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EFFECT OF MICROBIAL BIOSTIMULANTS ON THE MORPHOLOGY AND PRODUCTIVITY OF CUCUMBER PLANTS (*Cucumis sativus* var. Poinsett) UNDER GREENHOUSE CONDITIONS

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ABSTRACT

Objective: The objective of this study was to investigate the effect of microbial biostimulants on the morphology and fruit of cucumber plants (*Cucumis sativus* var. Poinsett) under greenhouse conditions.

Theoretical Framework: Cucumber production is gaining importance among farmers due to its beneficial and nutritional potential; this, coupled with an increase in demand, leads to higher yields and higher incomes in a shorter period. However, new, sustainable alternatives have been sought to improve fruit quality through plant growth-promoting microorganisms.

Method: Cucumber seeds (var. Poinsett) were inoculated directly onto the root with 10 mL of the inoculum. The microorganisms used were: *Trichoderma harzianum*, *Bacillus amyloliquefaciens*, *Debaryomyces hansenii*, and Mix (a combination of the three microorganisms). A completely randomized block experimental design was established with 5 treatments and 20 repetitions for each treatment with a total of 100 plants as experimental units. The variables evaluated were plant height, stem diameter, root length, root volume, plant yield, total sugars, and chlorophyll. The data were analyzed using SAS for Windows, version 9.1.

Results and Discussion: According to the statistical results, treatment T1 (*Trichoderma harzianum*) was the one that achieved the best results on the study variables: plant height, stem diameter, yield per plant, quantification of total sugars (°Brix) and chlorophyll, followed by T2 (*Bacillus amyloliquefaciens*). The beneficial potential of various species of Trichoderma has been demonstrated. They can promote the growth of roots, stems and leaf area in plants because they favor the increase in nutrient absorption (Rodríguez-García and Vargas-Rojas, 2022).

Originality/Value: This study contributes to the literature by reporting the potential of various microorganisms as agricultural production alternatives, which have a lower environmental impact while simultaneously improving economic efficiency and enhancing crop qualities.

Keywords: Beneficial Microorganisms, *T.harzianum, B.amyloliquefaciens, D. Hansenii*, Growth Promotion.

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EFEITO DE BIOESTIMULANTES MICROBIAIS NA MORFOLOGIA E PRODUTIVIDADE DE PLANTAS DE PEPINO (*Cucumis sativus* var. Poinsett) EM CONDIÇÕES DE ESTUFA

RESUMO

Objetivo: O objetivo deste estudo foi investigar o efeito de bioestimulantes microbianos na morfologia e no fruto de plantas de pepino (Cucumis sativus var. Poinsett) em condições de estufa.

Estrutura teórica: A produção de pepino está ganhando importância entre os agricultores devido ao seu potencial benéfico e nutricional; isso, juntamente com o aumento da demanda, leva a maiores rendimentos e maiores rendas em um período mais curto. No entanto, novas alternativas sustentáveis têm sido buscadas para melhorar a qualidade do fruto por meio de microrganismos promotores do crescimento das plantas.

Método: Sementes de pepino (var. Poinsett) foram inoculadas diretamente na raiz com 10 mL do inóculo. Os microrganismos utilizados foram: Trichoderma harzianum, Bacillus amyloliquefaciens, Debaryomyces hansenii e Mix (uma combinação dos três microrganismos). Foi estabelecido um delineamento experimental em blocos casualizados com 5 tratamentos e 20 repetições para cada tratamento com um total de 100 plantas como unidades experimentais. As variáveis avaliadas foram altura da planta, diâmetro do caule, comprimento da raiz, volume da raiz, rendimento da planta, açúcares totais e clorofila. Os dados foram analisados usando o SAS para Windows, versão 9.1.

Resultados e Discussão: De acordo com os resultados estatísticos, o tratamento T1 (Trichoderma harzianum) foi o que obteve os melhores resultados nas variáveis do estudo: altura da planta, diâmetro do caule, rendimento por planta, quantificação de açúcares totais (°Brix) e clorofila, seguido pelo T2 (Bacillus amyloliquefaciens). O potencial benéfico de várias espécies de Trichoderma foi demonstrado. Eles podem promover o crescimento de raízes, caules e área foliar em plantas porque favorecem o aumento da absorção de nutrientes (Rodríguez-García e Vargas-Rojas, 2022).

Originalidade/Valor: Este estudo contribui para a literatura ao relatar o potencial de vários microrganismos como alternativas de produção agrícola, que têm menor impacto ambiental, ao mesmo tempo em que melhoram a eficiência econômica e melhoram a qualidade das culturas.

