

Effect of microbial biostimulants on seedlings and fruits of jalapeño pepper (*Capsicum annuum* L.) produced in macrotunnel

Efecto de bioestimulantes microbianos en plántulas y frutos de chile jalapeño (*Capsicum annuum* L.) producidos en macrotúnel

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ABSTRACT

The intensive use of chemical fertilizers has caused environmental pollution and public health problems. A strategy that guarantees nutrient use efficiency with reduced rates of synthetic fertilizers is the use of microbial biostimulants. This study aimed to evaluate the effect of three microbial biostimulants on jalapeño pepper seedlings and fruits under protected macrotunnel conditions. The treatments evaluated were: 1) Genifix®, 2) *Trichoderma*, 3) Bio-Terra®, and 4) control. For the seedling evaluation, 40 seeds per treatment were germinated. The response variable was taken 29 days after sowing, which was the dry weight of the seedling and root. A randomized complete block design with four replications (blocks) was used for the fruit evaluation, with six bell pepper plants in each block. The response variables were weight, equatorial, and polar diameter of 20 fruits taken at random. Macrotunnel production in weight of total fruit per cut and per block was also considered. The Genifix® product produced seedlings with higher dry weight, with an average increase of 39.9 % in seedlings and 40.8 % in roots. Genifix® and *Trichoderma* had a significant effect on fruit weight and equatorial diameter. In production, Genifix® and *Trichoderma* biostimulants showed the best results. In conclusion, the use of the evaluated biostimulants presents an effective option to enhance the quality and yield of jalapeño peppers while minimizing the need for extensive fertilization in the crop.

KEY WORDS: *Bacillus*, *Trichoderma*, mycorrhizae, vegetables, protected production.

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RESUMEN

El uso intensivo de fertilizantes químicos ha provocado problemas de contaminación ambiental y de salud pública. Una estrategia que garantiza la eficiencia del uso de nutrientes con tasas reducidas de fertilizantes sintéticos es el uso de bioestimulantes microbianos. El objetivo del trabajo fue evaluar el efecto de tres bioestimulantes microbianos sobre plántulas y frutos de chile jalapeño en condiciones protegidas de macrotúnel. Los tratamientos evaluados fueron: 1) Genifix®, 2) Trichoderma, 3) Bio-Terra® y 4) testigo. Para la evaluación en plántulas se pusieron a germinar 40 semillas por tratamiento. La variable de respuesta se tomó a los 29 días después de la siembra, la cual fue el peso seco de plántula y raíz. En la evaluación de frutos se utilizó un diseño en bloques completos al azar con cuatro repeticiones (bloques), en cada bloque se consideraron seis plantas de chile. Las variables de respuesta fueron peso, diámetro ecuatorial y polar de 20 frutos tomados al azar. También se consideró la producción del macrotúnel en peso del total de frutos por corte y por bloque. El producto Genifix® originó plántulas con mayor peso seco, con un incremento promedio del 39.9 % en plántula y del 40.8 % en raíz. Genifix® y Trichoderma tuvieron un efecto significativo en el peso y diámetro ecuatorial de los frutos. En producción, los bioestimulantes Genifix® y Trichoderma fueron los que presentaron los mejores resultados. El uso de los bioestimulantes evaluados es una opción para incrementar la calidad y producción del chile jalapeño con una fertilización mínima del cultivo.

PALABRAS CLAVE: *Bacillus*, *Trichoderma*, micorrizas, hortalizas, producción protegida.

Introduction

In Mexico there is a record of 29 types of cultivated bell peppers, four of these varieties contribute 77.9 % of the national production volume, in which jalapeño pepper stands out, contributing 31.2 %; followed by morrón, with 21.7 %; poblano, with 13.7 %, and serrano, with 11.3 %. For this reason, jalapeño peppers are one of the most economically and culturally important vegetables in Mexico (SADER, 2020). SIAP (2021) reports a national production of 5,853,581.23 tons of bell peppers. Currently, vegetable production faces significant challenges. It must meet global demands for high productivity while adopting management practices that ensure environmental protection. This shift is increasingly directed towards sustainable agriculture.

Plant nutrition is a key factor in yield; the jalapeño pepper crop requires a dose of N-P-K 220-80-50 per hectare applied to the soil, so a deficiency of these nutrients correlates with significant losses in crop production (Abdelaziz *et al.*, 2008).

