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Effects of microbial and organic bio inputs on growth and yield of vegetables of economic interest

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ABSTRACT

Microbial populations carry out important processes and benefits for plants, including direct activities such as biological nitrogen fixation, phosphate solubilisation, production of indole acetic acid, production of ACC deaminase and siderophores. This study aimed to evaluate the plant growth promoting effect on Capsicum annuum L. of the fungi Trichoderma sp, *Bacillus cereus* and organic biofertiliser. The evaluations were carried out under greenhouse conditions in which suspensions prepared from Trichoderma sp, and Bacillus cereus and organic bio-input were applied as suspensions, which were applied on seedlings of Capsicum annuum. The variables evaluated were plant length, dry weight of the parts areas, root length, dry weight of the root and stem diameter. The highest values found for the variables evaluated corresponded to the organic bio-input, followed by the bio-input prepared with Trichoderma sp fungus strains and finally the suspension with *Bacillus cereus* bacteria.

Key words. Bacteria, fungus, organic fertilizer, growth and productivity.

1. INTRODUCTION

As stated by (Pazos-Rojas et al., (2016), in Colombia, the agricultural sector is characterized by the trend towards monoculture, which requires a high demand for nutrients, minerals and biomass production, which is determined by the demand for nitrogen (N), on which in turn the demand for other macronutrients depends. Nitrogen is the main nutritional limiting factor in crops, because its source in the soil is organic matter, which mineralizes only 1 to 3% of total nitrogen per year. Similarly, another important nutrient for development is phosphorus (P), which is found in the soil as a soluble form in low concentrations, varying its content in the soil up to depletion in acid soils.

There is a need to amend soils to correct the deficiency of the element phosphorus in order to obtain a response to nitrogen. This forces producers in the agricultural sector to intensify the use of fertilizers and chemical products to increase plant yields, but their indiscriminate use has had adverse effects on the environment (Pazos-Rojas et al., 2016).

Likewise, according to Jiménez, (2011), the agricultural sector has become one of the main producers of pollution, degrading the physicochemical and biological properties of the soil, due to the indiscriminate use of chemical fertilizers, which are highly toxic, altering microbial communities, contaminating surface and groundwater, and polluting the environment.

As pointed out by Bashan et al. (2014), current trends in agriculture propose a sustainable and environmentally friendly production system that minimizes the use of agrochemicals and increases productivity in the agricultural sector. The use of biofertilisers or bio inoculants and plant growth promoting microorganisms (PGPM) play a fundamental role, as their functions in various biological processes have the ability to positively affect plant growth, their interactions in the rhizosphere with the roots provide nutrients or protection in various environments.

Babu et al, (2014), show that among the key beneficial microorganisms in the rhizosphere, fungi of the genus Trichoderma, a cosmopolitan filamentous microorganism characterized by inhabiting soils and decaying wood, have proven to be a biotechnological tool presenting great benefits for agriculture, Among these are growth-promoting activity from siderophore production, phosphate solubilisation, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, indole acetic acid (IAA), phytase activity and acid phosphatase under biotic or abiotic stress.

Similarly, Velasco-Jiménez et al., (2020), point out that rhizobacteria establish root-rhizosphere relationships in which the parties involved have developed abilities that allow adaptation, promoting plant development through biological nitrogen fixation, phosphate solubilisation, plant defensive response against pathogen attacks and abiotic stress sources.

According to Mediana et al. (2010), organic fertilizers are a crucial element for the regulation of many processes related to agricultural productivity; their main functions are well known, such as substrate or growing medium, cover or mulch, maintenance of the original levels of soil organic matter and complement or replacement of synthetic fertilizers; this last aspect is of great importance, due to the boom in their implementation in clean and ecological production systems. According to Libreros, (2012), organic fertilizers are the material resulting from the natural decomposition of organic matter by the action of microorganisms present in the environment, which digest the materials, transforming them into other beneficial materials that provide nutrients to the soil and, therefore, to the plants growing in the soil. It is a controlled and accelerated process of waste decomposition, which can be aerobic or anaerobic, resulting in a stable product of high value as a soil improver.

