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Effect of different application forms of efficient microorganisms on the agricultural productive of two bean cultivars

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Efecto de diferentes formas de aplicación de microorganismos eficientes en la productividad agronómica de dos cultivares de fríjol

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ABSTRACT

Keywords:
Biofertilizer
Foliar application
Soil inoculation
Yield

The use of Efficient Microorganisms (EM) can be an effective alternative to improve plant growth and yield in the bean cultivation. Therefore, different forms of application of efficient microorganisms were evaluated in the production of two cultivars of the common bean from November of 2013 to March of 2014. Two factors were studied; the first one was comprised of the two cultivars, Velazco Largo (VL) and Cuba Cueto (CC-25-9-N). The second factor consisted of four treatments with EM; without EM (control), soil inoculation (100 mL L^{-1}), foliage applications (100 mL L^{-1}), and the combined soil inoculation (100 mL L^{-1}) plus foliar applications (100 mL L^{-1}). The experiment was carried out in a randomized block design, in factorial outline 2×4, with three repetitions. The agronomic indicators were evaluated as the number of leaves per plant, the height of plants, number of pods per plant, number of seeds per pod, the mass of 100 seeds (g) and the yield (t ha^{-1}). The results showed that the different forms of application of efficient microorganisms stimulated the agronomic indicators evaluated in both crops. The associated applications between the inoculation of the soil and foliage applications of efficient microorganisms provided better results, producing increments in the yield of 1.13 t ha^{-1} in VL and 2.15 t ha^{-1} in CC-25-9-N.

RESUMEN

Palabras clave:
Biofertilizante
Aplicación foliar
Inoculación al suelo
Rendimiento

El uso de Microorganismos Eficientes (ME) puede ser una alternativa efectiva para mejorar el crecimiento y el rendimiento del cultivo de fríjol. Por lo tanto, fueron evaluadas diferentes formas de aplicación de microorganismos eficientes en la producción de dos cultivares de fríjol común, de noviembre de 2013 a marzo de 2014. Se estudiaron dos factores, el primero conformado por dos cultivares de fríjol común, Velazco Largo (VL) y Cuba Cueto (CC-25-9-N), y el segundo compuesto por cuatro tratamientos con ME; sin ME (0), inoculación al suelo (100 mL L^{-1}), aplicaciones foliares (100 mL L^{-1}) y la inoculación al suelo (100 mL L^{-1}) más aplicaciones foliares (100 mL L^{-1}), distribuidos en un diseño en bloques al azar, en esquema factorial 2×4, con tres repeticiones. Fueron evaluados los siguientes indicadores agronómicos, número de hojas por planta, altura de las plantas, número vainas por planta, número de semillas por vaina, masa de 100 semillas (g) y el rendimiento (t ha^{-1}). Los resultados mostraron que las diferentes formas de aplicación de microorganismos eficientes estimularon los indicadores agronómicos evaluados en ambos cultivares, siendo la aplicación asociada entre la inoculación al suelo y aplicaciones foliares de microorganismos eficientes la que proporcionó mayores resultados al producir incrementos en el rendimiento de 1,13 t ha^{-1} en VL y 2,15 t ha^{-1} en CC-25-9-N.

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For the next 40 years, food security will be a great challenge for the world because of the continuous increase of the population and the high consumption index growth (Ma *et al.*, 2016). The bean *Phaseolus vulgaris* L. (Fabaceae) is the most important grain consumed by humans in the world. In nutritional terms, these seeds are a source of protein, minerals, and vitamins (García-Fraile *et al.*, 2012).

Many agricultural soils are lacking enough quantities of one or more essential nutrient for these plants; as a result, their growth and development can be limited. The farmers become dependent on chemical products, as sources of fertilizers, to avoid this problem and to obtain better yields (Glick, 2012). Although the chemical fertilizers help the plants to grow, their environmental effect can be adverse, relating to fertilizers rich in phosphorus, potassium, and nitrogen (Adesemoye and Kloepper, 2009).