Palavras-chave: Microrganismos Benéficos, *T.harzianum, B.amyloliquefaciens, D. Hansenii*, Promoção do Crescimento.

EFECTO DE BIOESTIMULANTES MICROBIANOS EN LA MORFOLOGÍA Y PRODUCTIVIDAD DE PLANTAS DE PEPINO (*Cucumis sativus* var. Poinsett) BAJO CONDICIONES DE INVERNADERO

RESUMEN

Objetivo: El objetivo de este estudio fue investigar el efecto de bioestimulantes microbianos en la morfología y fruto de plantas de pepino (*Cucumis sativus* var. Poinsett) bajo condiciones de invernadero.

Marco Teórico: La producción de pepino está ganando importancia entre los agricultores debido a su potencial benéfico y nutricional; esto, unido a un incremento en la demanda elevada con un mayor rendimiento e ingresos en un corto periodo de tiempo. Sin embargo, se ha buscado nuevas alternativas sustentables para mejorar la calidad del fruto mediante microorganismos promotores del crecimiento vegetal

Método: Las semillas de pepino (Var. Poinsett) fueron inoculadas directamente a la raíz con 10 mL del inóculo. Los microorganismos que se utilizaron fueron: *Trichoderma harzianum*, *Bacillus amyloliquefaciens, Debaryomyces hansenii,* Mix (combinación de los tres microorganismos). Se estableció un diseño experimental de bloques completamente al azar con 5 tratamientos y 20 repeticiones por cada tratamiento con un total de 100 plantas como unidades experimentales. Las variables evaludas fueron: altura de la planta, diámetro del tallo, longitud radical, volumen radical, rendimiento de la planta, azúcares totales y clorofila. Los datos fueron analizados con el programa estadístico SAS para Windows versión 9.1.

Resultados y Discusión: De acuerdo con los resultados estadísticos el tratamiento T1 (*Trichoderma harzianum*) fue el que logró mejores resultados sobre las variables de estudio: altura de la planta, diámetro de tallo, rendimiento por planta, cuantificación de azúcares totales (°Brix) y clorofila, seguido de T2 (*Bacillus amyloliquefaciens*). Se

ha demostrado el potencial benéfico de diversas especies de *Trichoderma* presentan capacidad de promover el crecimiento de raíces, tallos y área foliar en plantas debido a que favorecen el incremiento en la absorción de nutrientes (Rodríguez-García y Vargas-Rojas, 2022).

Originalidad/Valor: Este estudio contribuye a la literatura al reportar la capacidade de diversos microorganismos como alternativas agrícolas de producción, que tengan un menor impacto en el medio ambiente y a la vez mejoren la eficiencia de manera económica, además de potenciar las cualidades de los cultivos.

Palabras clave: Microorganismos Benéficos, *T.harzianum, B.amyloliquefaciens, D. hansenii,* Promoción de Crecimiento.

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1 INTRODUCTION

Cucumber (*Cucumis sativus*) is considered a plant of great economic importance due to its high consumption rate, as it serves both as a fresh and processed food. In several regions of the world, it is considered a species whose agricultural value lies in its seasonal production, which is why it needs to be developed primarily as a protected crop. It is one of the most consumed horticultural crops worldwide due to its nutritional value and high economic potential (Moreno et al., 2013). It is an export product that is cultivated and consumed in many regions of the world (Cruz, 2015).

Optimal crop development requires high levels of fertilizer and pesticide use, as these are essential elements for increasing agricultural yields. However, it has been proven that the indiscriminate use of these chemical inputs entails high costs, soil contamination, reduced biodiversity, increased risks of salinization, a significant decrease in soil energy reserves, and water pollution (González et al., 2018).

Currently, a new trend has emerged involving the use of beneficial microorganisms as an option to improve the yields of agricultural crops destined for food production (González and Fuentes, 2017). It is important to emphasize that inoculants with beneficial microorganisms are safe, and their effectiveness depends on careful handling to avoid reducing their benefits. However, in many developing countries, there are no industries dedicated to the production of inoculants, which further hinders their popularization in those areas. Furthermore, in many rural areas, there is widespread resistance to using bacteria and fungi as beneficial microorganisms in agriculture (Grageda-Cabrera et al ., 2012).

This study evaluated the effect of beneficial microorganisms on the morpho-productive production of cucumber (*Cucumis sativus) plants under greenhouse conditions.*

The potential of Trichoderma harzianum, Bacillus amyloliquefaciens , and Debaryomyces hansenii as plant growth promoters was quantified .