Taking into account the importance of the use of microbial and organic bio-inputs to improve soil conditions and the promotion of plant growth, as well as the implementation of environmentally friendly technologies, it was proposed to evaluate the effect on the promotion of growth and yield of Capsicum annuum L. using three treatments, two with microorganisms (a fungus and a bacterium) and an organic fertilizer prepared with microorganisms and organic plant residues.

2. MATERIALS AND METHODS

Fungus as a bio input. The fungus Trichoderma spp. used corresponds to the collection of the genomic bank of the microbiological research laboratory of the University of Sucre, which was isolated from soils from avocado crops in the municipality of Chalán, Sucre, Colombia, in the Microbiological Research Laboratory of the University of Sucre.

A stock solution of conidia was prepared from the Trichoderma spp. strain, by adding 10 ml of sterile distilled water to a 15-day incubation culture, for which the mycelium was scraped with a triangular loop to release as many spores as possible. The volume of the wash was then recovered in a 50 ml falcon tube using a 1000 ml micropipette and passed through sterile gauze to remove any remaining medium and mycelium from the solution. The spores were counted in a neubauer chamber and the volume and concentration required for inoculation were calculated.

Bacteria as bio input. The rhizobacterium identified as Bacillus cereus, obtained and purified from the germplasm bank of the Microbiological Research Laboratory of the University of Sucre, was used. The bacterial inoculum was prepared from culture stored at -20°C in nutrient broth with 15% glycerol, the bacteria were seeded by exhaustion and incubated for 24 hours at 30°C, after which time the purity of the bacteria was verified (figure 2). Inoculate were prepared in LB broth for 24 hours, a colony of the strain to be evaluated was taken and inoculated in a 150 ml Erlenmeyer flask for 24 hours with agitation at 150 rpm, then centrifuged at 7000 rpm for 5 minutes. The biomass was then resuspended in sterile distilled water until bacterial suspensions were obtained with a concentration equivalent to an absorbance of 0.2 ± 0.05 at 600 nm, which corresponds to approximately 1x 108 colony forming units (CFU) per mL (Zapata and Vélez, 2011).

Organic fertilizer (soil conditioner). The preparation of the soil conditioner followed the scheme as shown in figure 1. The volume of organic fertilizer used (20 mL of biofertiliser in one litre of distilled water). The form of supply consisted of applying directly to the soil around the roots of the seedlings sown.

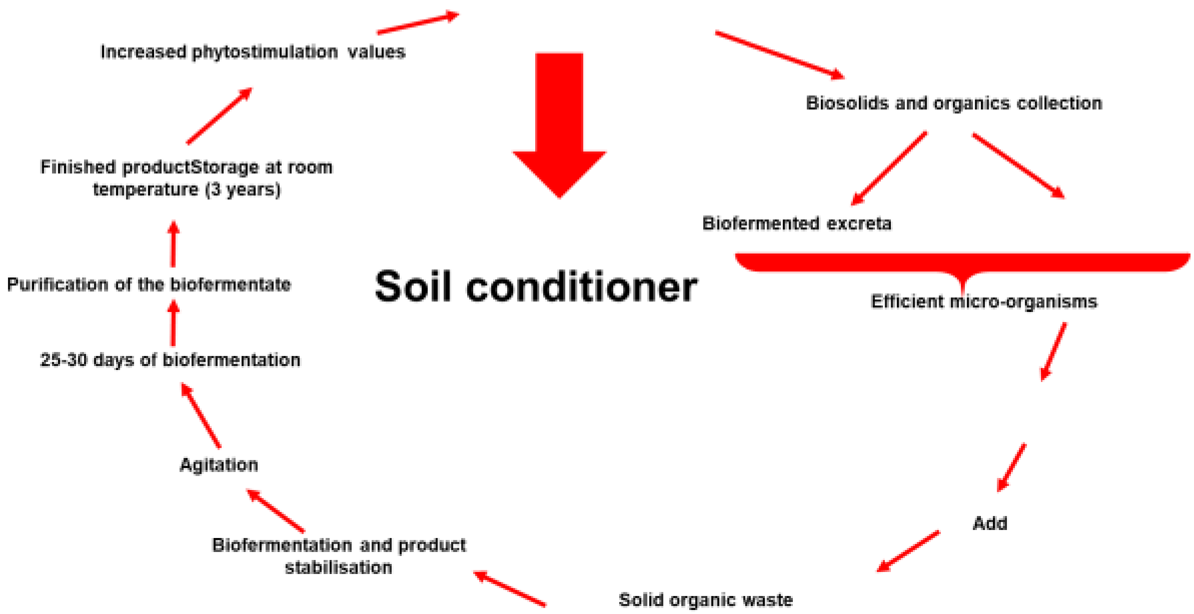

Figure 1. Schematic of the soil conditioner (organic fertilizer) preparation process.

