Revista Mexicana de Ciencias Agrícolas

REMEXCA

DOI: https://doi.org/10.29312/remexca.v15i5.3255

Article

Biostimulants and Steiner solution in growth and production of *Capsicum annuum* L.

Salomón Buelna-Tarín[1]
Celia Selene Romero-Félix[1,§]
Cosme Bojórquez-Ramos[2]
Gabriel Antonio Lugo-García[1]
Bardo Heleodoro Sánchez-Soto[2]

1 Colegio de Ciencias Agropecuarias-Facultad de Agricultura del Valle del Fuerte-Universidad Autónoma de Sinaloa. Calle 16 y Avenida Japaraqui s/n. Juan José Ríos, Ahome, Sinaloa, México. CP. 81110. (buelnatarin@gmail.com; gabriel-lugo9010@hotmail.com).

2 Departamento de Biología-Universidad Autónoma de Occidente. Blvd. Macario Gaxiola y carretera internacional México 15, Los Mochis, Sinaloa, México. CP. 81223. (cbojorquezr@gmail.com; bardosanchez@hotmail.com).

Autora para correspondencia: celiaromero82@hotmail.com

Abstract

The use of biostimulants is an alternative to improve the assimilation of nutrients by the plant, whether applied in irrigation water or foliarly. This research aimed to evaluate the effect of the Steiner nutrient solution and the foliar application of three biostimulants on growth and yield parameters of two cultivars of jalapeño pepper (*Capsicum annuum* L.) under greenhouse conditions. Four treatments were established under a randomized complete block design with four repetitions, in Juan José Ríos, Ahome, Sinaloa in October 2018. The variables evaluated were plant height, stem diameter, leaf dry weight, stem dry weight, fruit length, number of fruits per plant, total fruit weight, fruit weight per plant and number of flowers. The Giberelin 10 biostimulant had a significant effect on plant height. The Bronco cultivar, on average of treatments, was outstanding for presenting high values in the growth and yield parameters evaluated, except for stem diameter and fruit length, which were higher in the Forajido cultivar. The Forajido cultivar showed a higher plant height due to the effect of the Giberelin 10 treatment, while Bronco exhibited a higher number of fruits per plant with the Fiamin-fol treatment and a higher fruit weight per plant with the treatments of Maxi-Grow Excel and the Steiner nutrient solution (control).

Keywords:

growth, jalapeño pepper, nutrient solution, yield.

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Introduction

Chili pepper (*Capsicum annuum* L.) is one of the main vegetables grown worldwide, with a production of approximately 36 million tonnes (FAOSTAT, 2020). In Mexico, the most economically important vegetables are chili pepper and tomato, of which the former contributes 20.2% of the national production. Sinaloa is the largest producer of jalapeño pepper, with an approximate production of 127 517.29 t (SIAP, 2020).

Nevertheless, its production can be negatively affected by biotic and abiotic factors, within the latter we can mention extreme temperatures, soil salinity and low soil fertility; therefore, its production requires optimal conditions to obtain quality fruits and high export volumes, such as management practices, fertilization and water, as well as plant protection technologies such as shade mesh and greenhouses, which improves the growth and development of plants, which is reflected in the yield and quality of the fruit.

For a higher yield and quality, it is of utmost importance to know the nutritional requirements of the crop, as well as their relationship with the environmental factors where it is produced (Ramírez *et al*., 2017). According to the nutritional requirement of the jalapeño pepper crop, the base fertilization (kg t^{-1}) used for its management is nitrogen (N, 2.4-4), phosphorus (P_2O_5, 0.4-1), potassium (K_2O, 3.4-5.29), calcium (CaO, 0.55-1.8) and magnesium (MgO, 0.28-0.49) (Salazar and Juárez, 2013).

Commonly, the nutrient solution of Steiner (1984) is used as a reference due to its nutrient content, ionic balance, and rapid assimilation, which increases the yield of the plant by favoring its growth and fruit production (Luna-Fletes *et al*., 2021).

