Article

Biostimulants in the quality of habanero pepper fruits

Félix David Murillo-Cuevas[1]
Héctor Cabrera-Mireles[2§]
Jacel Adame-García[1]
Andrés Vásquez-Hernández[2]
Adrián de Jesús Martínez-García[1]
Rebeca Luria Moctezuma[1]

[1]National Technological Institute of Mexico-Úrsulo Galván. Highway Cd. Cardel-Chachalacas km 4.5, Úrsulo Galván, Veracruz, Mexico. CP. 91667. [2]Cotaxtla Experimental Field-INIFAP. Federal highway Veracruz-Córdoba km 34.5, Medellín de Bravo, Veracruz, Mexico. CP. 94992.

[§]Corresponding author: cabo.cabrera50@gmail.com.

Abstract

The production of habanero pepper is mainly done with chemical fertilization, which can be inefficient since much of the applied fertilizer is released into the environment and can often become unavailable to plants. One way in which the use of chemical fertilizers can be reduced and the absorption of nutrients by the crop can be improved is by using biostimulants in the fertilization of plants. The objective of the work was to evaluate three microbial biostimulants on seeds, seedlings and the quality of habanero pepper fruit under protected macro tunnel conditions. The work was carried out in 2021 at the Tecnológico Nacional de México, Úrsulo Galván campus. The habanero pepper seeds used were of the Jaguar variety provided by the Cotaxtla Experimental Field- National Institute of Forestry, Agricultural and Livestock Research. The treatments evaluated were: 1) T22®+mycorrhizae INIFAP®; 2) Mix®; 3) Genifix®; and 4) control. An evaluation of the treatments in seed germination, growth and biomass of seedlings and quality of habanero pepper fruits was carried out. There were no significant differences in seed germination, but in height and dry weight of seedlings at 20 days after inoculation, since the seeds inoculated with the biostimulant Genifix were the ones that reached the highest height and dry weight. In terms of fruit size and weight, the plants treated with the biostimulants produced significantly larger and heavier fruits than the control plants.

Keywords: *Bacillus*, *Trichoderma*, biofertilizers, vegetables.

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Introduction

Currently, the use of chemical fertilizers in agriculture is very inefficient; much of the applied fertilizer is released into the environment, washed out of the soil by runoff, and can often become unavailable to plants; through a chemical, physical or biological transformation (Sánchez *et al*., 2001; Daverede *et al*., 2004). So, farmers need to apply more chemical fertilizer than the plant really needs, and the rest is often released into the environment, polluting the air and water (Vance, 2001). In addition, the industrial production of chemical fertilizers contributes significantly to global CO_2 emissions (Vance, 2001).

The production of habanero pepper is carried out mostly with chemical fertilization (Reyes and Cortéz, 2017; Ramírez-Vargas *et al*., 2019), in various dosages, depending on the producer's economic resources and in many cases without the required technical advice (Grageda-Cabrera *et al*., 2012).

One way in which the use of chemical fertilizers can be reduced without harming plant nutrition is by improving nutrient uptake by crops by using biostimulants in plant fertilization (Halpern *et al*., 2015; Torres *et al*., 2016; Rodríguez-Hernández *et al*., 2020). Agricultural biostimulants are substances or microorganisms that are applied to plants with the aim of improving nutritional efficiency, tolerance to abiotic stress and crop quality, some of these biostimulants being commercial products that contain a mixture of these substances and microorganisms (du Jardin, 2015). Microbial biostimulants include mycorrhizal and non-mycorrhizal fungi, endosymbiotic bacteria, and plant growth-promoting rhizobacteria (Calvo *et al*., 2014; Halpern *et al*., 2015).

The use of microbial biostimulants in modern agriculture has considerably increased the interest in studying them and knowing their effects (Calvo *et al*., 2014), they have been evaluated as stimulants of corn and beans (Hernández *et al*., 2017), as biostimulants in passion fruit seedlings (Díaz *et al*., 2020) and horticultural crops under conditions of abiotic stress (Bulgari *et al*., 2019); as well as plant growth promoters and sustainable management of phytoparasite nematodes (D'Addabbo *et al*., 2019).

In addition, they have been evaluated in response to the agronomic characteristics of seedlings, plant and fruit quality in chili pepper (Candelero *et al*., 2015; Gamboa-Angulo *et al*., 2020). Biostimulants in horticulture need to be evaluated locally and temporarily, tools are needed to monitor the efficiency of biostimulants and thus define mechanisms that optimize their use. The objective of this work was to evaluate three microbial biostimulants on the quality of fruit and production of habanero pepper var. Jaguar under protected macro tunnel conditions.