Greenhouse test. Commercial chilli pepper seeds were used, which underwent a surface disinfection process consisting of 3 washes with sterile distilled water, then left for 6 minutes in 6% hypochlorite, then rinsed with sterile distilled water twice, followed by washing with 70% ethyl alcohol for 6 minutes and finally rinsed four times with sterile distilled water until the remains of fungicide and disinfectants were eliminated. They were soaked for 24 hours in sterile distilled water and dried under the air of a laminar flow cabinet. The seeds were sown in 4 kg bags of fertilized soil per treatment, following the instructions given by Guigón-López et al.

Experimental design. The amount of inoculum used was: Trichoderma sp (108 spores/mL) and Bacillus cereus (108 CFU/mL). The inoculation of the microorganisms was carried out by spraying 1 ml of the culture of microorganisms per seedling. Five replicates and 10 experimental units were used for a total of 50 experimental units for each treatment evaluated. To maintain sufficient humidity, periodic irrigation with running water was applied to each of the seeds (Rojas-Badía et al., 2020) and weekly diagnostics of pests and diseases were carried out.

Parameters to evaluate. Data were taken 60 days after sowing the experiment, the measurable variables were: plant length, root length, foliage dry weight and fruit weight (Guigón-López et al., 2004).

Analysis of results. The Shapiro-Wilk test (p-value≥0.05) was used for the growth promotion and production variables to establish whether the data are normal. Then, an analysis was applied according to the result of the Shapiro-Wilk test, the ANOVA test was used if the data are normally distributed and if they are not normally distributed the Kruskal-Wallis test was used and the significant statistical differences were found with the Tukey rank multiple test, with this method there is a risk of 5.0% when saying that each pair of means is significantly different, when the real difference is equal to 0.

3. RESULTDS AND DISCUSSION

The results of the variable plant height are shown in figure 2. It is observed that the seedlings that showed greater height corresponded to the treatments where the inoculum with the fungus Trichoderma sp. was applied, followed by the treatment with soil conditioner and Bacillus cereus. When comparing the results of the treatments used, a significant difference was observed with the control, indicating that the microbial and organic bio supplies provide nutrients to the soil.