Nonetheless, taking into account some types of abiotic stress (extreme temperatures, water stress, etc.) that occur during the growth cycle of the crop, which cause losses in the quality and production of the jalapeño pepper, the use of biostimulants, which are defined as substances of organic origin, is also being implemented, which, when applied to the crops, stimulate their growth and development by influencing their physiological (such as stomatal conductance and photosynthetic activity by inducing greater CO_2 capture, which is reflected in greater dry weight accumulated in the plant's aerial and root organs) (Ikan *et al*., 2023) and biochemical processes (such as protein biosynthesis, chlorophyll biosynthesis, and the accumulation of metabolites responsible for stimulating the formation of reproductive structures such as flowers and fruits) (Rouphael and Colla, 2020), allowing better nutrient absorption and greater resistance to adverse climatic conditions (Batista *et al*., 2015; Du Jardin, 2015; Veobides *et al*., 2018; Drobek *et al*., 2019).

It has been observed that with the use of different biostimulants, formulated with amino acids, hormones and beneficial microorganisms, the quality of the fruits is improved and their production is increased in a sustainable way (Para#ikovi# *et al*., 2011); in chili crops it has been shown that greater biomass production is obtained (Murillo *et al*., 2015), and the number of flowers, plant height, number and size of fruit set (Juárez, 2014; Pichardo *et al*., 2018), as well as fruit weight (Al-Said and Kamal, 2008; Campo *et al*., 2015; Popko *et al*., 2018) is increased, thereby improving quality and production.

In the state of Sinaloa, inorganic products (macro and micronutrients) are commonly used as base fertilization, in addition to foliar applications of nutrients to be absorbed and used more efficiently by the plant at critical times and thus increase crop yields (Trejo *et al*., 2007; Fernández, 2015).

In this sense, there is little information regarding the effect of the foliar application of biostimulants in crops of jalapeño pepper, on their growth and yield, an aspect that should be studied since their application could be considered as a good production strategy to obtain high yields with a lower impact on the environment (Para#ikovi# *et al*., 2011). This research aimed to evaluate the effect of the Steiner nutrient solution and the foliar application of three biostimulants on growth and yield parameters of two cultivars of jalapeño pepper (*Capsicum annuum* L.) under greenhouse conditions.

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Materials and methods

Experimental area

The study was carried out at the facilities of the Faculty of Agriculture of Valle del Fuerte, located in Juan José Ríos, Ahome, Sinaloa, Mexico at 25° 45' 57" north latitude, 108° 49' 23" west longitude, and an altitude of 10 m. A tunnel-type greenhouse with a metal structure and polyethylene cover (75% light transmission) was used. The temperature inside the greenhouse was determined with a Steren digital thermometer; during the crop growth cycle, maximum and minimum temperatures were recorded, which ranged between 38 and 22 °C, respectively.

Plant material

Seeds from two cultivars of jalapeño pepper (*Capsicum annuum* L.), Forajido from the Enza Zaden company and Bronco from the Vilmorin company, were used. The Forajido cultivar is characterized by strong plants with concentrated production of intermediate to early ripening, bright dark green fruits, uniform in shape and size, ideal for the process industry due to their characteristics of thick wall and smooth skin without corking (Enza Zaden, 2020).

The Bronco cultivar is a variety that is characterized by being a robust plant of medium to tall size, with good coverage and excellent production. It produces fruits of approximately 10 cm in size, with thick and compact walls, dark green of high quality, highly tolerant to bacterial attack and has an intermediate cycle of 80 to 85 days to harvest after transplanting (Vilmorin, 2020).

Cultural work

Sowing was carried out on October 10, 2018. The seedbed was established in 242-cavity polystyrene trays in peatland soil substrate (Peat moss BM2 euro Berger®). The trays were irrigated every day with water only and every 10 days they received irrigation with 50% Steiner nutrient solution. At 45 days after sowing (das), the transplantation was carried out in hydroponic bags (white/black in color, cal. 600, 35 x 35 cm), with a capacity of 10 L of substrate, the bags were filled with peat moss substrate (BM2 Berger®).

