ARTÍCULO DE INVESTIGACIÓN

Effect of fungal microorganisms on promoting the growth of pepper (*Capsicum annuum* L.) seedlings under controlled conditions

Efecto de microorganismos fúngicos en la promoción del crecimiento de plántulas de pimiento (*Capsicum annuum* L.) en condiciones controladas

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ABSTRACT

The need for food production worldwide is constantly increasing, generating a deterioration of the environment, which requires the implementation of less harmful crop management techniques, one of these strategies is the use of microorganisms with the potential to increase plant growth. The evaluation of the effect of inoculation in pepper (*Capsicum annuum* L.) seeds of nine fungal strains (HC1, HC2, HC3, HC4, LBH1, LBH33, LBH59, LBH9 and VP) was proposed. The seeds were sown in two types of tray sizes (128 and 200 alveoli) and the percentage of germination, stem length and diameter, root length, aerial and root dry weight were measured after five weeks of growth. The results show that the HC2 and LBH9 strains have the greatest potential to increase the variables evaluated under the study conditions. Just as there are strains that increase the growth of plants, there are also those that inhibit it, which makes it necessary to evaluate them in different crops to generate a proper management of them, optimizing the use of resources and, in turn, producing crops with better development of aerial parts and roots and, consequently, higher productivity.

Keywords: biofertilizers, crops, seed trays.

RESUMEN

La necesidad de producción de alimentos en todo el mundo aumenta constantemente generando un deterioro del medio ambiente, que requiere la implementación de técnicas de manejo de cultivos menos dañinas, Una de estas estrategias es el uso de microorganismos con el potencial de aumentar el crecimiento de las plantas. Fue propuesto la evaluación del efecto de la inoculación en semillas de pimentón (*Capsicum annuum* L.) con nueve cepas de hongos

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(HC1, HC2, HC3, HC4, LBH1, LBH33, LBH59, LBH9 y VP). Las semillas se sembraron en dos tipos de bandeja de siembra (128 y 200 alvéolos) y el porcentaje de germinación, longitud y diámetro del tallo, longitud de la raíz, peso seco aéreo y de la raíz, se midieron después de cinco semanas de crecimiento. Los resultados muestran que las cepas HC2 y LBH9 tienen el mayor potencial para aumentar las variables evaluadas bajo las condiciones del estudio. Del mismo modo que hay cepas que aumentan el crecimiento de las plantas, hay otras que las inhiben, lo que hace necesario evaluarlas en diferentes cultivos para generar un manejo adecuado de las mismas, optimizando el uso de los recursos y, a su vez, produciendo cultivos con un mejor desarrollo de las partes aéreas y las raíces y, en consecuencia, una mayor productividad.

Palabras claves: biofertilizantes, cultivos, bandejas de siembra.

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INTRODUCTION

The rhizosphere is known as the fraction of soil next to the roots of plants (Kuppe *et al.*, 2022; Prasad *et al.*, 2019), where microorganisms are attracted by various metabolites that are exuded by the roots. Most of these nutrients not only benefit from nutrient secretion but also have a positive influence on plants through mechanisms involved in the stimulation of plant growth, such as the germination of seeds, rooting, and elongation of the stem, among other phenological and physiological characteristics, due to the production of compounds such as vitamins and phytohormones (Kloepper *et al.*, 1991; Santoyo *et al.*, 2019), in addition to the dissolution and mineralization of phosphates, sulfur oxidation, and increased availability of nitrogen (Franco, 2008; Raj *et al.*, 2005; Reyes *et al.*, 2002; Saharan and Nehra, 2011). The relationships established in this context most of the time between beneficial microorganisms and plants are of the mutualistic type (Bastías *et al.*, 2022).

One of these mutualistic relationships that occur in the soil involves fungi and bacteria that are closely related to the roots of plants and that produce substances that regulate plant growth (Celis and Gallardo, 2008; Santoyo *et al.*, 2019); for example, Chang *et al.* (1986) refers to *Trichoderma harzianum* and its mechanisms of action that are based mainly on the stimulation of plant growth, which manifests from the earliest stages of seedling development and allows greater advantages when transplanting (Jamiołkowska and Michałek, 2019; Galeano *et al.,* 2002).