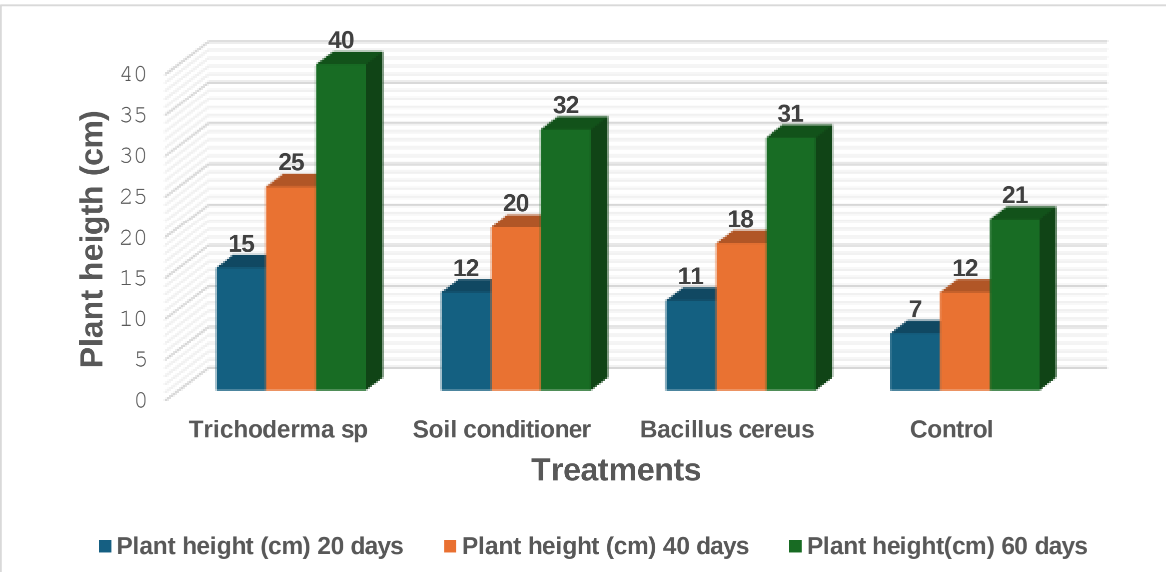

Figure 2. Plant height results by treatments in different phenological periods of the crop.

Figure 3 shows the results of root length of chilli pepper seedlings in three phenological periods of the crop. The figure shows that the treatments with soil conditioner showed greater promotion of root growth, followed by Trichoderma sp. and Bacillus cereus, compared to the control where no organic or microbial fertilizer was applied.

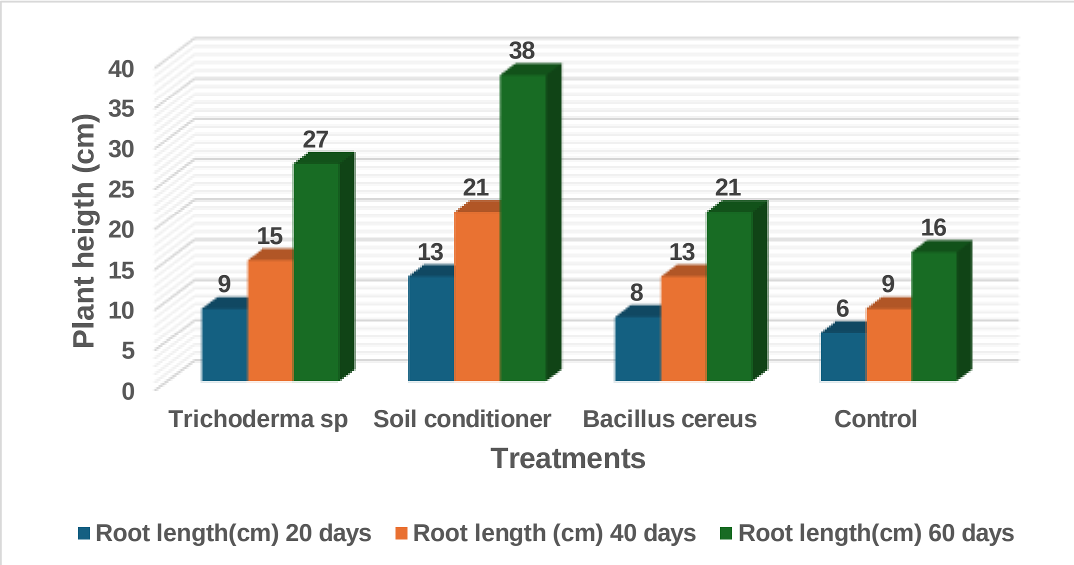

Figure 3. Root length results by treatments in different phenological periods of the crop.

Figure 4 shows the results of the dry weight of the foliage evaluated 60 days after the start of the experiment. The results show that the treatment with Bacillus cereus showed the highest dry weight in foliage followed by Trichoderma sp. and the soil conditioner compared to the control where the lowest dry matter yield in foliage was presented.