Materials and methods

Study area

The work was carried out in 2021 at the Tecnológico Nacional de México, Úrsulo Galván campus at the coordinates of 19° 24' 43.12" north latitude and 96° 21' 32.66" longitude west, located in the municipality of Úrsulo Galván, in the coastal central region of Veracruz. The climate of this region is classified as Aw (tropical wet-dry) by the Köppen-Geiger system, defined as warm subhumid with rainfall in summer, with a temperature range between 24 and 26 °C and a precipitation range between 1 100 and 1 300 mm (INAP, 2013).

Plant material

The habanero pepper seeds used were of the Jaguar variety inoculated with rhizophagus provided by INIFAP's Cotaxtla Experimental Field. The seeds were germinated in trays in the Cotaxtla Experimental Field.

Biostimulants

The biostimulants that were used were products based on *Trichoderma* spp., and nitrogen-fixing bacteria *Bacillus* spp. The treatments evaluated were: 1) T22; 2) Mix; 3) Genifix; and 4) control (Table 1).

Table 1. Treatments used in the evaluation of biostimulants in seeds and plants of habanero pepper (*C. chinense* var. Jaguar).

Treatments	Active ingredient	Enterprise	Dose
T22®+ INIFAP® mycorrhizae	*Trichoderma harzianum* + mycorrhizae	PHC and INIFAP	0.5% (w/v)
MIX®	*T. harzianum*, *T. viride*, *T. asperellum*, *T. koningli*	Organisms beneficial	0.5% (w/v)
Genifix®	*Bacillus* sp. JVN5, *B. megaterium* strain VVM1, *Bacillus* sp. FDMC4, *B. subtilis* strain JAG3, *B. megaterium* strain EAV2	TecNM, Úrsulo Galván C*ampus*	20% (v/v)
Control	Water		

Evaluation of biostimulants in habanero pepper seeds. In trays with Peat Moss substrate, 100 seeds of habanero pepper Jaguar variety were inoculated with each of the treatments. For treatments 1 and 2, 10 ml of T22 and Mix were applied, respectively. Treatment 3 was inoculated with 1 mL of Genifix. The response variables were germinated seedlings, seedling height at 20 days after inoculation and dry weight of seedlings (65 °C for 72 h) at 20 days after inoculation.

Evaluation of biostimulants in habanero pepper plants. A macro tunnel 3 m wide by 30 m long, lined with anti-aphid mesh, was used. Inside the macro tunnel, two beds were built with compost mixed with soil and black-white mulch, the beds were 90 cm wide and 30 cm high, separated from each other by an alley no less than 40 cm wide, the planting frame was one plant every 25 cm, which gave a total of 120 plants per bed and 240 per macro tunnel. An irrigation system of four water outlets and 30 m of 6 000 caliber tape was used for each bed, connected to the main line with four bypass valves to control the irrigation of the crop. The experimental design was in randomized complete blocks with four repetitions. In each experimental block, the biostimulants were applied monthly to the soil, directed to the neck of the plant (drench).

Culture management consisted of the application of humic acids (10%) at 15 days after transplantation (DAT); through the irrigation system, at 20, 50, 90 and 120 DAT, chemical fertilization was applied in drench, 20 ml per plant and foliar applications of micronutrients. At 80 DAT, boron/calcio was applied (Table 2). At 115 DAT, Bayfolan® foliar fertilizer (0.2%) was applied for flowering induction.

Table 2. Chemical fertilization applied to the soil directed to the neck of the plant.

Ingredient	Trade name	Dose
Phosphorus/nitrate	DAP + urea	1 g DAP + 1 g urea in 20 ml^{-1}
Micronutrients	PoliQuel	Foliar 2 L ha^{-1} in 200 L of water
Boron/calcium	Boron/calcium	Foliar 2 L ha^{-1} in 200 L of water

The fruits of four cuts, at 111, 118, 136 and 146 days, were used. The response variables were: height and weight of seedling at 20 days after inoculation, weight and quality of fruit (equatorial diameter and polar diameter) and weight of 20 fruits chosen at random by treatment. Statistical analysis. To compare the effect of biostimulants on the germination of habanero pepper seeds, a non-parametric Friedman analysis was performed and to compare the effect on seedling height, seedling weight, weight and fruit quality, an Anova and a Tukey's comparison of means $\alpha= 0.05$ were performed. In addition, the cut + biostimulant interaction was also analyzed. Statistical analyses were performed with the InfoStat software version 2020.