A less aggressive practice to the environment used to improve the nutrition of the plants has been the use of microorganisms as biofertilizers in the plants' cultivation. The rhizobacteria, which are plant growth promotors (PGPR), stood out as biofertilizers because these microorganisms adapt and grow quickly around the roots of the plants (Ahirwar *et al.*, 2015; Ul Hassan and Bano, 2015).

The response of the plant to the inoculation of PGPR varies considerably according to the rhizobacteria's species; the host, the soil type, and the density of the inoculum, the environmental conditions, and the inoculation method could affect such response (Shah *et al.*, 2017). The method of PGPR incorporation has an influence on the establishment and the permanency of bacterial populations in the rhizosphere and indirectly affects the growth promotors (He *et al.*, 2016).

On the other hand, in soils managed by organic inputs, exudates from bacteria, fungi, decomposed cells as well as plant and animal residues boost the soil's organic matter, which in turn improve the soil structure, function, and quality (Vejan *et al.*, 2016). The process of colonization, for some bacteria, is slow and not very effective because their growth and distribution through the rhizosphere depend on the soil's humidity, pH, temperature, microbial antagonism, competition space, perspired radicals, as well as the bacteria's physiological state. According to these factors, solo cells can proliferate quickly and invade the roots. A great number of bacteria will be able to promote the growth of the plants in an effective way (Gupta *et al.*, 2015; Pathak *et al.*, 2017).

The efficient microorganisms (EM) is a discovered and developed technology by professor Teuro Higa (Higa and Parr, 1994). They found that the success of its effect was in its mixture. From then on, this technology has been investigated, developed, and applied in a multitude of agricultural and environmental areas. It is used in more than 80 countries worldwide (Arias, 2010). Authors like Pedraza *et al.* (2010) state that the fundamental principle of this technology consists of the introduction of a group of beneficent microorganisms to improve the physical-chemical conditions of the soil.

The use of EM has been favorable for agriculture. Diverse studies have reported beneficial effects when they are introduced to common bean production (Calero *et al.*, 2016, 2017, 2018). They also have improved and benefitted the farmers (Luna and Mesa, 2016). It is well-known that bean production is low and is essential in the diet of the Cuban residents. Therefore, it was considered convenient to test the following hypotheses. Firstly, the application of EM could stimulate and increase the morphometric and productive parameters in two cultivars of common bean; and secondly, it is possible to maximize the beneficial effect of EM on increasing yields by the combined soil inoculation and foliar spraying. It was evaluated whether EM supplied in different forms increases the productivity of two cultivars of common bean, Velazco Largo and Cuba Cueto.

MATERIALS AND METHODS

Plant grow conditions

The experiment was carried out at the Collective farmer "Martires de Taguasco" (22°6'17.588'' N; 79°22'33.544'' W) in Sancti Spíritus, Cuba. The varieties, Velazco Largo (VL) and Cuba Cueto (CC-25-9-N) were donated by the Provincial Company of Seeds of Sancti Spíritus, with 96 to 97% germination, respectively. VL presented red-colored grains, a potential yield of 2.3 t ha^{-1}, a habit of growth type I, and a cycle of 72 to 77 days. CC-25-

9-N presented black colored grains, a yield of 2.7 t ha^{-1}, a habit of growth type III, and a cycle between 75 and 80 days. The cultivation was carried out in November of 2013 and the harvest in March of 2014. The cultivation was done manually at 0.60 m between rows and 0.07 m between plants. The climatic variables during the development of the research were registered by the Municipal Station of Hydraulic Resources of Cabaiguan, Sancti Spiritus, Cuba. The daily average temperature was 23.22 °C, the relative humidity 77.65% and the accumulated precipitation of 98.56 mm. The soil was classified as Brown Carbonated Sialitic by following the method by Hernández *et al.* (2015), being denominated as Cambisol (FAO, 2015).

