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Article

Tomato response to reduced doses of mineral fertilization with microbial biostimulant in a high tunnel

Jacel Adame-García[1]
Félix David Murillo-Cuevas[1,§]
José Antonio Fernández-Viveros[1]
Adriana Elena Rivera-Meza[1]
Héctor Cabrera-Mireles[2]

1 Tecnológico Nacional de México-Instituto Tecnológico de Úrsulo Galván. Carretera Cardel-Chachalacas km 4.5, Úrsulo Galván, Veracruz, México. CP. 91667. Tel. 296 9625029. (jacel.ag@ugalvan.tecnm.mx; jose.fv@ugalvan.tecnm.mx; adriana.rm@ugalvan.tecnm.mx).

2 Campo Experimental Cotaxtla-INIFAP. Carretera Federal Veracruz-Córdoba km 34.5, Medellín de Bravo, Veracruz, México. CP. 94992. Tel. 229 1521077. (cabo.cabrera50@gmail.com).

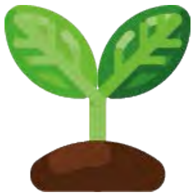
Autor para correspondencia: felix.mc@ugalvan.tecnm.mx.

Abstract

The tomato is one of the most important crops in Mexico, requiring considerable amounts of nitrogen, phosphorus and potassium to achieve optimal yields. Mineral doses are increasingly overused, which is inefficient, and much of it is released into the environment, causing contamination of bodies of water and soil. The research aimed to evaluate the effect of low doses of mineral fertilization combined with a microbial biostimulant on the weight and dimensions of tomato fruits and production per plant. The work was conducted in Úrsulo Galván, Veracruz, in the autumn-winter-spring cycle (2023-2024) under protected conditions using a high tunnel. The study employed a biostimulant formulated with five bacterial strains (*Bacillus subtilis* FDMC1, *Stenotrophomonas* sp. JAG2, *Bacillus wiedmannii* JAG3, *Priestia megaterium* JAFV4, and *P. megaterium* AERM5) and a minimum chemical fertilization with NPK (165-120-90) at different percentages (100%, 75%, 50% and 25%) with and without biostimulant, except for 100%, which was a control without biostimulant. A randomized complete block design was used and 240 plants were established using a planting frame of one plant every 25 cm in a triangular pattern. The reduced doses of fertilization, without applications of biostimulants, significantly affected the yield and quality of tomato fruits. The applications of the biostimulant with small doses of chemical fertilization at 50% and 75% significantly increased the production and dimensions of tomato fruits. The applications of the biostimulant resulted in a saving of 25% in chemical fertilization, which achieved production and quality of tomato fruits equal to that achieved with 100% fertilization.

Keywords:

bacteria, low inputs, vegetables.



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Introduction

In Mexico, there is a planted agricultural area of 12 622 755.43 ha with a production of 56 948 979.85 t, of which vegetables contribute approximately 20% of the production; regarding tomatoes, 3 220 048.24 t are produced (SIAP, 2023). It is one of the most important crops for the economy and food of our country (Lara *et al*., 2022). Tomato cultivation requires substantial amounts of nitrogen, phosphorus, and potassium (NPK) to achieve optimal yields (Díaz-Vázquez *et al*., 2023). NPK fertilizer influences production parameters, morphological parameters, phenolic compounds, flavonoid content, lycopene, and β-carotene of tomato fruits (Bentamra *et al*., 2023).

Since chemical fertilization is so important for tomato production, farmers tend to increase the doses and use of synthetic fertilizers (Burcea *et al*., 2016). Even though synthetic fertilizers are one of the most important factors that help boost agricultural production, their abuse causes serious problems of groundwater contamination and soil degradation (Galindo *et al*., 2020). Thus, it is crucial to use strategies with ecological approaches to reduce the use of chemical fertilizers and, at the same time, increase nutrient efficiency, yields, and crop quality.

One such strategy is the use of plant biostimulants, which are used as a management tool in low-input vegetable crops (Sánchez-Sánchez *et al*., 2022; Chafai *et al*., 2023). Biostimulants improve plant and fruit development through mineral solubilization, nitrogen fixation, and phytohormone production (Caulier *et al*., 2019). They improve tolerance to biotic and abiotic stress by activating genes that activate defense systems, producing enzymes, amino acids, and organic acids (Kaushal *et al*., 2023). Microbial biostimulants provide benefits to plants, but one of the fundamental characteristics they must have is to preserve crop quality and yield in low-input systems. In this sense, the purpose of this study was to analyze how these stimulants influence plant growth, quality, and productivity.