Given this background, there has been excessive use of synthetic fertilizers in vegetable production, which in many cases is inefficient, since a large part of the fertilizer applied escapes into the environment through leaching, polluting water bodies and soil (Daverede *et al.*, 2004; da Silva *et al.*, 2020). On the other hand, the rising costs of synthetic fertilizers and the environmental pollution they generate have led to the development of strategies to reduce the excessive use of chemical fertilizers. Microbial biostimulants are one of the most recent strategies that guarantee nutrient use efficiency in crop production with reduced rates of synthetic fertilizers (Wozniak *et al.*, 2020).

Microbial biostimulants are products made with microorganisms such as mycorrhizal and non-mycorrhizal fungi, endosymbiont bacteria, and plant growth-promoting rhizobacteria, which are applied to plants to improve nutritional efficiency, abiotic stress tolerance, and/or crop quality characteristics, regardless of their nutrient content (du Jardin *et al.*, 2015). Among the microorganisms most commonly used as plant biostimulants are *Trichoderma* fungi and *Bacillus* bacteria. Fungi of the *Trichoderma* genus colonize roots and transfer nutrients to plants (Behie & Bidochka, 2014), promoting vegetative growth and development through the formation of iron-chelating siderophores and growth-regulating hormones that act as stimulants in primary meristematic tissues in young plant parts (Fiorentino *et al.*, 2018). This induces an increased tolerance to abiotic stress and efficiency in nutrient use, growth, and organ morphogenesis in crops (Shoresh *et al.*, 2010; Colla *et al.*, 2015). Bacteria of the genus *Bacillus* are plant growth promoters and stimulate plant growth by activating various mechanisms within the plant cell. *Bacillus* species can solubilize different chemical substances; they are also involved in auxin synthesis, siderophore production, nitrogen fixation, and bacteriocins synthesis (Lee *et al.*, 2009; Badía *et al.*, 2011). These bacteria can produce organic compounds, fix nitrogen, and solubilize phosphates through enzymes such as nitrogenases and phytases, with positive effects on promoting plant growth and increasing productive potential (Corrales *et al.*, 2017). In addition, they positively influence plant growth through the synthesis and excretion of phytohormones such as auxins or cytokinins (Anguiano *et al.*, 2019), as well as with the production of compounds that strengthen plant immunity, such as jasmonic acid, salicylic acid, and phytoalexins (Ahmad *et al.*, 2008).

Recently, microbial biostimulants made with *Trichoderma* and *Bacillus* have gained interest in horticulture to improve the quality of crops with reduced or minimal traditional fertilization (Parađiković *et al.*, 2018; Sánchez-Sánchez *et al.*, 2022; Adame-García *et al.*, 2023). Jalapeño pepper holds significant importance in Mexico due to its substantial production volume and value, reaching 799,388.29 tons and 6,344,778.97 pesos, respectively (SIAP, 2021). Given its importance, the bell pepper crop has been employed for evaluating *Trichoderma* spp. and *Bacillus* spp. strains to enhance the quality and yield of crops. In habanero pepper plants, the *Trichoderma* strain Clombta increases height, stem diameter, aerial biomass, and root volume (Larios *et al.*, 2019), and mixed applications of *T. virens* and *T. koningiopsis* increase, on average, 14.53 % in plant height, 11.20 % in stem diameter, 12.97 % in root dry biomass, 33.13 % in fruit number, and 25.77 % in fruit weight, compared to control plants with 50 % chemical fertilization (Cristóbal-Alejo *et al.*, 2021).

In Serrano pepper, treatments with *Trichoderma* have shown high yields in HS-52 and Coloso varieties (15.67 and 13.89 t ha⁻¹) (Espinoza-Ahumada *et al.*, 2019). Additionally, compatibility between *T. asperellum*, *T. lignorum*, and *T. harzianum* species stimulated an increase in yield per plant by 86 % and fruit number by 79 % (Gallegos *et al.*, 2022). *Bacillus* strains produce, in habanero pepper, an increase in leaf area, seedling dry biomass, plant height, and root volume (Sosa-Pech *et al.*, 2019; Gamboa-Angulo *et al.*, 2020), in addition to stimulating plant development and fruit weight (Adame-García *et al.*, 2021). In a greenhouse, the *B. subtilis* strain CBMT51 increases fruit number and crop yield by 79.5 % and 58.8 %, respectively. Moreover, it improves some growth variables such as final height (56 %), the number of shoots (92 %), and total dry biomass (86 %) (Mejía-Bautista *et al.*, 2022). In the jalapeño pepper, plants inoculated with *B. subtilis* strain ITC-N67 showed an increase in stem diameter and root volume, while inoculation with *B. cereus* strain ITC-BL18 increased the number of flower buds, root fresh biomass, and total fresh biomass (Peña-Yam *et al.*, 2016).