Experimental design and treatments

The experimental design adopted was randomized blocks, factorial outline at 2×4. Two factors were studied; the first was comprised of two cultivars of common bean, VL and CC-25-9-N. The second consisted of four treatments with EM: control absence of EM (0), 100 mL L^{-1} of EM via soil inoculation (I), 100 mL L^{-1} of EM via foliar applications (F) and the combined application of via soil inoculation (100 mL L^{-1}) and foliar spraying (100 mL L^{-1}) (I+F). They were repeated three times, which formed 24 experimental parcels of 9.60 m^2. The useful area was 3.36 m^2, and the total area was 0.23 ha. The inoculation to the soil with EM was carried out before depositing the seeds, subsequently proceeded by the sowing. The foliate application was carried out with the support of a manual sprayer (ECHO MS-21H) of 7.60 liters of capacity, applying 40 L ha^{-1} of both application mode via soil and foliar spraying, in the vegetative (V4) and reproductive (R5 and R6) stages.

Bioproduct characteristics

The inoculation of efficient microorganisms EM-50 was acquired at the Labiofam of Sancti Spíritus, composed by *Bacillus subtilis* nato B/23-45-10 (5.40×10^4 colony-forming units (CFU) mL^{-1}), *Lactobacillus bulgaricum* B/103-4-1 (3.60×10^4 CFU mL^{-1}), and *Saccharomyces cerevisiae* L-25-7-12 (22.30×10^5 CFU mL^{-1}), with certificate of quality emitted by Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar (ICIDCA), code R-ID-B-Prot-01-01. The means used to obtain this bio preparation were industrial waste obtained from molasses, whey milk, rice waste mixed with leaf litter collected from a bamboo area. The methodology of Olivera *et al.* (2014) was followed.

Agro-technical management

The soil preparation, pest management, irrigation (by spraying), among other agro-technical cultivation practices were carried out following the recommendations and instructions from Faure *et al.* (2014), highlighting that no mineral or organic fertilizers were applied at any moment of the cultivation, and the practices of cleaning/weeding were carried out manually.

Plant growth parameters

The evaluated variables corresponded to the recommended descriptors for the growth and development stages of the crop (CIAT, 1987). The samplings were carried out on the effective area of the plots, and 45 plants per treatments (15 replications) were evaluated. The indicators were the number of leaves per plant (LP) and the plant height (PH): from the cultivation date until evaluation, up to 50% of the plants presented the floral clusters (R5). All the trifoliate leaves per plant were counted (the support of a calibrated ruler was used). The number of pods per plant (PP) was evaluated when concluding the crop cycle (R9): the resultant pod count per parcel carried out on all plants was used to determine the average. The number of seeds per pod (SP) was determined by counting all the seeds contained within. The mass of 100 grains (M100) (g) was the result of the average of four samples of 100 grains in each experimental unit. The yield (t ha^{-1}) was obtained by converting the production of each parcel (kg) to t ha^{-1}.

Statistical analysis

It was used a factorial analysis to test the main effects of the four levels of EM application (EM) and two bean cultivars (VL and CC-25-9-N) and their interactions (EM × CV). Data obtained for the applied treatments were analyzed assuming normality and significance of variance with the Shapiro-Wilk and Fisher tests (P<0.05), respectively. Once these assumptions were verified, data were subjected to a two-way ANOVA. Mean values were compared using the test of Multiple Ranges of Tukey (P<0.05). All analysis was performed using the statistical software AgroEstat® (Barbosa and Maldonado, 2015).

RESULTS AND DISCUSSION

Effect of EM applications in the morphological parameters

There was a significant (*P*<0.05) and interactive effect of EM on the plant height of the two cultivars of common bean VL and CC-25-9-N (Figure 1). CC-25-9-N was superior to VL in the absence or presence of EM treatments. In both cultivars, all the EM treatments increased PH compared with the control (EM (0)). Especially, the EM (I+F) treatment surpassed the cv. VL by 5.12 cm and in the CC-25-9-N by 4.15 cm concerning the EM (I) and EM (F) treatments, and it increased both cultivars' height up to 14.11 and 8.86 cm, respectively, compared with control (EM (0)). Positive effects were achieved with the EM (I+F) treatment because it increased PH in the cv. VL by 66%, while the cv. CC-25-9-N by 59%, compared with that of the EM (0) treatment in both crops.