Pest control was carried out with the commercial chemicals: Sivanto (Flupyradifurone 17.09%, 2.5 ml L^{-1}), Aben 1.8% (abamectin 1.8%; 2.5 ml L^{-1}) and Controla 480 CE (chlorpyrifos ethyl 44.5%; 5 ml L^{-1}), while manzate 200 (mancozeb 80%, 5 ml L^{-1}) and Curamycin 500 (streptomycin 2.19% + oxytetracycline 0.23%; 1.5 g L^{-1}) were used for disease control.

Treatments and experimental design

Four treatments and two hybrids of jalapeño peppers (Forajido and Bronco) were evaluated under a randomized complete block design with four repetitions. The experimental unit consisted of four polyethylene bags with one seedling per bag. The treatments were three commercial biostimulants (Maxi-Grow Excel, Fiamin-fol, and Giberelin 10) and the Steiner nutrient solution as a control.

The application of biostimulants was carried out via foliar at 20, 30 and 40 days after transplantation (dat), while the control (Steiner nutrient solution without application of biostimulant) was applied to the irrigation water every third day at concentrations of 50, 70 and 100% from 0 to 47 dat, from 48 to 70 dat, and 71 dat until the end of the experiment, respectively.

It should be noted that the plants that were under the effect of the biostimulants also received the application of the Steiner nutrient solution during their growth cycle. Irrigations were applied once a day manually, on average 500 ml per bag was applied in the initial stage of establishment and 1 L per bag was applied in the development, flowering, and fruiting stages. The nutritional content of each treatment was as follows.

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Control

The Steiner nutrient solution (Steiner, 1984) was used as a control treatment, with a composition of: 12, 1 and 7 me L^{-1} of NO_3^-, $H_2PO_4^-$ and SO_4^{-2} for anions and 7, 9 and 4 me L^{-1} of K^+, Ca^{2+} and Mg^{2+} for cations, also including the microelements: 1.33, 0.62, 0.02, 0.11, 0.05 and 0.04 ppm of Fe, Mn, Cu, Zn, B, and Mo for a 100% concentration solution. The commercial fertilizers used were: KNO_3, $Ca(NO_3)_24H_2O$, KH_2PO_4, $MgSO_47H_2O$, K_2SO_4, and for microelements: $FeSO_47H_2O$, $MnSO_44H_2O$, Cu SO_45H_2O, $ZnSO_47H_2O$, $Na_2B_4O_710H_2O$, and $Na_2MoO_42H_2O$.

Maxi-Grow Excel (5 ml L^{-1} water

Combination of organic extracts 0.5625 g L^{-1}, auxins 0.45 mg L^{-1}, gibberellins 0.5 mg L^{-1}, cytokinins 7.5 mg L^{-1}, nitrogen (N) 0.033 g L^{-1}, phosphorus (P_2O_5) 0.0665 g L^{-1}, potassium (K_2O) 0.0665 g L^{-1}, calcium (Ca) 0.01 g L^{-1}, magnesium (Mg) 0.02 g L^{-1}, copper (Cu) 0.0665 g L^{-1}, iron (Fe) 0.086 g L^{-1}, manganese (Mn) 0.0665 g L^{-1}, and zinc (Zn) 0.132 g L^{-1}.

Fiamin-fol (5 ml L^{-1} water

Free amino acids 5.1 g L^{-1}, nitrogen (N) 3.3 g L^{-1}, urea nitrogen 0.75 g L^{-1}, alpha-amino nitrogen 0.85 g L^{-1}, protein nitrogen 2.6 g L^{-1}, organic matter 11.85 g L^{-1}, organic carbon 6.85 g L^{-1}, boron (B) 0.03 g L^{-1}, copper (Cu) 0.032 g L^{-1}, iron (Fe) 0.58 g L^{-1}, manganese (Mn) 0.295 g L^{-1}, molybdenum (Mo) 0.0085 g L^{-1}, zinc (Zn) 0.058 g L^{-1}, peptides 5.05 g L^{-1}, and C.B.P. excipient 12.6465 g L^{-1}.