This potential has allowed a large number of species of microorganisms with specific functions in agriculture to improve plant productivity (Antón, 2004) through plant nutrition programs in the field or in protected systems. This new form of management promotes viable alternatives to reduce production costs and the environmental impact associated with chemical fertilization. According to various studies, this technology allows for an increase in the yield of crops between 17% and more than 50%, as well as an improvement in soil fertility and a reduction in the population of microorganisms damaging crops (Liu *et al.*, 2020; Morales, 2013; Rai *et al.*, 2020).

Part of the success in the use of rhizospheric microorganisms is related to the type of crop with which one works, by presenting in many cases specificity between microorganisms and plants (Thomson *et al.*, 2010) as well as the viability, diversity and functioning of these soil microorganisms (Khenaka *et al.,* 2019). These findings therefore show that soil fertility depends not only on its chemical composition but also on the quantitative and qualitative nature of the microorganisms that inhabit it, among other conditions (Giri *et al.*, 2005; Shukla, 2019). The literature contains a large amount of information on the beneficial effects of the use of microorganisms on the growth and production of a wide range of crops, such as tomato, corn (do Amaral *et al.*, 2022), rice (Lee *et al.,* 2019), pepper (Linu *et al.,* 2019), and potato (Trdan *et al.,* 2019). Among others, the most common of these include the choice of microorganisms, potential for the crop and its incorporation in crop management, starting with the seed (Cisternas-Jamet *et al.*, 2019).

The objective of this study was to evaluate the effect of different strains of fungal microorganisms on the morphological characteristics of pepper plants cultivated in protected environments, with a view to determining the optimal plant-fungus ratio recommended for this growth stage. Furthermore, the investigation incorporated the utilization of trays of varying dimensions, with the aim of ascertaining the impact of the volume available for root growth on the seedlings.

MATERIALS AND METHODS

Fungal microorganism selection and inoculation of seeds. Nine fungal strains were selected, all of them selected with the criterion of phosphate dissolution under laboratory conditions, and with potential to promote plant growth, being isolated from different plant rhizospheres:

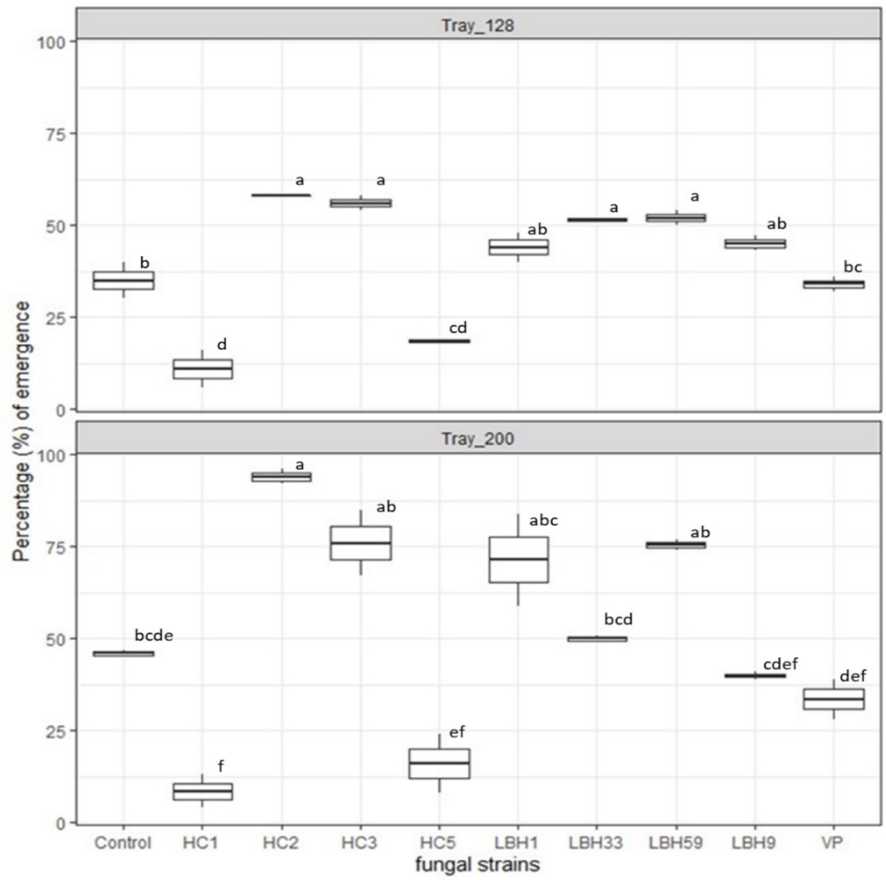

Figure 1. Percentage (%) of emergence of pepper plants according to treatment evaluated in trays with 128 and 200 alveoli. Different letters represent significant difference (p_value < 0.05) for each variable by tray type.

Penicillium spp strain HC1, HC2, HC3 and HC5 from the rhizosphere of coffee plants (*Coffea arabica* L.); *Penicillium* spp strain LBH01, *Aspergillus* sp. strain LBH09 and *Trichoderma* spp strain LBH33 from vetiver plants (*Chryzopogon zizanioides*); a strain of *Bauberia bassiana* strain LBH59; and one of *Trichoderma* sp. strain VP, which were obtained from the strain collection of Grupo de Investigación en Biotecnología Agrícola y Ambiental, Universidad Nacional Experimental del Táchira.

All the strains were seeded in Petri dishes with potato dextrose agar (PDA) for activation. In this phase, the viability, purity and activity of the fungus were verified by means of the texture and coloration on both sides of the Petri dish, in addition to the production of resistance structures. A solution with each fungal strain was prepared at a concentration of 10^7 conidia.ml^{-1}.

The pepper seeds were washed with running water to eliminate the chemical products present and then with sterile distilled deionized water to eliminate the residual traces of these products. They were then disinfected by immersion in 80% ethanol for two minutes and washed five consecutive times with sterile distilled and deionized water to remove residual ethanol. finally, they were allowed to drain under sterile conditions in a laminar flow chamber.

The disinfected seeds were placed in the different solutions of pre-prepared fungal conidia, and sodium alginate (2%) was added to the suspension as an adherent. The suspensions were placed in an orbital shaker for one hour, at a temperature of 25°C. Finally, the inoculated material was transported to a laminar flow chamber, allowing the seeds to drain through a sterile medical gauze strainer until the seeds were dried at the ambient temperature of the laboratory (25°C).

Growth of pepper plants in trays of different sizes

The seeds were placed in horticultural trays of 128 and 200 cells, one tray per fungus strain, which were filled with commercial substrate previously sperilized. Subsequently, seven days after sowing, the evaluations began with the percentage of emergence. After 5 weeks, stem diameter,