The wide range of microbial biostimulants and their varying effects on crop quality provide compelling evidence for considering them as efficient alternatives in agricultural practices to enhance crop quality. The selection of the most beneficial biostimulants under specific conditions should be determined in simultaneous trials of several biostimulants under these specific conditions. Thus, the objective of this work was to evaluate the effect of three microbial biostimulants on jalapeño bell pepper seedlings and fruits under protected macrotunnel conditions.

Material and Methods

Location of the study site

The work was carried out at the Tecnológico Nacional de México, Campus Úrsulo Galván at coordinates 19° 24' 43.12" N and 96° 21' 32.12" W, situated in the municipality of Úrsulo Galván, in the central coastal region of Veracruz, Mexico. The climate of this region is classified as Aw (tropical humid-dry tropical) by the Köppen-Geiger system and modified by García (1981). It is defined as a warm sub-humid with rainfall in summer, featuring a temperature range between 24 and 26 °C and a precipitation range between 1100 and 1300 mm (INAP, 2013).

Plant material and microbial biostimulants

Seeds of jalapeño pepper variety "Dante F1" from Harris Moran® were used. All seeds were germinated in trays with peat moss substrate and inoculated with mycorrhizae (*Rhizophagus intraradices*) from INIFAP® before germination. The biostimulants evaluated were products based on *Trichoderma* spp. and *Bacillus* spp. which are fungi and bacteria, respectively. The treatments evaluated were: 1) Genifix®, 2) Trichoderma, 3) Bio-Terra®, and 4) control (Table 1).

Table 1. Treatments used in the evaluation of the effect of microbial biostimulants on jalapeño bell pepper seedlings and fruits under protected macrotunnel conditions.

Treatments	Active ingredient	Dose
Genifix®	<i>Bacillus</i> sp. JVN5 (10^{-8} CFU/mL), <i>B. megaterium</i> strain VVM1 (10^{-8} CFU/mL), <i>Bacillus</i> sp. FDMC4 (10^{-8} CFU/mL), <i>B. subtilis</i> strain JAG3 (10^{-8} CFU/mL), <i>B. megaterium</i> strain EAV2 (10^{-8} CFU/mL)	20 % (v/v)
Trichoderma	<i>Trichoderma harzianum</i> (10^{-11} spores)	1 Kg/200 L water
Bio-Terra®	<i>Rhizobium</i> sp. (6.0×10^{-8} CFU/L), <i>B. subtilis</i> (5.5×10^{-8} CFU/L), <i>B. thuringiensis</i> var. <i>krustaki</i> (3.0×10^{-8} CFU/L), <i>Beauveria bassiana</i> (2.08×10^{-10} spores/L), <i>Trichoderma harzianum</i> (5.0×10^{-10} spores/L)	0.5 Kg/200 L water
Control	Water	

Source: Own elaboration.

Seedling evaluation

For the evaluation of microbial biostimulants in jalapeño pepper seedlings, 40 seeds per treatment were germinated in trays with peat moss substrate, previously inoculated with mycorrhizae. Subsequently, 24 h after sowing, they were inoculated with 1 mL of each biostimulant solution (Table 1). A completely randomized experimental design with 40 replicates was used, considering each seedling as a replicate.

The treatments assessed corresponded to each of the biostimulants evaluated, and the control consisted of seeds without biostimulant application. The response variable was measured 29 days after sowing, specifically the dry weight of the seedling and root, which were placed in a drying oven at 65°C for 72 hours. To compare the effect of biostimulants on seedling and root weight, an ANOVA and a Tukey test with $\alpha=0.05$ for mean comparison were performed. Statistical analyses were conducted using Infostat software version 2020.

Evaluation in fruits

In the evaluation of jalapeño pepper fruits, a macrotunnel was used under protected conditions. The macrotunnel measured 3 m in width by 30 m in length (90 m²) and was lined with anti-aphid mesh, featuring a back cover and a double security door for access. The planting frame included one plant every 25 cm at three bolls, resulting in a total of 120 plants per bed and 240 per macrotunnel. A drip irrigation system and white mulch were employed. The average temperature

and relative humidity recorded during summer-autumn inside the macrotunnel were 26.8 °C and 79.0 %, respectively.