Gibberellin 10% (0.2 g L^{-1} water

Gibberellin (GA_3) 20 mg L^{-1}, inert ingredients 180 mg L^{-1}.

Growth and yield variables evaluated

Plant height (PH, cm), it was measured with a tape measure from the base of the stem to the longest branch; stem diameter (SD, mm), it was measured with a digital vernier (Steren®) and obtained from the base of the main stem to 2 cm above the substrate; leaf dry weight (LDW, g) and stem dry weight (SDW, g), samples were placed in paper bags in a wooden dryer with lamps, reaching a temperature of 65 °C until constant dry weight was obtained; fruit length (FL, cm), it was measured from the peduncle to the apex of the fruit with a digital vernier (Steren®); the assessment of NFP, TFW (g) and FWP (g) began with the fruit harvest at 70 dat when the first fruits with slight cracks on their surface were observed, making a total of five cuts, one per week for five weeks (up to 105 dat).

In each cut, the fruits harvested per plant (NFP) were counted and weighed with a Scale digital scale, with which the FWP (g) was obtained; to obtain the TFW (g), at the end of the harvest, the weight of the fruits obtained in each cut for each treatment was added. The FWP was obtained by averaging the TFW between the number of fruits for each treatment; and number of flowers per plant (NF), all flowers that were in anthesis were recorded only during the fifth week of cutting or harvesting fruits.

Statistical analysis

An analysis of variance (Anova) was performed with SAS version 9.2 for Windows (SAS Institute, 2009) in combined form (Yijk = m + NTi + Cj + NTCij + B(i)j + Eijk), to determine the differences between treatments (T), cultivars (C) and CXT interaction. Tukey's test, based on the honest significant difference (HSD, $p \leq 0.05$), was used for the comparison of means.

Results and discussion

The results of the analysis of variance (Anova) are shown in Table 1; the Anova showed highly significant statistical differences ($p \leq 0.01$) between treatments for the PH and FWP variables, and

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significant statistical differences ($p \leq 0.05$) for NFP; between cultivars, highly significant statistical differences ($p \leq 0.01$) were observed for PH, SD, LDW, SDW and TFW and significant differences ($p \leq 0.05$) in FL and NF, and in the interaction of cultivar by treatment, significant differences were observed for PH and NFP, while for the rest of the variables, no statistical significant differences were observed ($p > 0.05$).

Table 1. Mean squares of the analysis of variance for growth and yield variables of two jalapeño pepper cultivars under the effect of four treatments.

Source of variation	df	PH	SD	LDW	SDW	FL	NFP	TFW	FWP	NF
Repetition	3	9.69 ns	0.17 ns	0.61 ns	2.29 ns	0.46ns	6.26 ns	8747.06 ns	1.86 ns	1.59 ns
Treatment (T)	3	50.75**	0.04 ns	0.94 ns	8.31 ns	0.2 ns	51.68**	46463.84 ns	15.48**	7.39 ns
Cultivar (C)	1	258.84**	3.98**	6.68**	58.91**	3.01*	30.51*	374500.16**	1.64 ns	16.14*
CXT	3	24.52*	0.34 ns	0.7 ns	2.11 ns	0.54ns	26.8ns	10487.72 ns	1.43 ns	5.62 ns
Error		4.88	0.14	0.33	2.68	0.57	2.15	23736.34	1.64	2.56
CV (%)		4.45	3.78	6.76	11.33	16.33	20.59	17.26	9.07	96.92

Df= degrees of freedom; PH= plant height (cm); SD= stem diameter (mm); LDW= leaf dry weight (g); SDW= stem dry weight (g); FL= fruit length (cm); NFP= number of fruits per plant; TFW= total fruit weight (g); FWP= fruit weight per plant (g); and NF= number of flowers (includes only the NF counted at the last fruit cut, 105 dat). **($p \leq 0.01$), *($p \leq 0.05$).