It is well-known that the soil inoculation with microorganism solubilizers/dissolvers of phosphate (bacteria, fungi, and actinomycetes with the capacity to break down minerals) can be fixed in the soil and can be used by the plants for their nutrition (Beltrán, 2014). These microorganisms show other activities that promote the vegetable growth such as supplying gibberellins, cytokines, ethylene, symbiotic nitrogen fixation; which are considered a potential and efficient bioagents to improve plant growth (Banerjee *et al.*, 2010). In both cultivars, all EM application was effective to increase the plant height compared with the control (EM (0)), especially the EM (I+F) treatment (Figure 1). This beneficial effect of the application of EM via soil or foliar spraying increasing the PH in beans plants as previous reported (Calero *et al.*, 2016, 2017).

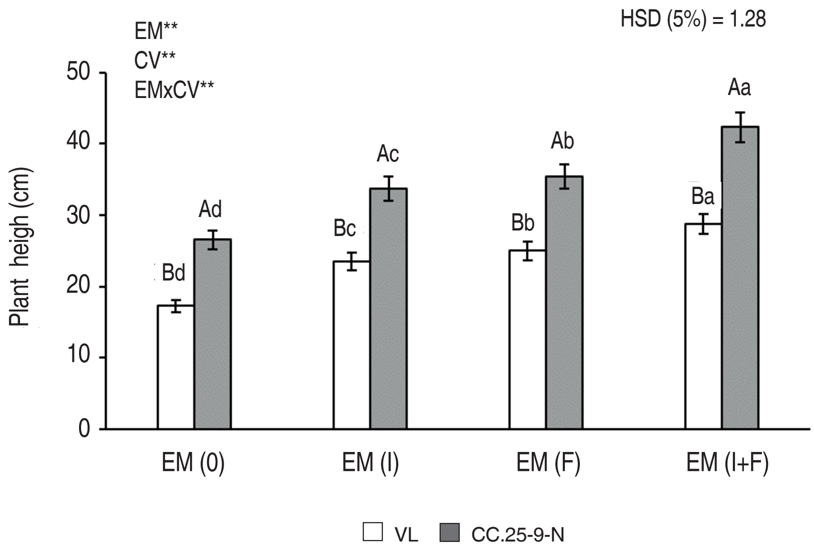

Figure 1. Plant height average of two bean cultivars (VL and CC-25-9-N), in function of EM treatments, without EM (0), soil inoculation EM (L), foliar applications EM (F) and the combined soil inoculation and foliar root applications EM (I+F).
Different small letters indicate significant differences between EM treatments at the same cultivar; different capital letters indicate significant differences between cultivars at the same level of EM (*P*<0.05). ***P*< 0.01; EM × CV, efficient microorganism–cultivar interaction; HSD, honest significant difference.

The comparison of means revealed that the interaction between EM and CV was significant (*P*<0.05) on the number of leaves per plant in both bean cultivars (Figure 2). The cv. CC-25-9-N had a better response than the cv. VL in the production of the LP in absence or presence of EM.

The favorable response in the crops, under the influence of different applications of EM on the number of leaves per plant (Figure 2), suggests that the cv. CC-25-9-N showed a better genetic expression for this character regarding the cv. VL on the physiologic stage R6. The form of application of EM (I+F) surpassed the individual treatments and the control (without application).