Seedlings, 29 days after sowing in a tray, were transplanted into soil with the following physical and chemical characteristics: pH 6.85, EC 81.3 μ S, DAP 1.2 g/mL, Sand 30.76 %, Silt 30.56 %, Clay 38.68 %, O. M. 2.11 %, K 0.17 Cmol/Kg, Ca 12.09 Cmol/Kg, Mg 3.91 Cmol/Kg, N 0.12 %, and P 11.5 mg/L.

Fruits from three harvest (90, 105, and 140 days after transplanting (ddt)) were used. In all treatments, a traditional minimum fertilization of the crop was applied (68 kg/ha nitrogen and 11 kg/ha phosphate as P₂₀₅). In the case of phosphate/nitrate, it was applied as a drench (50 mL per plant) every 30 days. Micronutrients were applied every 20 days via foliar, and boron/calcium were applied via foliar at the beginning of flowering and then every 20 days (refer to Table 2). Soil applications of humic acids (Hortihumus®, 10 %) were also made, initially at 15 ddt and subsequently every 30 days. All applications were continued until the end of the production cycle.

Table 2. Chemical fertilization applied to the soil directed to the plant collar in each of the treatments.

Ingredient	Commercial name	Dose
Phosphate/Nitrate	DAP + Urea	1g DAP + 1g Urea per plant in 50 mL of water
Micronutrients	Bayfolan®	2L ha ⁻¹ in 200 L of water
Boron/Calcium	Zinc® Cape	2L ha ⁻¹ in 200 L of water

Source: Own elaboration.

A randomized complete block design was used with four replications (blocks) distributed across two beds. In each block, there were six bell pepper plants. In each treatment, the biostimulants (Table 1) were applied monthly to the soil, and directed to the plant collar (drench).

The response variables included the weight, equatorial diameter, and polar diameter of 20 fruits randomly selected from the six plants within each block. Macro-tunnel production was also assessed based on the total fruit weight per cut and block.

To compare the effect of biostimulants on jalapeño pepper fruit weight and dimensions, as well as yield, an ANOVA and a Tukey $\alpha=0.05$ comparison of means were performed. Statistical analyses were performed with Infostat software version 2020.

Results and Discussion

Seedling evaluation

The biostimulant Genifix® had a significant effect on the increase in seedling dry weight and root dry weight of jalapeño pepper, with an average increase of 39.9 % (0.92 g to 1.53 g) in seedling dry weight and 40.8 % (from 0.90 g to 1.52 g) in root dry weight (Table 3). More vigorous seedlings and seedling roots were obtained when seeds were treated with Genifix® (Figure 1). The *Trichoderma* and Bio-Terra® products did not show significant effects on the increase in seedling and root dry weight of jalapeño pepper, although, with the *Trichoderma* biostimulant, there was a numerical increase in the weights (Table 3).

Most evaluations on microbial biostimulants in green peppers have been conducted on habanero cultivar (Candelero et al., 2015; Larios et al., 2019; Sosa-Pech et al., 2019; Cristobal-Alejo et al., 2021; Murillo-Cuevas et al., 2021; Mejia-Bautista et al., 2022), providing limited information for other pepper varieties such as serrano (Espinoza-Ahumada et al., 2019; Cabanzo-Atilano et al., 2020), morrón (Adame-García et al., 2023) and jalapeño (Peña-Yam et al., 2016; Angulo-Castro, et al., 2018; Camacho-Rodríguez, et al., 2022). Thus, the obtained data provide new information on the effects of the biostimulants evaluated in the jalapeño cultivar.

Bacterial biostimulants have been evaluated in jalapeño pepper, yielding varied results. Applications of *Serratia plymuthica*, *S. marcescens*, and *Pseudomonas tolaasii* have been shown to increase the height and number of leaves in jalapeño pepper seedlings (Angulo-Castro et al., 2018; Camacho-Rodríguez et al., 2022). However, it has also been reported that jalapeño pepper plants with applications of *Enterobacter ludwigii*, *S. quinivorans*, *Lysinibacillus sphaericus*, *Aeromonas media*, and *P. poae* did not show significant differences in height and stem diameter compared to control plants (Guevara-Avenida et al., 2014).