2 THEORETICAL FRAMEWORK

Agricultural activities and the growing global demand for food have resulted in environmental consequences by disrupting the ecological balance and soil quality. An additional challenge is the excessive and indiscriminate use of chemical fertilizers due to overexploitation. This has encouraged the search for alternative agricultural approaches that have a lower environmental impact while simultaneously improving economic efficiency and enhancing crop quality. Along these lines, biotechnologies, such as biofertilization, have emerged as a solution to mitigate negative effects on the environment, optimize the use of available resources on farmland, and offer a valuable option to replace, in whole or in part, mineral fertilizers (Afanador, 2017).

Microbial biostimulants contain beneficial microorganisms such as bacteria and fungi that inhabit roots and establish relationships with plants. The benefits of these plant-microorganism interactions include increased growth and production, as well as increased availability and/or uptake and assimilation of nutrients, even under abiotic stress conditions (Hasanuzzaman et al., 2021).

3 METHODOLOGY

3.1 GEOGRAPHICAL LOCATION OF THE EXPERIMENT

The experiment was carried out at the Faculty of Agricultural Sciences of the University of Veracruz located in Circuito Gonzalo Aguirre Beltrán in the municipality of Xalapa in the state of Veracruz, located at a latitude: 19°30'58.3" North and 96°55'06.4" West longitude at an altitude of 1400 meters above sea level.

3.2 BIOLOGICAL MATERIAL

For the activation of beneficial microorganisms, a specific culture medium was used; in the case of *Trichoderma harzianum* , Potato Dextrose Agar (PDA) was used , and for the

inoculation of *Debaryomyces hansenii and Bacillus amyloliquefaciens,* trypticase soy (ST) was used . The microorganisms were incubated for a period of 5 days at a temperature of 28º C.

3.3 VEGETATIVE MATERIAL

Cucumber seeds of the Poinsett variety were used. This seed has a 95% germination viability. It is a strong and vigorous plant, excellent fruit setting, adapts well to warm climates and not too hot conditions, and has cylindrical, dark green fruits approximately 18 to 25 cm long. To obtain high-quality cucumber seedlings, the seedbed was prepared using a substrate composed of peat moss, agrolite, and compost (2:1:1). The substrate was previously disinfected by applying ANIBAC 580®.

3.4 TRANSPLANTATION AND INCULATION WITH BENEFICIAL MACROORGANISMS

The cucumber plants were transplanted 30 days after sowing the seeds. Seedlings with the best morphological characteristics were chosen: large leaves, thick stems, and the greenest and most uniform leaves. Once the best 100 were selected, they were transplanted and watered thoroughly. Inoculation was carried out 5 days after transplanting, with 10 mL of each of the microorganisms to be evaluated applied at a concentration of 10^{8}. The application was made in a circular motion, surrounding the entire stem toward the root zone.

3.5 EXPERIMENTAL DESIGN AND VARIABLES EVALUATED

A completely randomized block design was established with 5 treatments: T0) Control, T1) *Trichoderma harzianum,* T3) *Bacillus amyloliquefaciens* , T4) *Debaryomyces hansenii* and T5) Mix (combination of the 3 beneficial microorganisms); with 20 replicates each, having a total of 100 plants as an experimental unit. At the conclusion of the experimental stage at 90 days after transplantation (DAT), the variables evaluated were: plant height, stem diameter, root length, root volume, plant yield, total sugars and chlorophyll. An analysis of variance was performed with the data obtained at the end of the experiment, the data were analyzed with the statistical program SAS for Windows version 9.1.

4 RESULTS AND DISCUSSIONS

According to the statistical analysis, for the plant height variable, no significant statistical differences were obtained (Figure 1A), with T1 (*Trichoderma harzianum*) being the one that obtained the highest average with 294.7 cm, followed by T2 (*Bacillus amyloliquefaciens*) (293.2 cm) and T3 (*Debaryomyces hansenii*) (288.05 cm). For their part, Gámez et al. (2019) reported that the application of *Bacillus amyloliquefaciens* in banana presented a significant increase in plant height (12.46 cm) compared to the control (8.78 cm). While authors such as Alvarado-Carrillo et al. (2018) mention that organic substrates, together with the positive effect of mycorrhizae on nutrient absorption, manifests itself in greater plant growth, which can guarantee greater productivity. This is attributed to the fact that among the beneficial mechanisms of growth-promoting bacteria are nitrogen fixation, synthesis of phytohormones, vitamins and enzymes, as well as phosphorus solubilization and organic phosphate mineralization (Moreno *et al* ., 2018).