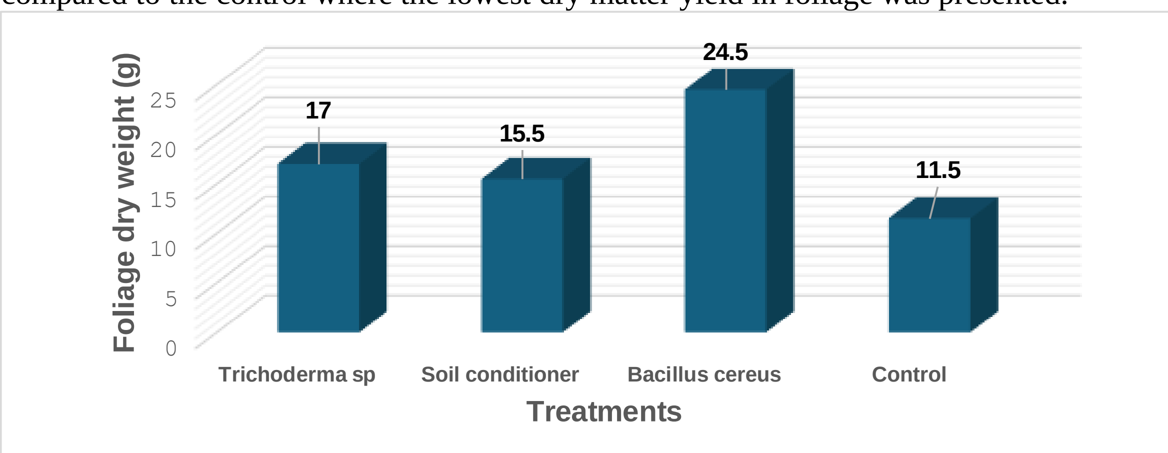

Figure 4. Results of the variable dry weight of foliage by treatment.

Figure 5 shows the results of the fruit weight of chilli peppers, showing that the treatment with soil conditioner presented the highest value of fruit weight, followed by Bacillus cereus and in third place the treatment with Trichoderma sp. The results also show that the control presented the lowest values of fruit weight.

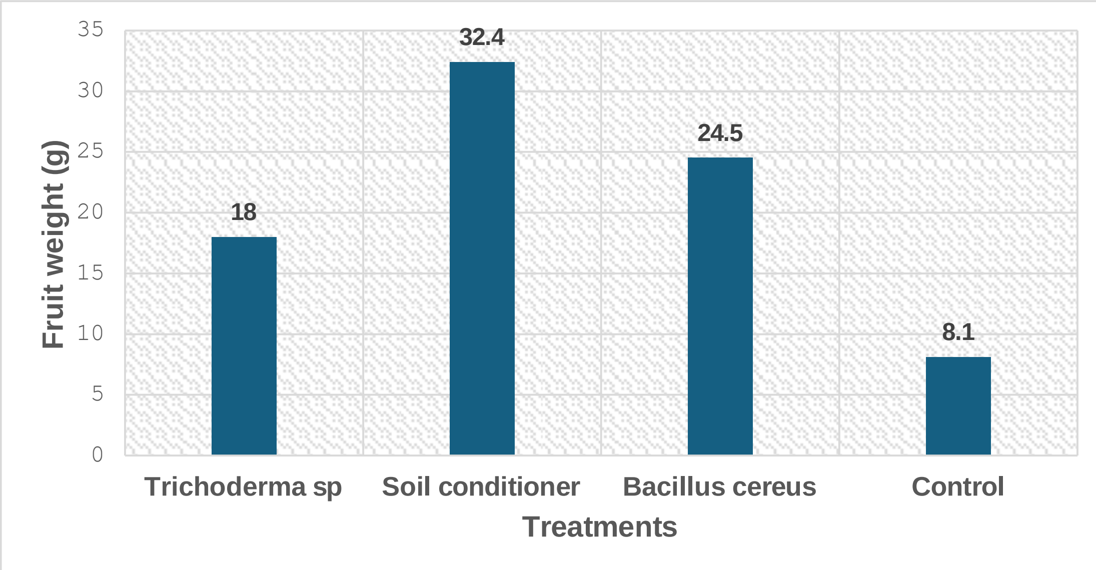

Figure 5. Results of the fruit weight variable by treatment.

As stated by Puentes et al., (2010), in nature there are microorganisms that favor root development, the fixation of atmospheric N, the solubilisation of soil P and the production of secondary metabolites, directly influencing the availability of nutrients and the stimulation of plant growth, which supports the results obtained in this study, where statistically significant differences were observed for the variables evaluated with respect to the treatments where microbial bio inputs were used with respect to the control.

All variables showed statistically significant differences between treatments, with the soil conditioner treatment showing the best results, positively affecting the overall growth of C. annuum seedlings. The soil conditioner, a combination of fermented biosolids with beneficial soil microorganisms, plays an important role in protecting the plant against pathogens, which improves crop yields and protects soil quality.

