prior sowing. After plant germination (7 days), a 5 ml dose of EM, dissolved in 16 L of water, was applied to the leaves.

Table 2: Experimental scheme

Treatments	Efficient Microorganisms (EM)	Application manner	Application frequency	Dose I/ha
T1	Inoculation of the seed before sowing, with a foliar application every 7 days.	Seed and leaf immersion	Foliar every 7 days.	1.5
T2	Foliar application after germination.	Foliar	Foliar every 7 days.	1.5
T3	Inoculation of the seed before sowing, without foliar application of efficient microorganisms (EM).	Seed immersion	Sowing	1.5
T4	Control	Control	No application	0

Sowing took place on January 12, 2014, within the optimum season for the crop (MINAG, 2012), manually, 1 cm deep, by direct sowing (0.70 m x 0.70 m), and 0.49 m^2 of vital growth space between plants.

Irrigation and other tilling and sanitary actions were performed according to the Technical Instructive for Beans (MINAG, 2012). Harvest was made 75 days after crop germination. The calculation of plot surface area for each treatment was evaluated. Humidity was 14%. Weight was determined with a certified digital scientific balance.

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The indicators evaluated and measured were,

- Plant height: It was measured from the soil surface to the growth point, on days 20, 30 and 60, using a 50 cm ruler.
- Stem thickness: The lowest third of the plant stem was measured with a gauge caliper.
- Pods per plants: Count was made 7 days before harvest.
- Seeds per pods: Ten pods from five plants per treatments were selected for seed counts.
- Yields: All the plants from the calculation area in every plot were harvested, the yields were expressed in t/ha.

Information was processed with SPSS software, version 11.5, for Windows. The means were compared using the Duncan's Multiple Range test, with 5% error probability (Duncan, 1965).

RESULTS AND DISCUSSION

Table 3 shows the behavior of plant height. Significant differences were observed in growth dynamics on days 20, 30, and 60 in the treatments evaluated. The greatest average value of height was observed in treatment 1 (seed inoculation before sowing, with foliar application every 7 days), with statistical differences from the control (22.7 cm higher on the 60th day); treatments 2 (T2) and 3 (T3) showed similar values, though they differed (7.42 and 7.9 cm, respectively) from the control. This result demonstrated that the application of biostimulators that secrete beneficial substances promote plant growth and development.

Similar results to this were achieved by Pérez (2012) and Cisnero (2013), when they evaluated the use of biostimulants in plant growth, corroborated by López & Montejo (2013), who used organic enhancers to increase crop yields in urban and suburban agriculture, in the municipality of Camagüey.

Table 3: Plant height (cm)

Treatments	20 days	30 days I	60 days
T1	7.4a	14.5^{a}	35.8^{a}
T2	4.55^{b}	8.74^{b}	20.52^{b}
T3	4.45^{b}	8.77^{b}	21.0^{b}
Control	3.22^{c}	6.62^{c}	13.1^{c}
ESx	0.1355	0.1264	0.5123

The analysis of stem thickness is shown in table 4, where treatment 1 had the best results, according to the dynamics of evaluations. It differed 2.2 mm from the control 60 days after. Treatments 2 and 3 did now show any significant differences between them, but they differed from the control in 1.35 and 1.08 mm, respectively. All the treatments were statistically significant in relation to the control, which produced the lowest index (3.07 mm). These results coincided with the reports of plant growth and development, which evidenced that the application of treatment 1 produced the highest possible stem thickness. The results of this trial were similar to the ones achieved by Nápoles (2013), upon evaluating the utilization of biostimulators of plant growth, corroborated by Saborit (2013), who used these products for foliar immersion to reproduce guava seedlings (*Psidium guajava L.*) through scion.

Table 4: Behavior of stem thickness (mm)

Treatments	20 days	30 days I	30 days
T1	3.37[a]	4.0 [a]	4.0 [a]
T2	2.3 [b]	3.4 [b]	4.42[b]
T3	2.2 [b]	3.2 [b]	4.15[c]
Control	2.2 [b]	3.2 [b]	4.15[c]
ESx	0.1402	0.1220	0.0528

The analysis of pods per plant showed highly significant differences between the treatments evaluated, which are shown in table 5. Treatment 1 (T1) showed revealing differences in relation to the other variants studied, 8.5 pods more than the control. Treatments 2 and 3 did not differ significantly between them. Compared to the control, there were 3 pods more per plant, on average. The lowest results were observed in the control, where no efficient microorganisms were applied. The results of this trial were similar to the ones reported by Pérez (2012) and Cisnero (2013) in beans, cultivars Delicias rojo 364 and CC-25-8.
These results corroborated the reports of Poey, Olivera, Calero, Melendez & Sánchez (2012), who said that the utilization of efficient microorganisms for this crop favored an increase in the number of pods per plants. Moreover, previous research done by Calero, Fuentes & Olivera, (2011), showed the best results with the use of the above-mentioned dose.

Table 5: Number of pods per plants (U)

Treatments	Pods per plants
T1	16,0[a]
T2	11.0 [b]
T3	10.5 [b]
Control	7.5c
ESx	0.35

The average number of seeds per pod is one of the most important indicators of bean yields. The study showed the differences between the variables used in the research (table 6); the highest mean was observed in treatment 1 (T1), with marked differences in relation to the other treatments. Compared to the control, it had 3 beans more per pod. Treatments 2 (T2) and 3 (T3) did not show any significant differences between them. However, they were 1.3 beans more per pod than the control, which had the lowest values. These results were better than the ones reported by Torres (2006), who achieved maximum and minimum values of 5.80 and 4.14, respectively, in a study of cultivars of black beans, in the municipality of Majibacoa. The outcome of this trial is similar to the reports made by Cisnero (2013), using biostimulants in foliar applications to beans (Delicias rojo 364). Furthermore, the highest values of beans per legume were accomplished by López & Pouza (2014).

Table 6: Behavior of seeds per pods (U)

Treatments	Seeds per pods
T1	8.1[a]
T2	6.3 [b]
T3	6.4 [b]
Control	5.1c
ESx	0.37

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Table 7 shows an analysis of yields. Treatments 1, 2, and 3 somehow influenced this factor, evidencing the dynamics of evaluations, with no significant differences among them. Regarding the control, the indexes observed were higher, due to its inferior results. It demonstrated that the utilization of biostimulators favored crop yields. The results achieved in this trial were better than the ones achieved by Leiva and Reyes (2012), who used two solutions of efficient microorganisms. Moreover, Pérez (2012) obtained yields of 0.86-2.75 t/ha in the edaphoclimatic conditions of the municipality of Vertientes.

This corroborates the reports made by Chailloux, Hernandez, Faure, & Caballero (1996), who claimed that this crop has potential yields of 4 t/ha^{-1}; however, the yields in Cuba are 0.63-0,7 t/ha^{-1}, as a result of nutritional deficiencies and pest attacks.

Table 7: Behavior of crop yields (t/ha)

Treatments	Crop yields
T1	1.82[a]
T2	1.73 [b]
T3	1.70 [b]
Control	0.71c
ESx	0.70

CONCLUSIONS

The utilization of efficient microorganisms as biostimulators for the production of *Phaseolus vulgaris L.* (cultivar Delicia rojo 364), increased agroproductive indicators, such as plant height, number of pods per plants, average of seeds per pod, and yields, compared to the control.

The economic analysis showed a positive agronomic effect of the efficient microorganisms used as biostimulants on the production of beans (cultivar Delicia rojo 364).

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