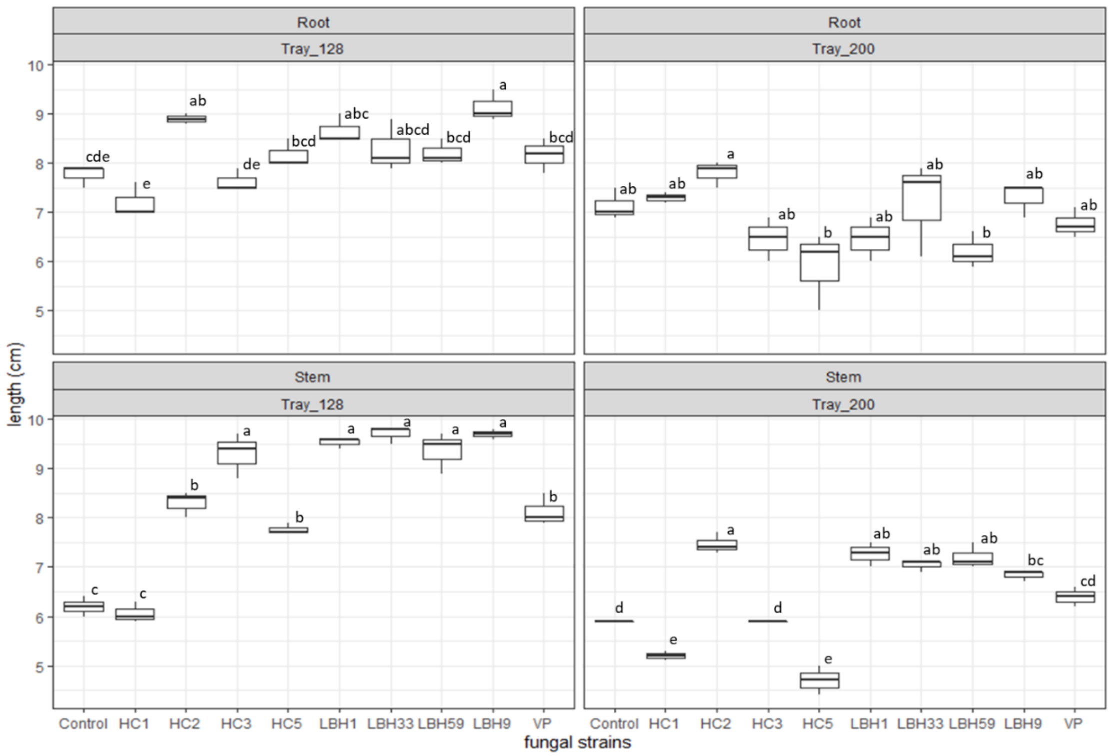

Figure 2. Effect of inoculation with fungal microorganisms on the length (cm) of the stem and on the root of pepper seedlings. Different letters represent significant difference (p_value < 0.05) for each variable by tray type.

stem and root length, and aerial and root dry weights of the seedlings were determined. The trays were kept in a greenhouse with constant irrigation management and temperatures ranging between 22 and 28°C. The aerial and root material, separately, was placed in labeled paper bags, dried and previously weighed on an analytical balance, and placed in an oven with forced air circulation at 70 °C for 72 hours for dry weight determination.

Statistical analysis

Three seedlings were randomly selected from the central part of each of the three trays, according to alveolus size, for each inoculated fungus strain, including the control (non-inoculated seed), for a total of nine experimental units per treatment. The results were analyzed descriptively, followed by an analysis of variance using the R-project/RStudio program.

RESULTS

Percentage of seedling emergence pepper

The percentage of emergence of the seedlings showed that there was an interaction between the evaluated factors and between the strains of the fungal microorganisms used and the size of the alveoli of the tray, suggesting that this variable responds randomly to both factors (Figure 1).

This led to the testing of media for each type of tray. Treatments that allow an increase in emergence compared to the control include, for example, treatments with strains 59, HC3 and HC2 in both types of trays. In contrast, there are treatments that seem to inhibit the emergence of seedlings due to the effect of the inoculated strain, such as strains HC1 and HC5. On the other hand, the favorable effect observed on the emergence of pepper plants with the use of trays containing 200 alveoli could be related to better drainage after irrigation, which was attributed to the lower proportion of substrate per alveolus.

Length of the stem, diameter of the stem and length of the root of pepper seedlings

The factorial statistical analysis revealed an interaction between the variables of stem length and diameter, and root length, with the strains and size of the alveoli. This indicates that modifying one of the conditions leads to alterations in the response, necessitating the evaluation of results based on tray size, akin to the approach employed for germination percentage. It is noteworthy that these three variables presented the highest values, especially in tray 128, compared to those of the 200 alveoli trays. A comparison with the control treatment revealed that, in both cases, the trays contained strains that enhanced the measured variable and others that inhibited it.