The results demonstrated the positive effects attained in the increment of the production of leaves per plant with the application of the different forms of efficient microorganisms. Consistently with other studies on beans, it was observed that the addition of EM (F) or EM (I) improved the number of trifoliate leaves, thus increasing yield production (Calero *et al.*, 2017, 2018, 2019a). This benefit of EM increasing the leaf production is known in different species grown under different conditions, such as onion (Liriano *et al.*, 2015), tomato (Olivera *et al.*, 2015), strawberry (Álvarez *et al.*, 2018) and tobacco (Calero 2019 c). On the other hand, it may be possible to increase the PP by the inoculation of soil or seeds with beneficial microorganisms. It also improves plant architecture by producing some substances that help in plant growth (Banerjee *et al.*, 2010).

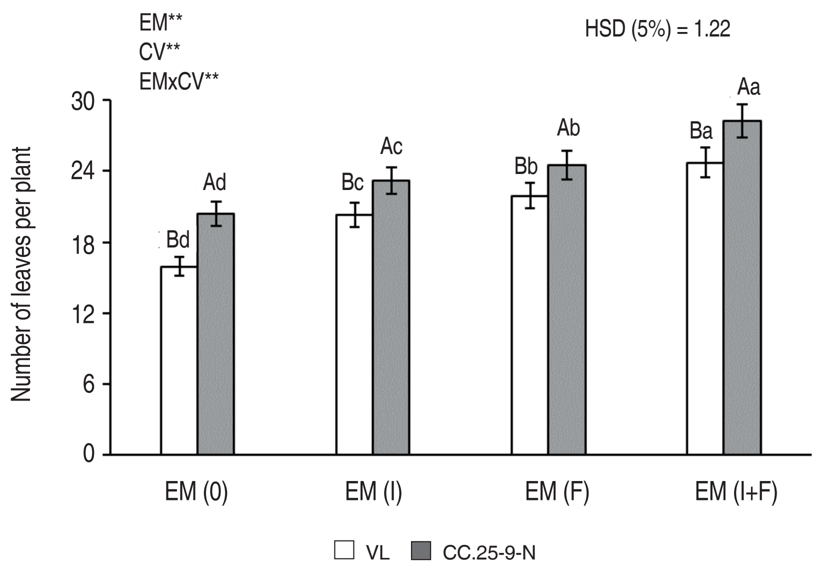

Figure 2. Number of leaves per plant of two bean cultivar (VL and CC-25-9-N), in function of EM treatments, without EM (0), soil inoculation EM (I), foliar applications EM (F) and the combined soil inoculation and foliar root applications EM (I+F).
Different small letters indicate significant differences between EM treatments at the same cultivar; different capital letters indicate significant differences between cultivars at the same level of EM (P<0.05). ** P< 0.01; EM × CV, efficient microorganism–cultivar interaction; HSD: honest significant difference.

Effect of EM applications in the agro-productive parameters

There were significant differences (P<0.05) and interactive effects of EM and CV on the number of PP, in both cultivars (Figure 3). The cv. Cuba Cueto was superior in the production of PP relative to the cv. Velazco Largo in absence or presence of EM. In both cultivars, the three forms of EM application (I, F, and I+F) increased the PP, compared with the absence of EM (Figure 3). The application of EM (I+F) treatment increased PP in the cv. VL by 110% and the cv. CC-25-9-N by 97% compared to the EM (0) treatment. These findings highlighted the superiority of the EM (I+F) treatment relative to the exclusive application of either the EM (I) or EM (F) treatments. However, EM (F) by itself was superior to the control (Figure 3).

Positive results with the application EM in the cultivation of the bean was achieved by Calero *et al.* (2017), where there was an increase of 22% of the PP concerning the absence of microorganism. Furthermore, Calero *et al.* (2016) increase the average of pods per plant by 30% with the soil inoculation and foliar applications of EM with the same bio-preparation. Similar results were obtained by Abdel-Fattah *et al.* (2016), who reported significant increments in the number of legumes per plant with the inoculation of seeds.