Materials and methods

The study was carried out at the Technological Institute of Úrsulo Galván, located at coordinates 19° 24’ 43.64’’ north latitude and 96° 21’ 32.42’’ west longitude, at an altitude of 13 m, in the municipality of Úrsulo Galván. The region has a climate classified as Aw (tropical wet-dry) according to the Köppen-Geiger system, characterized by warm sub-humid conditions with summer rains, temperatures fluctuating between 24° and 26 °C and annual rainfall varying between 1 100 and 1 300 mm.

Tomato seeds from Harris Moran® of the Atrevido F1 variety were used, a microbial biostimulant formulated based on a combination of bacterial strains (*Bacillus subtilis* FDMC1, *Stenotrophomonas* sp., JAG2, *Bacillus wiedmannii* JAG3, *Priestia megaterium* JAFV4 and *P. megaterium* AERM5) at a dose of 20% (v/v) and concentration of 10^6 CFU ml^{-1} of each strain. Seeds were inoculated with the INIFAP® mycorrhiza *Rhizophagus intraradices* before sowing in polyethylene trays containing peat moss substrate.

After 30 days, the seedlings were transplanted into a soil with the following characteristics: pH 6.84, EC 81.3 µS, BD 1.2 g ml^{-1}; with a composition of 30.76% sand, 30.56% silt and 38.68% clay, 2.11% organic matter, 0.17 cmol kg^{-1} potassium, 12.09 cmol kg^{-1} calcium, 3.91 cmol kg^{-1} magnesium, 0.12% nitrogen and 11.5 mg L^{-1} phosphorus. Production took place in a high tunnel 3 m wide by 30 m long (90 m^2), with drip irrigation and white mulch. Inside the high tunnel, an average temperature of 28.8 °C and 75% relative humidity were recorded.

A minimum chemical fertilization NPK (165-120-90) was used at different percentages (100%, 75%, 50%, and 25%) with and without biostimulant, except for 100% which was a control without biostimulant. The treatments evaluated were seven fertilization combinations: T1) 100% chemical (CF100); T2) 75% chemical + biostimulant (CF75+B); T3) 50% chemical + biostimulant (CF50+B); T4) 25% chemical + biostimulant (CF25+B); T5) 75% chemical (CF75); T6) 50% chemical (CF50); and T7) 25% chemical (CF25). The treatments were applied in drench (50 ml $plant^{-1}$) every 30 days. Every month, the biostimulant was incorporated into the soil, applied in the area near the neck of the plant (drench).

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In all treatments, foliar applications of micronutrients with commercial products (N 13%, P_2O_5 9%, K_2O 12%, Ca 0.17%, Cu 0.05%, Co 0.01%, B 0.07%, S 0.04%, Fe 0.08%, Mn 0.5%, Mg 0.18%, Mo 0.03%, Zn 0.2%, diluents and conditioners 64.31%) were made every 20 days. At the beginning of flowering and subsequently every 20 days, a foliar application of calcium and boron was made, using a commercial product (Ca 9%, B 2%, Zn 4%, and 85% diluents and conditioners). In addition, 15 days after transplantation (dat), humic and fulvic acids (both at 12%) were applied to the soil, repeating this dose every 30 days, until the end of the production cycle.

A randomized complete block design was used, each block was considered as a bed of the high tunnel of 30 m length by 1 m width. In which 240 plants were established with a planting frame of one plant every 25 cm in a triangular pattern. Two replications per treatment (blocks) and 40 experimental units per treatment for fruits and 12 for production per plant. Each treatment included 13 plants, fruits from three cuts (123, 148, and 168 dat) were used. From the six central plants, 20 fruits were taken at random to record the weight and dimensions of the fruits, and the total fruits were taken to weigh them and determine the production per plant per treatment.

The response variables were weight (g), equatorial and polar diameter (cm) of the fruits, as well as the production of the high tunnel in weight (g) of the total fruits per plant per treatment. Data normality was assessed with the Shapiro-Wilk and Kolmogorov-Smirnov goodness-of-fit tests. Data were transformed to √#+0.5 to stabilize variance and use an analysis of variance (Anova). In cases where significant differences were identified (*p*Ò0.05), a Tukey means comparison test was used; the original means of the data are presented. InfoStat software version 2020 was used for data analysis.