Table 2 shows the comparison of means for the growth and yield variables under the effect of four treatments on average of the two cultivars, plant height was significantly higher with the Giberelin 10 treatment, the number of fruits per plant was higher with the Fiamin-fol treatment, followed by Giberelin 10 and the fruit weight per plant was higher with Maxi-Grow Excel, Steiner solution (control) and Fiamin-fol (Table 2).

Table 2. Average values for growth and yield on average of two cultivars of jalapeño pepper under the effect of four treatments.

Treatment	PH	SD	LDW	SDW	FL	NFP	TFW	FWP	NF
Control	47.91 b	10.08 a	8.57 a	14.67 a	5.17 a	11.96 b	943 a	15.1 a	2.42 a
Maxi-Grow Excel	47 b	9.96 a	8 a	13.17 a	4.83 a	11.04 b	994 a	15.5 a	1.58 a
Fiamin-fol	49.33 b	10.13 a	8.57 a	14.33 a	4.67 a	17.54 a	843 a	14 a	2.54 a
Giberelin 10	53.92 a	10.04 a	8.86 a	15.67 a	4.67 a	13.5 ab	792 a	11.6 b	0.29 a
Overall mean	49.65	10.05	8.52	14.46	4.83	13.5	893	14.1	1.65
DSH ($p \leq 0.05$)	3.88	0.64	0.9	2.78	0.91	4.72	261	1.98	2.64

PH= plant height (cm); SD= stem diameter (mm); LDW= leaf dry weight (g); SDW= stem dry weight (g); FL= fruit length (cm); NFP= number of fruits per plant; TFW= total fruit weight (g); FWP= fruit weight per plant (g); and NF= number of flowers (includes only the NF counted at the last fruit cut, 105 dat).

The higher plant height and the number of fruits per plant determined with the Giberelin 10 treatment can be explained by the gibberellic acid it contains, which acts as an endogenous regulator of growth, controlling processes of plant development, such as stem elongation, expansion of

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vegetative structures, formation of flowers and fruits, and the number of fruits and their growth (Zieslin and Algom, 2004; Pichardo *et al*., 2018).

The results determined in this study are consistent with those reported in previous studies when foliar applications of gibberellin-based products were carried out, in which higher plant height (El-al and Faten, 2009; Maboko and Du Plooy, 2015; Sanjay and Singh, 2019) and number of fruits were obtained in chili cultivars (Pichardo *et al*., 2018; Ahmed *et al*., 2022).

In addition to this, there was also a high yield in number of fruits per plant and weight of fruits per plant with the Fiamin-fol treatment used as a source of amino acids, which are precursors of hormones that contribute to the growth and development of the plant and the formation of reproductive structures (flowers and fruits) that are part of the yield, this is due to the close relationship they have with the photosynthetic process, chlorophyll synthesis and nutrient absorption (Shahrajabian *et al*., 2022) and this translates into greater remobilization and translocation of assimilates for fruit formation and weight (Romero *et al*., 2015).

Similarly, in other studies, in different plant species, it has been shown that applying different doses of amino acids via foliar stimulates vegetative growth and increases some yield parameters (such as the number and weight of fruits) in common beans (Zewail, 2014; Romero-Félix *et al*., 2023), pepper (Campo *et al*., 2015; El-Hamady *et al*., 2017; Aly *et al*., 2019), tomato (Hernández *et al*., 2021; Salim *et al*., 2021), squash (Abd El-Aal *et al*., 2010), and chamomile (El-Attar and Ashour, 2016).

The weight of fruits per plant was also higher with the Maxi-Grow Excel treatments, which contains growth regulators, and with the Steiner solution or control; this effect can be attributed in part to the nutritional composition of these treatments, in which macro and microelements are present, which act in interaction with the crop, promoting plant growth, higher quality yields, and increased production levels (Terry *et al*., 2012; Luna-Fletes *et al*., 2021).