Results and discussion

The seeds of habanero pepper treated with the biostimulants Genifix and Mix registered 94% germination, unlike the seeds treated with the biostimulant T22 and the control, which only obtained 90 and 87% germination respectively; however, these differences were not significant ($T^2= 1.16$, $p= 0.3254$) in the Friedman statistical analysis. As for the height of the seedlings at 20 days after inoculation, the seeds inoculated with the biostimulant Genifix were the ones that reached the highest height, they were significantly different ($F_{3, 959}= 405.97$, $p= 0.0001$) from the seeds inoculated with the other treatments (Table 2).

Seeds inoculated with the biostimulant Mix did not show significant differences in seedling height with respect to the control (Table 2). For the dry weight of seedlings at 20 days after inoculation, the seeds inoculated with the biostimulant Genifix were the ones that achieved the highest weight of seedlings and were significantly different ($F_{3, 959}= 18.75$, $p= 0.0001$) from the seeds treated with the biostimulant T22 and the control (Table 3).

Table 3. Effect of three biostimulants on the height and weight of habanero pepper seedling.

Biostimulants	Height (cm)	Weight (g)
Genifix	6.53 ±0.04 a	0.037 ±0.004 a
Q22	6.04 ±0.12 b	0.029 ±0.001 c
Mix	3.82 ±0.12 c	0.035 ±0.001 ab
Control	3.68 ±0.08 c	0.032 ±0.004 bc
CV (%)	19.01	12.02

Means with a common letter are not significantly different ($p> 0.05$).

For the variable polar diameter of the fruit, the plants treated with the biostimulants had fruits significantly larger ($F_{3, 4028}$=98.44, p= 0.0001) than the control plants (Table 4). When comparing only biostimulants, it was observed that the fruits of plants treated with the biostimulant T22 were, on average, larger than the fruits of plants treated with the biostimulant Mix (Table 4). The biostimulant Genifix did not show significant differences in relation to the other two biostimulants (Table 4).

When analyzing the cut + biostimulant interaction, it was found that the first three cuts + any of the biostimulants resulted in significantly larger fruits ($F_{9, 4028}$= 8.48, p= 0.0001) than the controls in any of the cuts. The best interaction that occurred was cut one + biostimulant T22. In the variable equatorial diameter of fruit, significant differences ($F_{3, 4028}$= 66.82, p= 0.0001) between the treatments were registered; however, the results were reversed in terms of biostimulants, the fruits of plants treated with the biostimulant Mix were those that presented a larger equatorial diameter compared to the fruits of plants treated with the biostimulant Genifix (Table 4).

The stimulant T22 did not obtain statistical differences in relation to the other two biostimulants (Table 4). When comparing the biostimulants with the control, the results were like the polar diameter of the fruit, since the plants treated with any of the biostimulants had wider fruits than those obtained in the control plants (Table 4). As for the interaction, cut two + biostimulant Mix was the one that generated the widest fruits and was significantly different ($F_{9, 4028}$= 9.18, p= 0.0001) from all cut + control interactions.

Table 4. Effect of three biostimulants on polar diameter and equatorial diameter of habanero pepper fruits.

Biostimulants	Polar diameter (cm)	Equatorial diameter (cm)
T22	4.13 ±0.03 a	3.12 ±0.02 ab
Genifix	4.03 ±0.02 ab	3.06 ±0.02 b
Mix	3.99 ±0.02 b	3.15 ±0.02 a
Control	3.53 ±0.03 c	2.82 ±0.02 c
CV (%)	8.75	7.49

Means with a common letter are not significantly different (p> 0.05).

Regarding the variable weight per fruit, the plants treated with the biostimulants produced significantly heavier fruits ($F_{3, 4028}$= 69.43, P= 0.0001) than the control plants and no statistical differences between the biostimulants were obtained (Table 5). The interactions of cuts one, two, and three + any biostimulant registered significantly heavier fruits ($F_{9, 4028}$= 2.25, p= 0.0169) than interactions that included any cut + the control.

For the sample weight of 20 fruits, the plants treated with the biostimulants Genifix and Mix recorded the highest sample weights and were significantly different ($F_{3, 819}$= 17.9, p= 0.0001) from the control (Table 5). As for the interaction, cut three + biostimulant Genifix or Mix were significantly different ($F_{9, 819}$= 3.32, p= 0.0005), with the highest sample weight of 20 fruits, from the controls in the four cuts. Also, the interaction cut four + biostimulant T22 had a greater sample weight of 20 fruits than the controls in any of the cuts.