Obtained results indicate that the product Genifix® formulated with bacterial strains of the genus *Bacillus* stimulates the development of jalapeño pepper seedlings. In contrast, products formulated with *T. harzianum* fungi failed to stimulate seedling development. Similar results were obtained in habanero peppers, where inoculation with Genifix® increased seedling weight by an average of 1.5 %, while *Trichoderma*-based biostimulants did not show significant differences compared to the control (Murillo-Cuevas et al., 2021).

Table 3. Effect of microbial biostimulants on seedling and root dry weight of jalapeño pepper.

Treatments	Dry weight (g)	
	Seedling	Root
Genifix®	1.53 ± 0.04 ^a	1.52 ± 0.05 ^a
Trichoderma	0.96 ± 0.06 ^b	0.98 ± 0.04 ^b
Bio-Terra®	0.80 ± 0.04 ^c	0.82 ± 0.04 ^c
Control	0.92 ± 0.03 ^{bc}	0.90 ± 0.04 ^{bc}
C.V. (%)	24.80	24.36

Different letters indicate statistical differences ($p < 0.05$) between treatments. Data are presented in $\bar{x} \pm E.E.$, C.V. = Coefficient of Variation. Source: Own elaboration based on results.

The effect of the biostimulant Genifix® on the increase in dry weight of seedlings and roots of jalapeño pepper seedlings may be due to the properties related to the promotion of plant growth that the bacteria of the genus *Bacillus* present in its composition have since these can produce indoleacetic acid (Sosa-Pech *et al.*, 2019; Mejía-Bautista *et al.*, 2022). This acid is a hormone that regulates cell growth, tissue differentiation, and mediates phototropism and gravitropism. It also plays a role in stimulating root and xylem formation (Vega-Celedón, 2016).

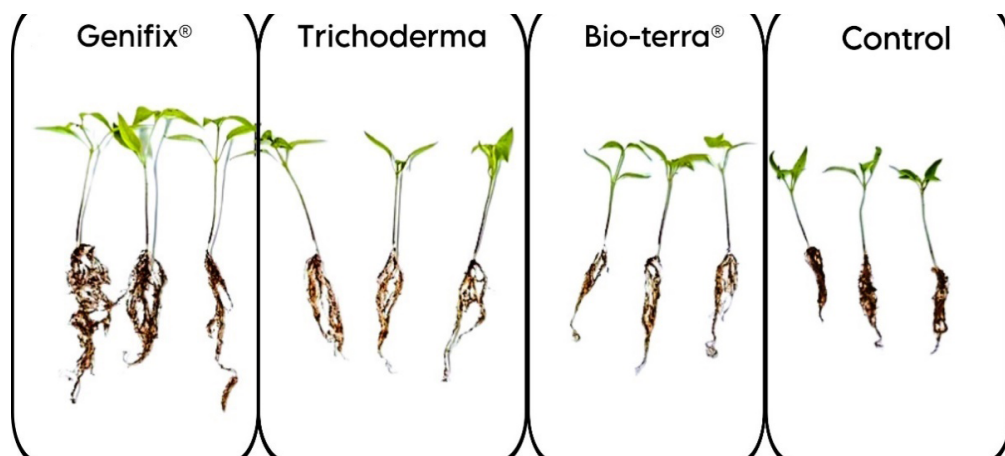


Figure 1. Jalapeño pepper seedlings treated with microbial biostimulants.

Source: Own elaboration based on results.

Some authors reported *Bacillus* spp. strains as growth promoters in *C. annuum*, increasing seedling biomass by up to 32 % (Ogugua et al., 2018). In addition, *B. subtilis* strain CBMT51 has been reported to promote the growth of habanero pepper seedlings, increasing biomass by 34.6 % (Mejía-Bautista et al., 2022). Likewise, it has been observed that *Bacillus* strain CBCC57 increases the dry weight of habanero pepper seedlings by 33 % (Sosa-Pech et al., 2019). Concerning the product Genifix®, two of its *Bacillus* strains, JAG3 and FDMC4 increase, on average, up to 30 % the dry weight of habanero pepper seedlings (Adame-García et al., 2021). These results are reflected in the performance of the product in the jalapeño variety, obtaining increases above those reported for *Bacillus* bacteria in habanero peppers

The effects of biostimulants based on the genus *Trichoderma* can vary because they can be formulated with strains or species that possess different attributes for the stimulation of plant growth, in addition to biochemical differences that allow one strain to be better than another in promoting plant growth (Ortuño et al., 2013). For example, significant differences have been reported between two *Trichoderma* strains, SP6 and Clombta, with the latter stimulating a greater increase in aerial biomass (fresh= 0.8 g plant⁻¹ and dry= 0.13 g plant⁻¹) and root volume (fresh= 0.13 g plant⁻¹ and dry= 0.04 g plant⁻¹) of pepper var. Chichen Itza (Larios et al., 2019).