Results and discussion

The total average of the three fruit cuts maintained significant differences ($p < 0.05$) between the dimensions and weight of the fruits obtained from the plants with and without applications of the microbial biostimulant at doses of 50 and 75% fertilization (Table 1). Furthermore, reduced doses of chemical fertilization significantly affected the average fruit weight and size in the absence of biostimulant applications (Table 1 and Figure 1).

Table 1. Average effects of the application of a microbial biostimulant with reduced doses of chemical fertilization on the size and weight of tomato fruits from three fruit cuts.

Treatments	Fruit weight (g)	Fruit diameter	
		Polar (cm)	Equatorial (cm)
CF 100	132.04 ±2.27a	5.99 ±0.05a	3.61 ±0.03ab
CF75+B	136.33 ±2.27a	6.04 ±0.05a	3.7 ±0.03a
CF50+B	131.21 ±2.3a	5.91 ±0.06a	3.59 ±0.03b
CF25+B	100.66 ±2.27b	5.32 ±0.06b	3.28 ±0.03c
CF75	100.86 ±2.27b	5.35 ±0.05b	3.19 ±0.04c
CF50	93.05 ±2.27b	5.21 ±0.05b	3.2 ±0.03c
CF25	84.02 ±2.27c	4.93 ±0.05c	3 ±0.03d
CV (%)	11.66	4.61	4.59

CF100= chemical 100%; CF75+B= chemical 75% + biostimulant; CF50+B= chemical 50% + biostimulant; CF25+B= chemical 25% + biostimulant; CF75= chemical 75%; CF50= chemical 50%; CF25= chemical 25%. CV= coefficient of varia tion. Data are presented in $\bar{x}$ ±SE. Different letters within each column indicate significant differences according to Tukey's test ($p < 0.05$).

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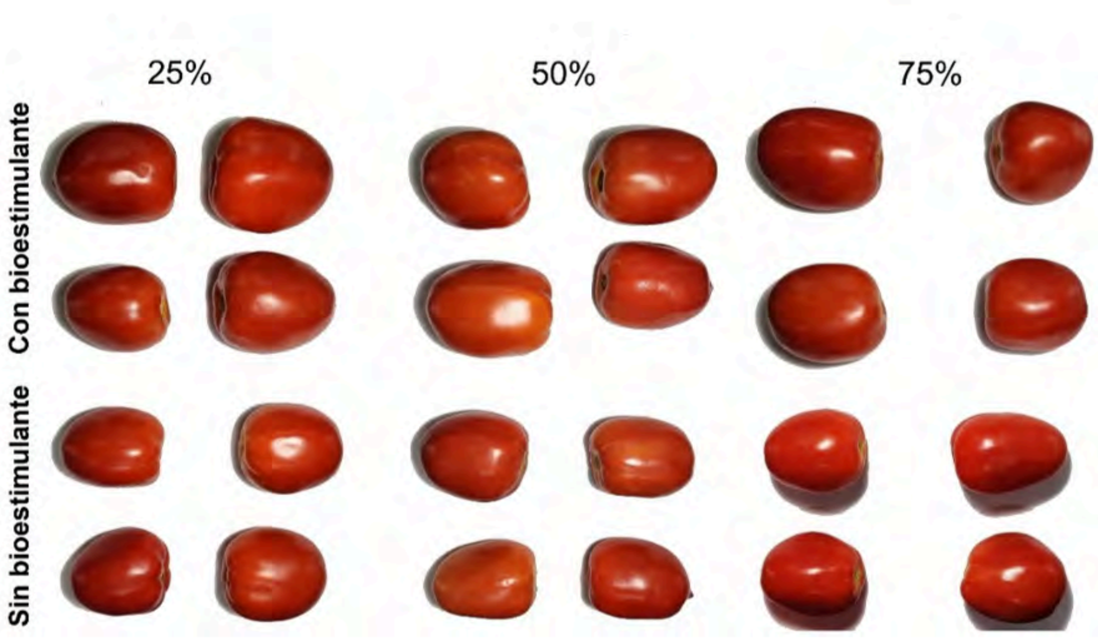
Figure 1. Sample of fruits produced from plants with different doses of chemical fertilization with and without microbial biostimulant.