Table 5. Effect of three biostimulants on weight per fruit and total weight of habanero pepper fruits.

Biostimulants	Weight (g) x fruit	Weight (g) x 20 fruits
T22	10.18 ±0.11 a	300.74 ±13.45 a
Mix	10.07 ±0.1 a	305.38 ±14.44 a
Genifix	9.98 ±0.1 a	303.75 ±13.11 a
Control	8.27 ±0.12 b	186.83 ±13.49 b
CV (%)	15.46	34.5

Means with a common letter are not significantly different ($p > 0.05$).

The results corroborate what has been reported in other studies on the positive effects of biostimulants based on *Trichoderma* spp. and *Bacillus* spp. in vegetables (Diánez *et al*., 2018; Gamboa-Angulo *et al*., 2020; Rojas-Badía *et al*., 2020). However, in the germination of habanero pepper, the results indicated a null effect of biostimulants, which may be due to the fact that some strains of *Trichoderma* or *Bacillus* decrease or do not have a stimulating effect on the germination of habanero pepper as reported by Sosa-Pech *et al*. (2019) for *Bacillus* isolates CBCC57 and CBFRF5, which obtained a lower germination in relation to the control.

Variations in the effect of *Bacillus* strains on pepper germination have also been reported, indicating that the application of *Bacillus* sp. MA06 increased the germination percentage by 8%, but three *Bacillus* strains showed no significant difference from non-inoculated seeds (Luna *et al*., 2013).

On the other hand, Ezziyyani *et al*. (2004) obtained only 60% germination in pepper seeds treated with *T. harzianum* at 10 days. However, unlike these results, positive effects on the germination of chili pepper seeds treated with *T. harzianum* have also been reported, with germination percentages of 82 and 90.3% (Madhavi *et al*., 2006; Miguel-Ferrer *et al*., 2021) and regarding bacteria of the genus *Bacillus*, Kaymak *et al*. (2009) indicate that *B. megaterium* improved the percentage and germination rate in radish seeds.

Also in this research, it was demonstrated with these results that the biostimulant Genifix, based on *Bacillus* bacteria, has a significant effect on the development of habanero pepper seedlings, this because bacteria of the genus *Bacillus* promote and stimulate plant growth through the synthesis of hormones in the plant, such as cytokinins, ethylene and gibberellins (Rojas-Solís *et al*., 2013); as well as through nitrogen and phosphorus fixation (Corrales *et al*., 2017; Rodríguez-Hernández *et al*., 2020).

Likewise, Sosa-Pech *et al*. (2019) have reported that *Bacillus* isolates promote growth at the level of habanero pepper seedlings, among their treatments, CBCC57 and CBRF12 isolates promoted growth in plant height and leaf area. In addition, Kokalis-Burelle *et al*. (2002) indicated that the formulation LS256 (*Bacillus subtilis* GBO3 and *B. pumilis* INR7) promoted the growth of the stem, root and aerial part in chili pepper seedlings. For *Trichoderma*, Candelero *et al*. (2015) report strains that significantly improved seedling height, root length, root volume and total dry biomass of *Capsicum chinense*, which coincides with the results obtained from the biostimulant T22 in seedling height.

Regarding the quality of the fruit, the results showed that the biostimulants significantly improved the size and weight of the habanero pepper fruit compared to the control, which complements the information generated by Gamboa-Angulo *et al*. (2020) on the positive effects on the internal quality of chili pepper fruits in relation to lipid, protein and phosphorus content, when plants are bio-fertilized with *T. harzanium* and *B. subtilis*. There is little information on the effects of biostimulants on the dimensions and weight of fruits, which is necessary to establish and relate to nutritional characteristics. Microbial inoculants based on *Rhizophagus irregularis*, *Pseudomonas* spp. and *Azospirillum brasilense* have been evaluated in yield and fruit size of habanero pepper, reporting that the inoculation of *Pseudomonas* spp. to habanero pepper in transplantation increases growth, yield and fruit size (Reyes-Ramírez *et al*., 2014).

Conclusions

It was demonstrated with the results that the biostimulant Genifix had greater efficiency in stimulating the development of habanero pepper seedlings compared to the biostimulants T22 and Mix. The biostimulant T22 significantly improved the length of the habanero pepper fruits and the biostimulant Mix the width of the fruits. All biostimulants significantly increased the weight of habanero pepper fruit, without presenting significant differences between them. It is necessary to continue evaluating biostimulants in horticulture locally and temporarily to monitor the efficiency of the products and thus define mechanisms that optimize their use.

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