On the other hand, in the evaluation of 14 *Trichoderma* strains on habanero pepper seedlings, a varied effect of the strains could be observed, with some standing out more than others, the Th07-04 strain increased root length by 29.4 %, Th02-01 by 84.6 % in root volume and Th07-05 by 62.5 % in root biomass (Candelero et al., 2015). In addition, in xcat'ik pepper plants, microbial biostimulants based on *B. subtilis* and *T. harzianum* did not show significant differences with the control in relation to root dry biomass but did show differences in root volume (Gamboa-Angulo et al., 2020). Concerning bacteria of the genus *Rhizobium*, positive effects of *R. nepotum* have been reported to increase the weight of the aerial part of poblano pepper seedlings by more than 20 % (González et al., 2017). However, this was not the case for the product Bio-Terra® with *Rhizobium* sp. in this evaluation.

The little or no effect of *Trichoderma* and Bio-Terra® products on the development of seedlings and roots of jalapeño pepper seedlings may be attributed to the fungal and bacterial strains of these products being less physiologically and biochemically compatible with the jalapeño pepper crop in the microorganism-plant interaction and in the genetic recognition between them (Cano, 2011; Vázquez et al., 2000).

Evaluation in fruits

In the first jalapeño peppers harvest, plants treated with Genifix® had significantly heavier fruits than the control plants, increasing on average by 7.61 g (13.3 %) compared to the control plants (Table 4). Regarding the fruit dimension, all biostimulants increased the polar diameter, with a maximum average increase of 7.9 % by the Bio-Terra® biostimulant. For the equatorial diameter, only the Genifix® and *Trichoderma*-based products showed a significant increase of 11.4 % and 7.6 % respectively (Table 4).

The applications of the Genifix® and *Trichoderma* biostimulants in the second harvest of jalapeño peppers had significant effects on the increase in weight and equatorial diameter (Table 4). Genifix® increased the weight of the fruits on average by 3.57 g (5.9 %), and *Trichoderma* by 4.95 g (8.0 %) compared to the fruits of the control plants (Table 4).

For the third harvest, the Genifix® and Bio-Terra® biostimulants had significant effects compared to the fruits of the control plants, increasing the weight by an average of 7.12 g (10.6 %) and 3.96 g (6.2 %), respectively (Table 4), resulting in larger and heavier fruits (Figure 2). All biostimulants increased the polar diameter of jalapeño peppers, with a maximum increase of 7.7% by the Genifix® product (Figure 2). Only Genifix® and *Trichoderma* affected the increase in the equatorial diameter of the fruit (Table 4).

When analyzing the three harvests together, only the Genifix® and *Trichoderma* biostimulants affected the weight and equatorial diameter of the fruits (Table 4). Genifix® increased the weight by an average of 5.75 g (9.1 %), the equatorial diameter by 0.2 cm (5.2 %), and *Trichoderma* by 4.20 g (6.8 %) and 0.15 cm (3.9 %) in equatorial diameter, compared to the fruits of the control plants. For the polar diameter of the fruits, all biostimulants had a significant effect on its increase, with a maximum of 5.5% by the Genifix® product (Table 4).

The production in each of the three fruit harvests of jalapeño peppers under protected macrotunnel conditions with applications of microbial biostimulants varied, with higher production in the second harvest (Table 5). The highest production of jalapeño peppers was recorded in plants with applications of biostimulants. Plants treated with Genifix® had a higher production of jalapeño peppers (47,110 kg), followed by plants treated with *Trichoderma* (44,199 kg) and Bio-Terra® (40,191 kg) (Table 5). The average production, considering all three fruit harvests, was significantly higher in plants treated with Genifix® (15.70 kg), showing an increase of 28.1 %, and *Trichoderma* (14.73 kg) with an increase of 23.4 % compared to the control plants (Table 5).

Table 4. Effect of microbial biostimulants on jalapeño pepper fruit weight and diameter in three harvest cuts under protected macrotunnel conditions.