As reported by Romero (2012), organic fertilizers are composed of beneficial microorganisms, phosphorus, potassium, calcium, sulphur, zinc, organic carbon and other elements that have been as primary soil elements and it is emphasized that these minerals generated by the bio digestion of animals become potential fertilizer elements that help in all plant processes, Furthermore, Warnars and Oppenoorth (2014) indicate that this type of organic biofertiliser returns nutrients to the soil, thus achieving more fertile soils and favoring sustainable production.

The second most favourable treatment for the plants was that of Trichoderma sp, which, although it showed lower results for the variable weight compared to the soil conditioner, showed greater efficiency in the length of the plants with respect to the treatment with Bacillus cereus and significant differences with respect to the control. Trichoderma sp, this fungus helps the plant to have a better solubilisation of phosphate, micronutrients, cations and minerals, which the plant uses for its metabolism, thus increasing its root biomass, in addition, they produce secondary metabolites that fulfil the hormonal functions of auxins, which produces rapid growth and optimal performance (Tucci et al., 2011; Vinale et al., 2008). In addition, the Trichoderma genus possesses antagonistic mechanisms such as antibiosis, competition for space and nutrients, micro parasitism, stimulation of plant growth and induction of resistance, thanks to which they have potential in the sustainable development of agriculture, helping plant development in both nutrition and protection (Companioni González et al., 2019).

The treatment with Bacillus cereus showed lower results than the treatments with the soil conditioner and Trichoderma sp, however, Bacillus cereus compared to Trichoderma sp did not show statistically significant differences in any of the evaluated variables, but it did show significant differences compared to the control. Bacillus cereus stimulated the dry weight of the foliage compared to the other treatments and the control. This is because it has been shown that these bacteria have characteristics as plant growth promoters and are also antagonists of phytopathogens (Govelak et al., 2015). For the Bacillus cereus treatment, it was able to show better results, but there are factors that generally affect the bacteria when they are inoculated, after inoculation into the soil without a suitable carrier, the bacterial population decreases rapidly for most PGPB species.

The Bacillus genus is important for its broad physiological diversity profile (Restrepo-Franco et al., 2015). In the literature we can find several studies that agree with the results obtained, such as Kaymak et al. (2010), De Araujo et al. (2012) and Rojas-Badía et al. (2020) agree that Bacillus sp strains increased plant growth compared to the control, thanks to their physiological capabilities, so they can be used as growth-promoting microorganisms; likewise, the study by Chamorro-Anaya et al. (2020), report that the strain Bacillus cereus, proved to have growth-promoting activity, as they produce siderophores, fix nitrogen, solubilize phosphate and there is also ACC deaminase activity, factors that help plant growth.

The results of this study reflect the importance of microorganisms and the use of organic matter to help optimize the plant growth process, as all the treatments showed differences compared to the control in at least one of the variables. These alternatives can not only help improve crop yields, but also correct nutritional deficiencies in soils, increasing their fertility and minimizing the environmental impact of chemical fertilizers (Amparo, 2016).

4. CONCLUSION

The response variables plant height, root length, foliage dry weight and fruit weight showed the greatest increase in the present study, which indicates the importance of the fungus Trichoderma sp. and Bacillus cereus and the soil conditioner for the promotion of growth of seedlings of Capsicum annuum L. The treatment with organic bio input is a soil conditioner that activates the beneficial microbiota, offering a boost to crop productivity. Its formula includes fulvic and humic acids, which allows greater water absorption and nutrient retention, thus providing efficiency in its application with a lower dosage required. This innovative technology can be applied both foliar and soil, further enhancing uptake and efficacy.

In addition to the results obtained in this study, the soil conditioner has the capacity to restore the soil microbiota, which facilitates the mobilization of nutrients, promoting a more effective recovery for future sowings. It is important to note that this product is made from organic materials, guaranteeing nature-friendly agriculture.

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6. AUTHOR CONTRIBUTION. Alexander Perez Cordero: experiment execution, data analysis. Donicer Montes V and Yelitza Aguas M, conceptualization, writing - revision and editing. All authors have read and approved the manuscript.

7. CONFLICT OF INTEREST. All the authors of the manuscript declare that they have no conflictof interest

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