The number of seeds per pod was significantly different (P<0.05) in the individual effects and its interaction, being greater the production in the cv. CC-25-9-N than cv. VL. All the form of application of EM were significant (P<0.05) and superior in the production of seeds per pod concerning the absence of application (Figure 4). It existed optimum temperature, and good humidity for the development of the vegetables, and the treatment with the combined application of EM stimulated the production of this component, increasing the yield.

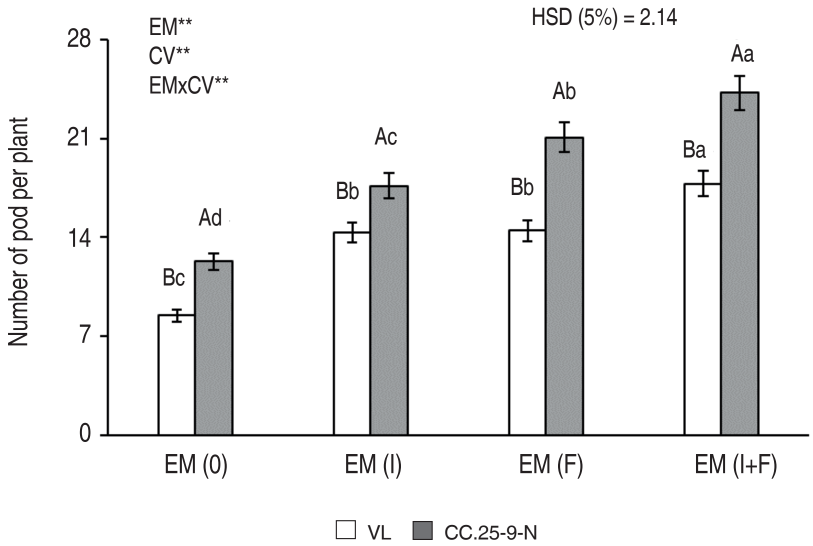

Figure 3. Number of pods per plant of two bean cultivars (VL and CC-25-9-N), in function of EM treatments, without EM (0), soil inoculation EM (I), foliar applications EM (F) and the combined soil inoculation and foliar applications EM (I+F).
Different small letters indicate significant differences between EM treatments at the same cultivar; different capital letters indicate significant differences between cultivars at the same level of EM (*P*<0.05). ***P*< 0.01; EM × CV, efficient microorganism–cultivar interaction; HSD, honest significant difference.

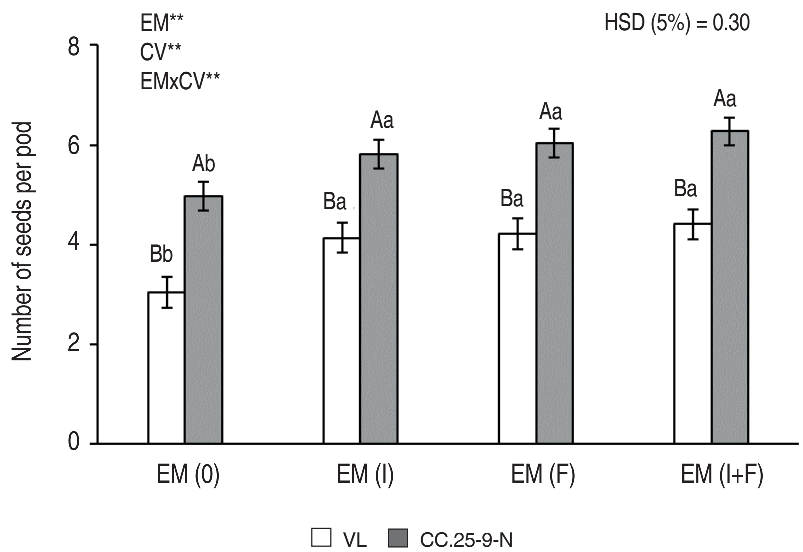

Figure 4. Number of seeds per pod of two bean cultivars (VL and CC-25-9-N), in function of EM treatments; without EM (0), soil inoculation EM (I), foliar applications EM (F) and the combined soil inoculation and foliar root applications EM (I+F).
Different small letters indicate significant differences between EM treatments at the same cultivar; different capital letters indicate significant differences between cultivars at the same level of EM (*P*<0.05). ***P*< 0.01; EM × CV, efficient microorganism–cultivar interaction; HSD, honest significant difference.