Treatments	Weight (g)	Pole diameter (cm)	Equatorial diameter (cm)
1st harvest cut			
Genifix®	57.43 ± 1.6 ^a	9.90 ± 0.14 ^a	3.68 ± 0.06 ^a
Trichoderma	55.93 ± 1.6 ^{ab}	9.84 ± 0.14 ^a	3.53 ± 0.06 ^{ab}
Bio-Terra®	53.03 ± 1.5 ^{ab}	10.0 ± 0.15 ^a	3.40 ± 0.08 ^{bc}
Control	49.82 ± 2.1 ^b	9.21 ± 0.18 ^b	3.26 ± 0.08 ^c
C.V. (%)	16.24	7.58	9.09
2nd harvest cut			
Genifix®	60.54 ± 1.1 ^a	10.05 ± 0.09 ^a	3.85 ± 0.03 ^a
Trichoderma	61.92 ± 1.2 ^a	9.98 ± 0.08 ^{ab}	3.86 ± 0.03 ^a
Bio-Terra®	58.44 ± 1.2 ^{ab}	9.67 ± 0.09 ^b	3.75 ± 0.04 ^{ab}
Control	56.97 ± 1.2 ^b	9.73 ± 0.09 ^{ab}	3.67 ± 0.04 ^b
C.V. (%)	16.94	7.46	7.84
3rd harvest cut			
Genifix®	67.47 ± 0.98 ^a	10.03 ± 0.07 ^a	3.95 ± 0.03 ^a
Trichoderma	63.18 ± 0.97 ^{bc}	9.54 ± 0.05 ^b	3.86 ± 0.04 ^{ab}
Bio-Terra®	64.31 ± 1.02 ^{ab}	9.71 ± 0.07 ^b	3.76 ± 0.04 ^{bc}
Control	60.35 ± 1.02 ^c	9.26 ± 0.07 ^c	3.74 ± 0.03 ^c
C.V. (%)	13.46	6.45	7.35
Total of the three harvest cut			
Genifix®	63.06 ± 0.74 ^a	10.02 ± 0.05 ^a	3.86 ± 0.02 ^a
Trichoderma	61.51 ± 0.73 ^{ab}	9.77 ± 0.06 ^b	3.81 ± 0.02 ^a
Bio-Terra®	59.99 ± 0.76 ^{bc}	9.75 ± 0.05 ^b	3.70 ± 0.04 ^b
Control	57.31 ± 0.78 ^c	9.47 ± 0.05 ^c	3.66 ± 0.03 ^b
C.V. (%)	16.30	7.24	8.42

Different letters indicate statistical differences ($p < 0.05$) between treatments. Data are presented in $\bar{X} \pm E.E.$,
C.V. = Coefficient of Variation.

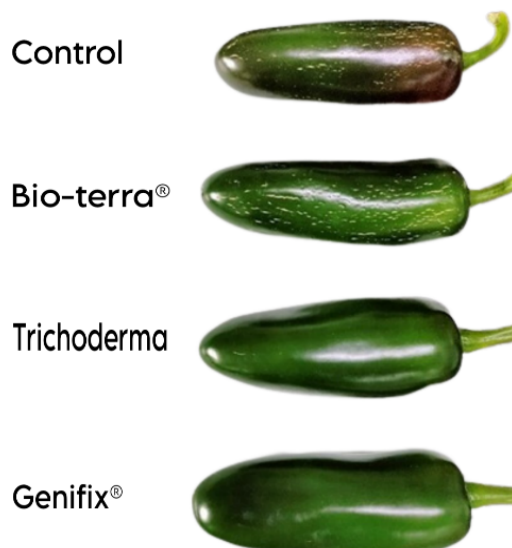


Figure 2. Jalapeño pepper fruits obtained from plants with applications of microbial biostimulants in the third harvest cut under protected macrotunnel conditions.

Source: Own elaboration based on results.

Table 5. Jalapeño pepper production in three fruit harvests under protected macrotunnel conditions in relation to the applied microbial biostimulants.

Treatments	Weight (Kg) per harvest cut			Total (Kg)	$\bar{x} \pm E.E$
	1 st	2 nd	3 rd		
Genifix®	15.215	16.820	15.075	47.110	15.70 ± 0.55 ^a
Trichoderma	14.524	14.942	14.733	44.199	14.73 ± 0.34 ^a
Bio-Terra®	12.195	14.599	13.397	40.191	13.40 ± 0.69 ^{ab}
Control	9.958	13.604	10.312	33.874	11.29 ± 0.73 ^b
C.V. (%)					9.23

Different letters indicate statistical differences ($p < 0.05$) between treatments. Data are presented in $\bar{x} \pm E.E.$, C.V. = Coefficient of Variation.