In this regard, Pichardo *et al*. (2018) used as treatments a commercial fertilizer rich in macro and microelements and different doses of gibberellins and observed a higher weight of fruits in jalapeño peppers of the Huichol variety with mineral nutrition than with gibberellins. Meneses-Lazo *et al*. (2020) also observed high values in the weight of fruits per plant when applying the Steiner solution in habanero peppers of the Orange variety under greenhouse conditions.

Some authors mention that the positive influence that mineral elements have on crops is partly due to the fact that they increase the synthesis of hormones by plants, improving and increasing the components of yield, such as the number of fruits set and the weight and size of fruits (Ramírez *et al*., 2005; Pichardo *et al*., 2018).

In Table 3, it was observed that the Bronco cultivar was outstanding for presenting high values in the parameters of growth and yield evaluated, except for stem diameter and fruit length, which were higher in the Forajido cultivar; Bronco presented greater plant height (6.26 cm), leaf dry weight (11%), stem dry weight (20%), total fruit weight (25%), and number of flowers (62%) compared to Forajido. On the other hand, Forajido showed greater stem diameter (8%) and fruit length (10%) than Bronco.

Table 3. Mean values for growth and yield of two cultivars of jalapeño pepper on average of four treatments.

Cultivar	PH	SD	LDW	SDW	FL	NFP	TFW	FWP	NF
Forajido	46.39 b	10.48 a	8 b	12.83 b	5.08 a	14.67 a	767.83 b	14.23 a	0.9 b
Bronco	52.65 a	9.63 b	9 a	16.08 a	4.58 b	12.35 a	1017.67 a	13.92 a	2.37 a
Overall mean	49.65	10.05	8.52	14.46	4.83	13.51	892.75	14.08	1.65
HSD (≤0.05)	2.01	0.33	0.47	1.45	0.47	2.45	135.9	1.03	1.37

PH= plant height (cm); SD= stem diameter (mm); LDW= leaf dry weight (g); SDW= stem dry weight (g); FL= fruit length (cm); NFP= number of fruits per plant; TFW= total fruit weight (g); FWP= fruit weight per plant (g); and NF= number of flowers (includes only the NF counted at the last fruit cut, 105 dat).

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Despite the fact that Forajido showed a higher value in the number of fruits per plant than the Bronco cultivar (although, statistically, it was the same in both cultivars), it had a lower total fruit weight; fruit weight is more related to yield than the number of fruits. Additionally, the differences between the two cultivars can be explained by the higher dry weight accumulated in the aerial part of the plant (SDW and LDW) and plant height of Bronco, which is related to greater availability and remobilization capacity of assimilates to the plant's demand organs (Romero *et al*., 2015), such as flowers and fruits.

The mean values between the two cultivars for plant height (49.65 cm), stem dry weight (14.46 g) and leaf dry weight (8.52 g) were lower than those reported by Alemán-Pérez *et al*. (2018) in Nathalie hybrid pepper at 72 days after transplanting, under greenhouse conditions in Ecuador. The effect of cultivar by treatment interaction is shown in Figure 1, where the Bronco cultivar exhibited higher plant height with the Steiner solution (control), Maxi-Grow Excel and Fiamin-fol treatments compared to Forajido in each of the treatments (Figure 1A), while the Forajido cultivar showed a higher number of fruits per plant with all treatments, except for Fiamin-fol, with which Bronco had higher NFP (Figure 1B).

Regarding the weight of fruits per plant, both cultivars showed equal weight of fruits per plant with the control, Maxi-Grow Excel and Fiamin-fol treatments, while Forajido had a higher weight of fruits per plant than Bronco due to the effect of the Giberelin 10 treatment (Figure 1C).