The biostimulant significantly increased ($p < 0.05$) the average fruit weight by 38.2 g (29.1%) with 50% fertilization and 35.5 g (26%) with 75% fertilization. The polar diameter of the fruit was also increased by 0.7 cm (11.8%) with 50% fertilization and 0.69 cm (11.4%) with 75% fertilization. Likewise, the equatorial diameter increased by 0.39 cm (10.9%) with 50% and 0.51 cm (13.8%) with 75% chemical fertilization. The effects of the microbial biostimulant on the external characteristics or quality in tomato fruit caliber (weight and size) show that bioinoculants have the ability to promote fruit weight increase in the variety studied.

The results of this work aim to surpass previous results with other strains of plant growth-promoting bacteria of the genus *Bacillus* in the same tomato variety, where the average increase in fruit weight was 16.4%, polar diameter 8.6% and equatorial diameter 7.7%, with a traditional minimum fertilization of 100%; these increases were significantly different from the control without biostimulant (Adame-García *et al*., 2023). It also exceeded the increase in equatorial diameter reported by Espinoza *et al*. (2017), who used inoculations of *Bacillus* species in tomato variety Afrodita, which was 4.5% compared to the control without biostimulant; however, in polar diameter, they report a greater increase (13.1%).

On the other hand, the increases obtained by Mena-Violante *et al*. (2009) in tomato variety Rio Fuego with the strain *Bacillus subtilis* BEB-13bs were lower in weight (16.2%) and equatorial diameter (4.2%) than those obtained in this work. However, there are works with higher increases when using other bacteria and tomato varieties, such as that reported by Palacio-Rodríguez *et al*. (2022) for the Top1182 variety, where *Bacillus paralicheniformis* strain LBEndo1 bacteria increased, compared to the uninoculated treatment, the equatorial diameter by 16.5% for flat soils and 15.2% for raised beds. In addition, they report that with *Pseudomonas lini* strain KBEcto4 bacteria, the equatorial diameter increased by 16.5% and 11.1% for flat soils and raised beds, respectively. Nevertheless, these works did not evaluate the effect of inoculants at different fertilization doses.

Production per tomato plant, in fruit weight, was also affected by reduced doses of chemical fertilization in the three productive cuts; however, when supplemented with biostimulant applications, the doses of 50 and 70% fertilization increased production, resulting statistically equal to the production obtained with 100% fertilization (Table 2).

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Table 2. Effects of the application of a microbial biostimulant and reduced doses of chemical fertilization on the weight of fruits per tomato plant in three cuts and total.

Treatments	Fruit weight (g) per plant in three fruit cuts			Total (g)
	1	2	3	
CF 100	562.4 ±36a	934.9 ±62.6ab	846.1 ±55.2ab	781.1 ±34.7ab
CF75+B	675.2 ±36a	947.2 ±62.6a	862.2 ±55.2a	828.2 ±34.7a
CF50+B	532.9 ±36.1ab	680.7 ±62.6bc	790.7 ±55.2ab	668.1 ±34.7bc
CF25+B	389.3 ±36bc	605.3 ±62.5c	625.2 ±55.3bc	539.9 ±33.1cd
CF75	394.3 ±36bc	642.3 ±62.6c	484.6 ±55.2c	507.1 ±34.7d
CF50	353.7 ±36.2c	572.8 ±62.6c	455.5 ±55.1c	460.7 ±34.7d
CF25	367.2 ±36c	484.9 ±51.1c	424.4 ±55.2c	434 ±32.1d
CV (%)	13.78	16.43	15.24	17.51

CF100= chemical 100%; CF75+B= chemical 75% + biostimulant; CF50+B= chemical 50% + biostimulant; CF25+B= chemical 25% + biostimulant; CF75= chemical 75%; CF50= chemical 50%; CF25= chemical 25%; CV= coefficient of varia tion; Data are presented in $\bar{x}$ ± SE. Different letters within each column indicate significant differences according to Tukey's test ($p < 0.05$).

In total production per tomato plant, analyzing the three cuts together, an increase of 207.4 g (31%) and 321.1 g (38.8%) was obtained when the biostimulant was used with doses of 50 and 75% chemical fertilization, respectively. The increases obtained were higher than those reported by Katsenios *et al*. (2021) in the Rio Grande variety, where the increase in the average weight of fruits per plant was 30.7% with inoculation with *Bacillus subtilis*, 28.81% with *Bacillus amyloliquefaciens*, 27.52% with *Priestia megaterium*, and 26.78% with *Bacillus licheniformis*, with respect to the control. However, increases greater than 38.8% with respect to the control are also reported using strains LBEndo1 (*Bacillus paralicheniformis*) and KBEcto4 (*Pseudomonas lini*) but in the Top1182 variety and without reducing fertilization doses (Palacio-Rodríguez *et al*., 2022).