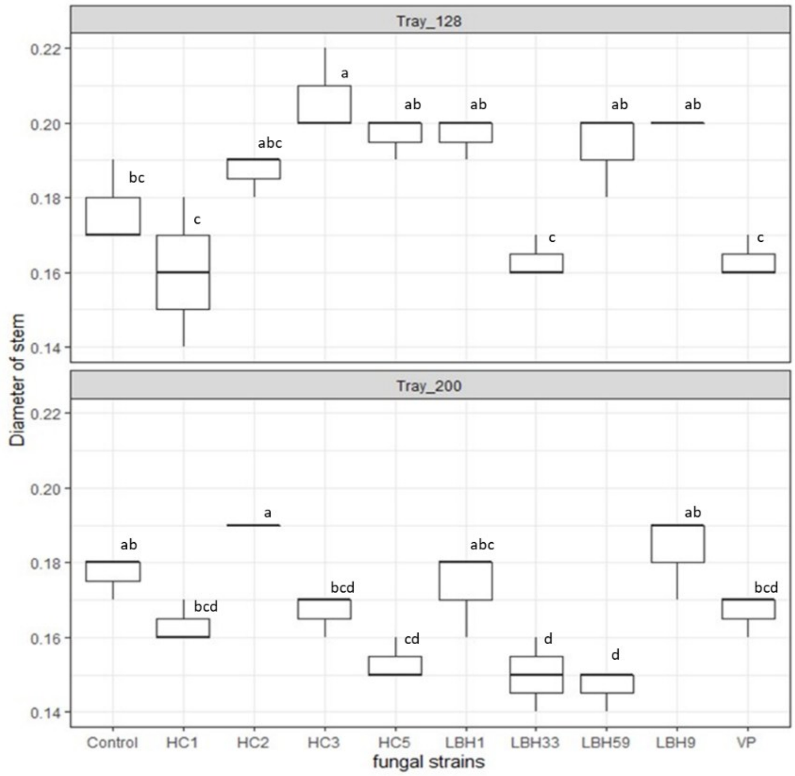

Figure 3. Effect of inoculation with fungal microorganisms on the diameter of the stem (cm) of pepper plants. Different letters represent significant difference (p_value < 0.05) for each variable by tray type.

In the 128 alveolar tray, stem length exhibited an increase in the presence of strains LBH33, LBH9, LBH1, LBH59 and HC3 (Figure 2) in comparison to the control and other strains. Furthermore, strain LBH9 resulted in increased root length, while strain HC3 led to augmented stem diameter (Figure 3). Conversely, strain HC1 exhibited an inhibitory effect on these variables in this type of trays (Figure 2 and 3).

In the case of 200-cell trays, stem length and diameter, and root length exhibited an enhancement in response to inoculation with fungal strain HC2 (Figures 2 and 3). Conversely, fungal strain HC5 exerted a inhibitory effect on stem and root length, while fungal strains LBH59 and LBH33 demonstrated an inhibition on stem diameter.

Dry weight of the aerial parts and roots of the pepper plants

The factorial analysis related to the dry weight of the aerial and root parts demonstrated no interaction between the factors evaluated, indicating a significant difference (p_value <0.05) for the size of the alveoli. The results demonstrate that the trays exhibiting smaller alveoli produced plants with greater weight, both aerial and root (Figure 4). This phenomenon may be attributed to the rapid identification of root limits by the roots, which stimulates increased root development towards the lower part of the alveolus in search of nutrients and water. Consequently, this may promote more vigorous aerial growth. Conversely, the increased planting density engenders competition among plants, thereby prompting accelerated growth in pursuit of light, culminating in enhanced aerial and root development.

When analyzing the type of tray separately, it was observed that for both the evaluated variables and the types of trays under study, there were no strains that were significantly different from those in the control treatment, and a numerical difference was presented on average. Among the dry weight of the aerial parts of the pepper plants planted in the trays of 128 alveoli, there were strains 59 and LHB9, while in the trays of 200 alveoli, except for strain HC5, all of which had average dry weights greater than that of the control. On the other hand, the dry weight of the roots was similar to that of the control plants, where for 128 trays, the average dry weight was greater for the HC2 and 59 strains, and for 200 trays, and the average dry weight was greater for the HC2 strain. It should be noted that depending on