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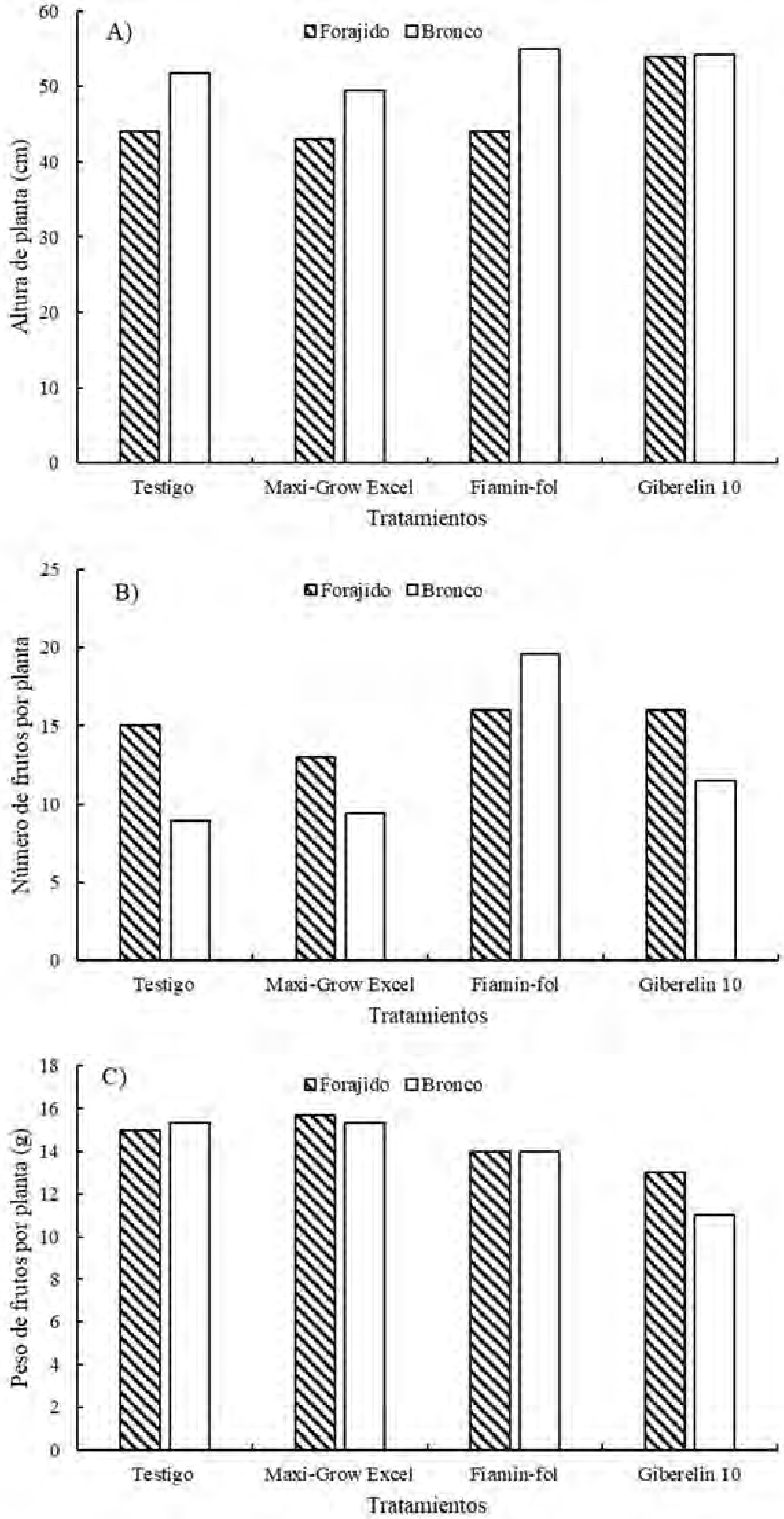
DOI: https://doi.org/10.29312/remexca.v15i5.3255

Figure 1. Interaction of cultivars x treatments for plant height (A) and number of fruits per plant.