Singh *et al.* (2011) indicate that the co-inoculation of bacteria and fungi with organic amendments could be an eloquent approach for sustainable management of soil fertility and crop production because they promote nitrogen fixation, the acquisition of main nutrients, and the development of branches and roots, and the improvement of the crops' yield and quality. It is demonstrated by the increments of the morphological indicators of the beans with the individual application of EM (Calero *et al.*, 2017). }

The mass of 100 seeds was significantly different (P<0.05) in the factors and its interaction. The cv. VL was superior compared with the cv. CC-25-9-N (Figure 5). The greatest value averages were reached with the application associated with EM (I+F), concerning the individual form of EM (I) and EM (F); there were increments of 14.11 g in the cv. VL and of 8.86 g in the CC-25-9-N compared with no application. It was demonstrated that the mass of 100 grains is closely related with the agricultural yield; in this sense, Ponce *et al.* (2002) indicated that this parameter contributes to defining crop norms, and it shows the number of seeds and the number of plants possible to achieve depending on the mass.

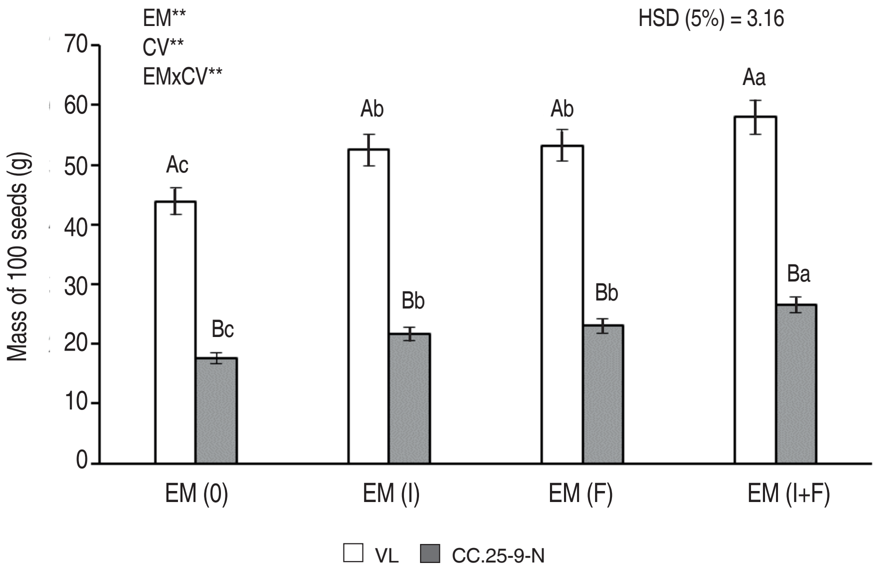

Figure 5. Mass of 100 seeds of two bean cultivars (VL and CC-25-9-N), in function of EM treatments, without EM (0), soil inoculation EM (I), foliar applications EM (F) and the combined soil inoculation and foliar root applications EM (I+F).
Different small letters indicate significant differences between EM treatments at the same cultivar; different capital letters indicate significant differences between cultivars at the same level of EM (P<0.05). **P< 0.01; EM × CV, efficient microorganism–cultivar interaction; HSD, honest significant difference.

This variable is decisive for the yield because it characterizes and clarifies the production of grains. According to the CIAT (1987), the cv. VL yields big grains because the mass average of 100 grains was superior to 40 g, while the cv. CC-25-9-N presented small grains because the mass of 100 seeds was inferior to 25 g. In this sense, Calero *et al.* (2017) obtained with the application of foliate singular EM a mass average of 100 seeds superior to the control. On the other hand, Calero *et al.* (2016) evaluated the application of several foliate bio-preparations of EM with the addition of biostimulants, reaching increments in a mass of 100 grains concerning the control.