Statistical analysis showed that no significant differences were found for the stem diameter variable (Figure 1B). However, the treatment with the highest average was T1 (*Trichoderma harzianum*) with 6.18 mm respectively, surpassing T2 (*Bacillus amyloliquefaciens) with 6.04 mm. The beneficial potential of various Trichoderma* species has been demonstrated with the ability to promote the growth of roots, stems and leaf area in plants because they allow an increase in the absorption of nutrients, stimulate the production of indole-acetic acid (IAA) and gibberellins (GA3), in addition to favoring the decrease in ethylene levels due to root colonization (Rodríguez-García and Vargas-Rojas, 2022).

Regarding root length (Figure 1C), the analysis indicates that there were significant differences between the treatments evaluated, with T2 (*Bacillus amyloliquefaciens*) standing out as the best average (55.45 cm), surpassing T3 (*Debaryomyces hansenii*) with 40.55 cm respectively. Beltrán-Acosta et al . (2023) obtained that the application of *B. amyloliquefaciens* (Bs006) increased root length by 16% in cape gooseberry (*Physalis peruviana L.*) seedlings under greenhouse conditions. It should be noted that Tejera et al . (2011) highlight that beneficial microorganisms have the ability to directly influence the root due to their nature and rhizospheric zone of influence.

As in the previous variable, for the root volume variable (Figure 1D), T2 (*Bacillus amyloliquefaciens*) showed a better result with an average of 29.05 cm^3 , surpassing treatment T1 (*Trichoderma harzianum*) with 18.2 cm^3 , for its part A ngulo-Castro et al. (2021) analyzed Bell pepper and jalapeño pepper plants inoculated with rhizobacteria and AMF where they

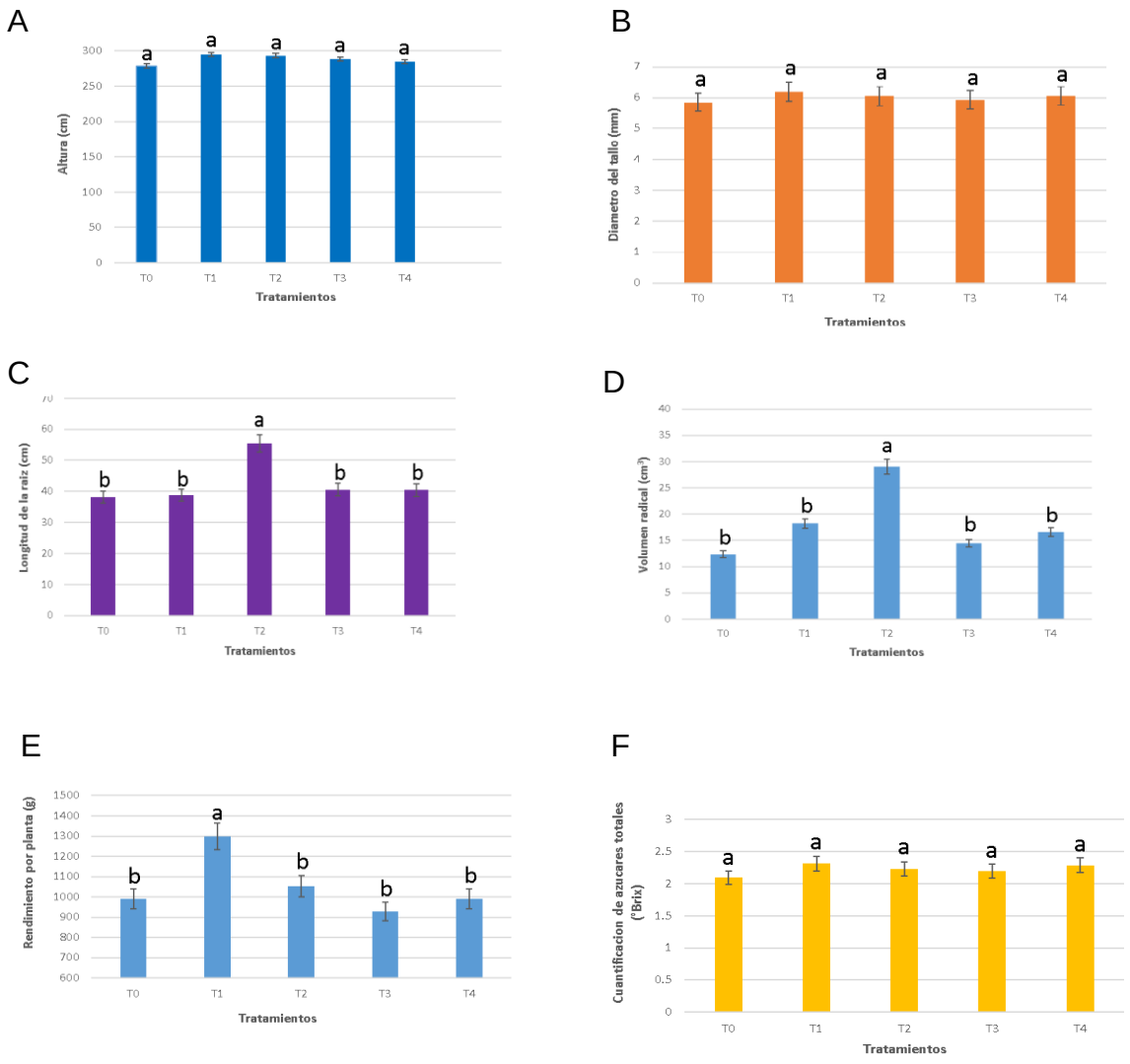
obtained that, for root volume, the treatment that gave the best result was the fertilized control in both plants. However, the best treatments with microorganisms in other variables were: *Bacillus pumilus* for pepper and *Pseudomonas tolaasii* for jalapeño pepper. For its part, *Debaryomyces hansenii* has been evaluated in hydroponic crops where its effect as a promoter in plant growth is highlighted, and it causes a greater proliferation of root hairs in cucumber and tomato plants (Bedolla et al., 2015).