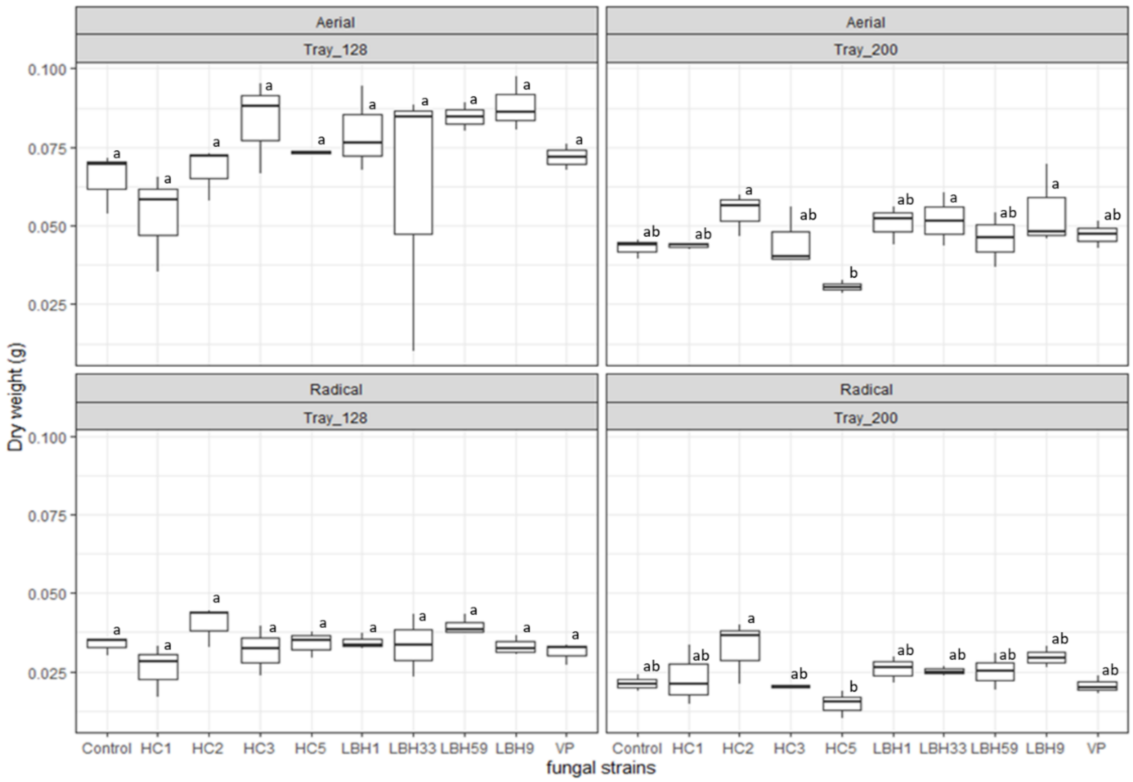

Figure 4. Effect of the inoculation of fungal microorganisms on the dry weight (g) of the stem and of the root of pepper seedlings. Different letters represent significant difference (p_value < 0.05) for each variable by tray type

the type of tray, a strain that gives lower averages to the control in both variables is presented for the trays of 128 the CH1 strain and for those of 200 the CH5 strain.

DISCUSSION

Percentage of seedling emergence pepper

The results of the effect on the inoculation of fungal microorganisms, of increase or inhibition of seed germination and emergence of seedlings, show the possible specificity of the strains with culture in this stage; likewise, different authors, who point out the capacity of certain microorganisms, for example, the *Penicillium* y *Trichoderma* strains (Odon *et al.,* 2020), of originating products such as gibberellins and indole acetic acid, among other compounds, which stimulate the germination of the seeds depending on the crop, are presented (Abdenaceur *et al.,* 2022; Tao *et al.,* 2019). Similarly, microorganisms have been reported to have negative effects on seed germination and the emergence of plants. An example of this phenomenon was presented by Reyes *et al*. (2008), who evaluated the effect of rhizospheric microorganisms on the germination of pepper seeds and reported that 10% of them inhibited the germination of plants, which could be related, among other conditions, to the production of substances such as phosphinothricin (Venkatachalam *et al*., 2010).