Regarding the evaluation with fertilization doses, there are some works that have reported increases greater than those obtained in this work; for example, Espinosa-Palomeque *et al*. (2019) report increases of 42.6 and 45.6% in the production per plant of the Moctezuma variety when inoculations of *Bacillus paralicheniformis* were carried out at a nutrient fertilization of 75% compared to that obtained with 75 and 100% of fertilization without inoculants, respectively. NPK fertilization doses influence biochemical, morphological, and production parameters of tomato (Bentamra *et al*., 2023). The results of this work indicate that, if there is no efficient complement in mineral nutrition, low doses of chemical fertilization affect fruit development and tomato production.

In such a way that, if the results of production per plant are extrapolated, with a saving of 25% in fertilization, production is reduced by 35% (60.85 t ha^{-1}) compared to production with 100% fertilization (93.73 t ha^{-1}); however, with the applications of the biostimulant, this reduction is eliminated and production is also increased by 6% (99.38 t ha^{-1}). When there is a 50% saving of fertilizer, production is reduced by 41% (55.28 t ha^{-1}); nevertheless, with biostimulant applications this reduction becomes smaller 14% (80.17 t ha^{-1}), using only 50% of the fertilization.

According to the above, it is important to determine the most appropriate dose of NPK fertilizer in combination with biostimulants for economically profitable conditions and more sustainable environments. For example, with the use of a biostimulant formulated with *Azospirillum* sp., *Azotobacter* sp., and *Rhizobium* sp., alone or combined with a commercial inoculum Micomix (*Rhizoglomus irregulare*, *Funnelliformis mosseae*, *Funnelliformis caledonium*, *Bacillus licheniformis*, and *Bacillus mucilaginosus*), always under conditions of reduced fertilization (30%), they had a production of tomato variety Rio Grande statistically equal to that obtained with a fertilization of 100% (Novello *et al*., 2024).

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The results of this work corroborate the efficiency of the microbial biostimulant in the development of tomato fruits with low doses of chemical fertilization under protected production conditions, which indicates that the bacterial strains that formulate the biostimulant are microorganisms capable of inducing some genetic, biochemical and/or physiological response that stimulates the growth and development of fruits.

The bacterial strains that formulate the biostimulant evaluated in this work were identified at the molecular level as *B. subtilis* FDMC1, *Stenotrophomonas* sp., JAG2, *B. wiedmannii* JAG3, *P. megaterium* JAFV4, and *P. megaterium* AERM5, which have not been evaluated separately. However, *B. subtilis* is classified as a plant growth-promoting rhizobacterium (PGPR), which improves nutrient availability, alters phytohormone homeostasis, and activates induced systemic resistance in plants (Blake *et al*., 2021). Bacteria of the genus *Stenotrophomonas* use various mechanisms to promote plant growth and development, such as nitrogen fixation, phosphorus solubilization, and phytohormone synthesis (Kumar *et al*., 2023). The rhizobacterium *B. wiedmannii* is very efficient and can solubilize inorganic phosphorus, fix atmospheric nitrogen, and produce phytohormones (Torres *et al*., 2024).

Finally, *P. megaterium* stimulates plant growth and tolerance to abiotic stress caused by salinity (Shi *et al*., 2023). Thus, the efficiency of the biostimulant lies in compensating for the low level of fertilization in tomato crops, and that may be due to the compatibility of the host plant with the microbial strains, since the physiological and biochemical compatibility of the microorganism-plant interaction is mainly due to the success of microbial biostimulants (Cano, 2011).

Conclusions

Reduced doses of chemical fertilization, without applications of the biostimulant, significantly affect the yield and quality of tomato fruits produced under protected conditions using a high tunnel. The applications of the microbial biostimulant result in a saving of 25% in chemical fertilization, achieving a production and quality of tomato fruits equal to that obtained with 100% fertilization. The applications of the biostimulant with reduced fertilization doses of 50 and 75% significantly increased the production and dimensions of the fruits compared to what was obtained without applications.

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