Our results confirm the biostimulant effects of *Bacillus* bacteria and *Trichoderma* fungi on the development of vegetable fruits and contribute new information about the benefits of these microorganisms in increasing the weight and size of jalapeño pepper fruits. This is particularly relevant as assessments of this nature have primarily been conducted on the habanero cultivar, where higher percentage increases in fruit weight have been reported than those obtained in this study for the jalapeño cultivar.

For example, the CBMT51 strain of *B. subtilis* increased fruit weight by up to 37% compared to the control (Mejía-Bautista et al., 2022), and the interaction of the Th05-02 strain of *Trichoderma virens* and Th41-11 of *T. koningiopsis* increased fruit weight by 25.8% compared to the control with 50% chemical fertilization (Cristóbal-Alejo et al., 2021). However, simultaneous evaluations of biostimulants based on the *Bacillus* and *Trichoderma* genera have reported varied results among products. For instance, Baktilis® (*B. subtilis*) outperforms Tricho-Bio® (*T. harzianum*) in increasing the production of xcat'ik chili, but neither of the two products affected fruit weight (Gamboa-Angulo, et al., 2020).

On the other hand, Genifix® (*Bacillus* spp.), T22® (*T. harzianum*), and MIX® (*Trichoderma* spp.) significantly increased the weight and dimensions of habanero pepper fruits without registering statistical differences between the products. They increased the weight by up to 18.8%, and the polar and equatorial diameters by 14.5% and 10.5%, respectively, with products based on the *Trichoderma* genus (Murillo-Cuevas et al., 2021). However, in bell peppers, the Genifix® product surpassed T22® and MIX®, increasing fruit weight by 17.1%, and the width and length dimensions by 9.2% and 12.1%, respectively, compared to biostimulants based on *Trichoderma* spp., which increased, on average, up to 10.5% in weight, 7.5% in width, and 15.1% in length of the fruits. Nevertheless, all biostimulants were significantly superior to the control (Adame-García et al., 2023).

The differences in fruit weight increase between the habanero and jalapeño varieties can be attributed to the fact that the habanero cultivar is more compatible with the microorganisms. Likewise, the variations among the products can be attributed to the different degrees of compatibility of the microbial strains with the host plant. The effect of inoculating microbial biostimulants on host plants largely depends on the physiological and biochemical compatibility of the microorganism-plant interaction and the genetic recognition between them (Cano, 2011; Vázquez et al., 2000). Other factors, such as the presence of other microorganisms in the rhizosphere and environmental conditions like temperature and humidity, can also play a role (Cano, 2011).

However, the results obtained with biostimulants indicate that using these products significantly benefits fruit development in jalapeño peppers. Therefore, microbial biostimulants are an efficient option to increase the productivity and fruit quality of the jalapeño cultivar, with a lower environmental impact by using biological products instead of increasing chemical fertilization.

All biostimulants were capable of increasing fruit weight and dimensions in at least one of the harvests. Nevertheless, Genifix® had a greater effect, possibly attributed to the ability of bacteria of the genus *Bacillus* to produce hormones such as auxins, cytokinins, and gibberellins,

promoting fruit growth and development (Ruiz-Cisneros *et al.*, 2019). This effect may also be influenced by genetic, biochemical, and physiological mechanisms directly affecting the fruit (Mena-Violante *et al.*, 2009).

Conclusions

The seedlings inoculated with the Genifix® product were the only ones that increased their biomass. Genifix® had the greatest biostimulant effect on the weight and size of jalapeño pepper fruits, followed by the biostimulants *Trichoderma* and Bio-Terra®. Jalapeño bell pepper yield was higher in plants with applications of Genifix® and *Trichoderma* biostimulants.

The use of microbial biostimulants is recommended to improve the quality and production of jalapeño peppers, ensuring robust seedling development, successful transplanting, plant growth, and favorable crop yields.

Authors contribution

JAG and FDMC designed and conducted the research; JAG and FDMC developed the methodology; RCC performed the field testing and variable collection; FDMC and HCM performed the analysis of results; JAG, FDMC, and JAFV performed the writing, preparation, drafting, revising, and editing of the manuscript. All authors of this manuscript have read and accepted the published version of the manuscript.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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