Length of the stem, diameter of the stem and length of the root of pepper seedlings

In various investigations, similar results have been found for the use of strains of microorganisms to increase and promote growth in different agricultural crops (Alvarez and Reyes, 2015; Chakraborty *et al*., 2010; Galeano *et al.,* 2002; Linu *et al*., 2019) or, on the contrary, their negative effect (Rokni *et al.,* 2021). It is important to note that the influence of each strain on the variables is random. This means that an increase in one variable can be accompanied by a greater or equal increase in another variable, or alternatively, a negative effect may be exerted on the second variable. The influence will depend on the effect of the microbial strain on plants (Diniz *et al*., 2009; Tao *et al*., 2019). According to the literature (Orozco-Mosqueda *et al*., 2023; Sokolova *et al*., 2011), this type of behaviour is related to the compounds generated by microorganisms, such as phytohormones and organic acids.

Dry weight of the aerial parts and roots of the pepper plants

Variables such as weight are related as part of the quality of the seedlings to be taken to the field and have a greater probability of establishment, which is possible with seed inoculation with growth-promoting microbial strains, similar results are presented by Reyes *et al*., (2008) when finding microorganisms with biofertilizing potential that increased variables of agronomic importance in bell pepper, even when other microbial strains inhibited some variables. This last inhibition may be related, among other factors, to the sensitivity of the seed to the substances generated by the microorganisms and/or to the microbial concentration that competes for nutritional resources (Cardarelli *et al*., 2022; Xiao *et al.,* 2020).

The response to microbial inoculation depends on the stage at which the inoculation was performed; for example, the results showed that the *Trichoderma* sp. strain can be inoculated into seeds and produce seedlings, although this approach is not among the best methods. In other investigations carried out with the *Trichoderma* sp. strain and rational applications of fertilizer, a tendency to increase yield per pepper plant under field conditions up to 18% was shown (Sánchez *et al.,* 2018). Similarly, when this strain was applied in combination with an *Enterobacter* sp. strain, the number of flower buds significantly improved by 62%, the height of the plant and the number of leaves by 23%, on average, compared to those in the control treatment (Sánchez and Reyes, 2018), which explains the effect that the inoculation of a microorganism can generate individually or in a microbial consortium on the growth of the plants according to the growth stage.

Additionally, the size of the alveoli of the tray is important for the response of both the plant and the inoculant (Vagnoni *et al*., 2014). These factors are related to the volume of the alveoli of the trays and the increase in restriction of the roots of the plants; therefore, plants that grow in small volumes undergo morphological and physiological changes in response to this reduction. These changes affect the amount of compounds exuded by the plant in the root system, which could in turn influence the effect of the inoculated strains, demonstrating the complexity of the system (Chapin *et al*., 2002).

CONCLUSIONS

The factor "valuable size of the tray" showed a significant difference in the morphological variables evaluated in the pepper seedlings and in the effect of the inoculated strains, revealing that the tray with 128 alveoli presented the best behavior.

While various strains can be inoculated at the tray level, various strains can be inoculated to improve the growth of paprika plants. In this sense, the strain LBH9 influences the length of the stem, the radical length and the dry weight of the aerial part of the plant, while for radical dry weight, the strain HC2 and the diameter of the stem are the strains. This raises the possibility and need to evaluate the use of these consortiums during the production of seedlings.

The presence of strains that inhibit the variables evaluated shows the need to evaluate the compatibility of crops with potential microorganisms for plant development, seeking to avoid production loss due to incompatibilities.

AUTHOR CONTRIBUTIONS

Conceptualization, Alexis Valery and Rossana Timaure; methodology, Valeria Fernandez, Alexis Valery and Luberto Sanchez; software, Rossana Timaure; validation, Veronica Rodriguez and Alexis Valery; formal analysis, Valeria Fernandez and Luberto Sanchez; research, Valeria Fernandez and Alexis Valery; resources, Luberto Sanchez; original drafting, Valeria Fernandez, Veronica Rodriguez and Alexis Valery; writing, revising and editing, Veronica Rodriguez and Alexis Valery; visualization, Alexis Valery; supervision, Alexis Valery; project management, Luberto Sanchez. Project management, Luberto Sanchez; project management, Luberto Sanchez; and fundraising, Luberto Sanchez.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of the data; in the writing of the manuscript; or in the decision to publish the results.

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