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It was expected that plant height would be higher with the Giberelin 10 treatment based on gibberellins, since among the main effects that gibberellins have is the elongation of the internodes; however, the response of plants to gibberellins is much more complex and depends on the environment, the species, and the hybrid (Castro *et al*., 2022).

In addition to the vegetative growth of the plant's organs, gibberellins applied in different concentrations in different cultivars of chili peppers stimulate the formation of reproductive structures, increasing the number of fruits and their weight, which contributes to increasing yield (Akhter *et al*., 2018; Mahindre *et al*., 2018; Pichardo *et al*., 2018; Sanjay and Singh, 2019; Ahmed *et al*., 2022), as was observed in this study in which Forajido had a higher weight of fruits per plant when receiving the application of gibberellins.

The higher plant height of the Bronco hybrid and the greater number of fruits per plant of Forajido due to the effect of the Steiner solution treatment may be due to the fact that it is a solution that is complete and balanced in nutrients, allowing an adequate absorption of all of them and a rapid assimilation of NO_3^- by the plant, which is reflected in an increase in crop yield (Alcántar and Trejo, 2010; Hawkesford *et al*., 2012) and growth (plant height) (González *et al*., 2019; Urbina *et al*., 2020).

Like the Steiner solution, the Maxi-Grow-Excel treatment also contains mineral elements that contribute to the improvement of physiological (photosynthesis, stomatal opening and conductance) and biochemical processes of the plant (synthesis of proteins and hormones), as well as plant growth regulators that are able to control the growth and biochemical activity of plants at the cellular level (Alcantara *et al*., 2019), which is why Bronco also showed high value in plant height and Forajido higher number of fruits per plant with this treatment; Graillet *et al*. (2014) evaluated a higher number of fruits per plant in habanero pepper (*Capsicum chinense* Jacq.) by using the commercial product Maxi-Grow as a hormone treatment.

Regarding the Fiamin-fol treatment, like the Steiner solution and Maxi-Grow Excel, it is composed of mineral elements, but also of amino acids, which influenced the increase in the plant height and number of fruits per plant of Bronco. Previous studies have shown an increase in growth and number of fruits per plant in *Capsicum chinense* L. cv. Fuoco della Prateria, when applying amino acids (Ertani *et al*., 2014).

On the other hand, Salim *et al*. (2021) demonstrated in plants of tomato cv 010 that foliar application of amino acids in combination with micronutrients stimulates vegetative growth and yield components such as number of leaves per plant, leaf fresh weight, number of fruits per plant, average fruit weight, fruit diameter and total fruit yield, outperforming control plants, 75 days after transplantation.

Conclusions

The Steiner nutrient solution and the foliar application of the biostimulants had a significant effect on the growth and yield parameters of the two cultivars evaluated; the Giberelin 10 treatment positively affected the plant height and the number of fruits per plant, while the Maxi-Grow Excel, Steiner solution (control) and Fiamin-fol treatments promoted an increase in the weight of fruits per plant. The Bronco cultivar was outstanding for presenting high values in growth (plant height, leaf dry weight, stem dry weight, and total dry weight) and yield (number of flowers and fruits) of the plant.

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Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v15i5.3255

Biostimulants and Steiner solution in growth and production of *Capsicum annuum* L.

Journal Information
Journal ID (publisher-id): remexca
Title: Revista mexicana de ciencias agrícolas
Abbreviated Title: Rev. Mex. Cienc. Agríc
ISSN (print): 2007-0934
Publisher: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias

Article/Issue Information
Date received: 01 February 2024
Date accepted: 01 May 2024
Publication date: 06 August 2024
Publication date: Jul-Aug 2024
Volume: 15
Issue: 5
Electronic Location Identifier: e3255
DOI: 10.29312/remexca.v15i5.3255

Categories

Subject: Articles

Keywords:

Keywords:

growth

jalapeño pepper

nutrient solution

yield

Counts

Figures: 1

Tables: 3

Equations: 0

References: 53

Pages: 0