Regarding the yield per plant variable (Figure 1E), significant differences were obtained between treatments, where T1 (*Trichoderma harzianum*) was positioned as the highest value with an average of 1,297.55 g per plant, surpassing T2 (*Bacillus amyloliquefaciens*) with 1,053.45 g. Veena et al. (2013) found a linear relationship between fruit traits and yield per plant and suggest that the crop improvement selection method could be focused mainly on fruit characteristics.

For the total sugars variable (Figure 1F) no statistically significant differences were found between treatments, however, the treatment that showed the highest percentage of total sugars in ripe fruits was T1 (*Trichoderma harzianum*) with an average value of 3.31 ° Brix, surpassing T4 (MIX) with 2.28 ° Brix. According to Galindo-Pardo et al. (2014) a percentage of total soluble solids of 2.5 ° Brix was found in cucumber, which is a very similar value to that reported by Moreno et al . (2013b) who mention that no significant changes occur during fruit development, as occurs with climacteric fruits. The small percentage of ° Brix observed can be explained by the fact that, in the initial stages of fruit growth and development, the total concentration of reducing and non-reducing sugars is very low.

Figure 1

A) Height of cucumber plants inoculated with beneficial organisms; B) Stem diameter; C) Root length; D) Root volume; E) Plant yield; F) Quantification of total sugars; G) Quantification of chlorophyll in cucumber plants. T0= Liquid control, T1= Trichoderma harzianum, T2= Bacillus amyloliquefaciens, T3= Debaryomyces hansenii, T4= MIX (Trichoderma harzianum + Debaryomyces hansenii + Bacillus amyloliquefaciens). Different letters above each bar show significant differences between treatments according to Tukey's test (P≤0.05).



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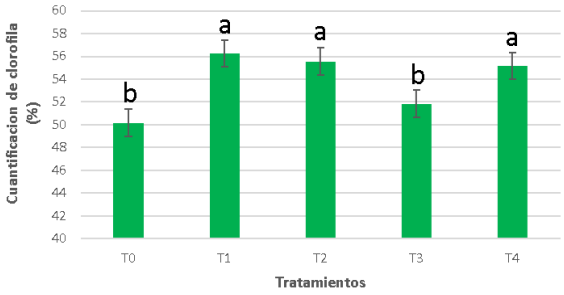

5 CONCLUSION

Beneficial microorganisms inoculated into cucumber plants using the liquid culture method contributed to plant growth and yield in each plant under a greenhouse system. *Trichoderma harzianum* stood out because it had a greater impact on the variables evaluated. Mixing these microorganisms in the same medium can be less efficient than applying them separately. Furthermore, plants that were not inoculated with yeast obtained the lowest values in most of the variables analyzed. The use of beneficial microorganisms in agriculture, and specifically *Trichoderma harzianum* and *Bacillus amyloliquefaciens* , stands out and at the same time promotes a great alternative as biofertilizers.

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