Different microorganisms can generate growth regulators that help plants to increase the growth of their upperparts. The inoculation of the soil and the seeds with bacterial promoters of vegetable growth can be a competent instrument for the management of cultivation systems (Benedetto *et al.*, 2017).

The different forms of EM application increased the yield of the cv. CC-25-9-N regarding cv. VL (Figure 6), with significant increments (P<0.05) for the factors EM and CV, as well as their interaction. The best results were reached with the application of associated EM (I+F) in both cultivars concerning the individual forms of EM (I) and (F). The mixture increases the yield up to 1.13 t ha^{-1} in the cv. VL and 2.15 t ha^{-1} in CC-25-9-N concerning the control, reaching increments of 145% in the cv. VL and 239% in the cv. CC-25-9-N.

Productivity is an important aspect to validate the investigation. The use of Plant Growth Promotors Microorganism (PGPM) helps to increase the yields of

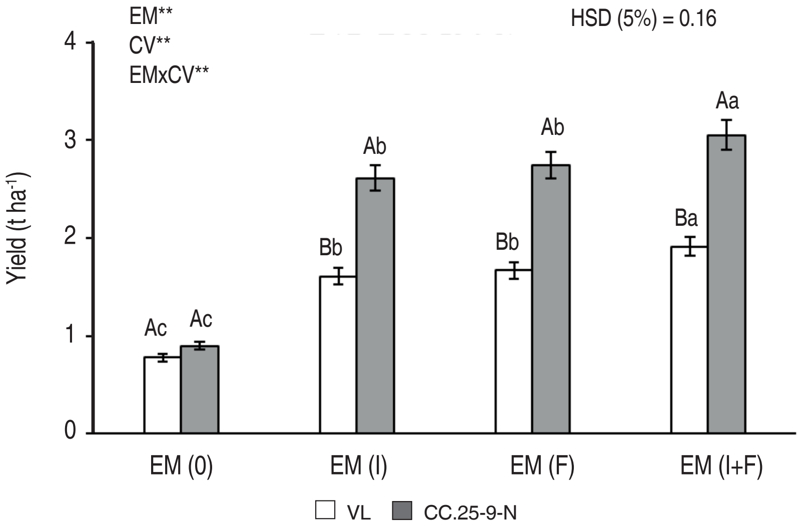

Figure 6. Yield average of two bean cultivars (VL and CC-25-9-N), in function of EM treatments, without EM (0), soil inoculation EM (I), foliar applications EM (F) and the combined soil inoculation and foliar root applications EM (I+F).
Different small letters indicate significant differences between EM treatments at the same cultivar; different capital letters indicate significant differences between cultivars at the same level of EM (P<0.05). **P< 0.01; EM × CV, efficient microorganism–cultivar interaction; HSD, honest significant difference.

crops (Zahedi *et al.*, 2016; Rashid *et al.*, 2016). Similar effects in the increment of the yield were achieved with the application of efficient microorganisms compared with the control (Calero *et al.*, 2017). In this respect, the application of foliate EM blended with bio-stimulants increased the yield to 78.90% regarding the yields obtained without EM application (Calero *et al.*, 2016).

CONCLUSION

The different forms of application of efficient microorganisms stimulated the agronomic indicators evaluated in both cultivars. The associate application between the inoculation of the soil and foliate applications of efficient microorganisms providing the best results, producing increments in the yield of 1.13 t ha^{-1} in the cv. Velazco Largo and 2.15 t ha^{-1} in the Cuba Cueto. It was found that the beneficial effects of EM application on improved bean productivity were amplified with the soil inoculation and foliar application of EM. This study indicates that further research on the methods of EM supply should be extended to other plant species.

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