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Biostimulants in the development of tomato and collard greens seedlings

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Abstract

The success of crop yields begins with the use of quality seedlings. Crops from vigorous plants respond better to phytosanitary treatments and management techniques, resulting in economic returns and better use of inputs. The objective of this study was to evaluate NPK fertilizer rates and rates of an NPK + auxin biostimulant on the effects over agronomic features of Collard Greens and salad tomato seedlings. The research consisted of two experiments in randomized block, with four replications each. Both experiments were in factorial scheme 2 x 4, consisting of the combination of two nutrient sources (NPK fertilizer 9-45-11 and biostimulant composed of NPK 9-45-11 + 400 mg kg $^{-1}$ of auxin IAA), and four rates, being then 50; 100; 150 and 200% of the recommended rate of biostimulant for tomato and 50; 75; 100 and 125% of the recommended rate for Collard Greens. The biostimulant favored the development of tomato seedlings, since it provides greater root dry mass accumulation and didn't promote seedling shedding, as it occurred in the application of NPK fertilizers. On the other hand, the Collard Greens seedlings didn't distinguish by the presence of auxin in the biostimulant, developing greater seedlings heights with NPK application. Concentrations of 50-200% of the recommended biostimulant fertilizer didn't interfere on root length, diameter and root dry mass of Collard Greens.

Keywords: auxin, *Brassica oleracea* var. *acephala, Solanum lycopersicum*, plant regulators

Introduction

Even with technological progress in seedlings production, there is still a deficiency of technical information on the best management practices to be adopted by the producers to obtain high-quality seedlings, especially considering the wide variation in physiological responses of each species (Gonçalves et al., 2012; Pinto et al., 2016).

To illustrate the impact of seedlings formation process, Thomas and Upreti (2014) observed that the initial growth management of tomatoes determines the incidence and severity of bacterial wilt caused by *Ralstonia solanacearum*, being the age of seedlings, considered strategy of disease management in the field.

The seedlings substrate plays a very important role in seedling cultivation. The good substrate provide appropriate roots growth support, adequate water retention, presence of oxygen and nutrients available for the plants, to develop throughout the period in the nursery (Meng et al., 2018).

The plants require increasing rates of nutrients to maintain their development. This makes mineral supplementation on substrates important to contribute to formation of high-quality plants. The lack of nutrients in seedlings formation of vegetables can cause delay of 16.6% in crop cycle and reduction of up to 70% on seedlings growth, and this compromises plants quality and reduce yield potential (Santos et al., 2000).

Recently, the use of products with action to stimulate root development are increasing, especially because the relation between root growths and higher absorption of water and nutrients through the roots. Such products, called biostimulants, tend to contribute to the hormonal balance of plants, accelerate the speed of emergency seeds and improve the performance of seedlings (Silva et al., 2014; Casadesús et al., 2019).

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Studies assessing the effect of biostimulants on production seedling of vegetables like lettuce (Soares et al., 2012), watermelon (Silva et al., 2014) and tomatoes (Casadesús et al., 2019; Dong et al., 2020; Alturk et al., 2020) found improvement in seedlings quality.

Despite reports in literature, there are still many details to understand. Researches need to clarify the behavior of plants growing in new conditions and which kind of interactions can be happen. In this context, the present study was conducted to assess different levels of NPK fertilizer and a NPK + auxin biostimulant on agronomic aspects of seedlings of Collard Greens and tomato.

Material and Methods

The tomato (*Lycopersicon sculentum*) experiment was conducted using the cultivar Caete (Blue Seeds, Holambra, Brazil), from January to March and, the Collard Greens (*Brassica oleracea* var. *Acephala*) experiment was realized using the cultivar Georgia (Isla Sementes, Porto Alegre, Brazil), from March to April of 2014. Both experiments took place at a greenhouse in the Institute of Agricultural Sciences from the Federal University of Uberlandia, Brazil.

The seedlings production was carried out in trays of expanded polystyrene, with dimensions of 0.34 x 0.68 x 0.06 m of width, length and height, respectively. The trays had 128 cells with a volume of 40 cm^3 each.

The seeds of tomato and Collard Greens were sown on the cells of the trays at 0.01 m depth, depositing three seeds per cell, on special substrate for seedling growth. After emergency of the plants, only the best seedling was left at each cell, for greater vigor.

The trays were laid on the greenhouse bench, supported on bricks, in order to allow drainage of excess water in the cells. Irrigation was performed twice a day, one time in the morning and other time in the afternoon.

The experimental design was a randomized block with four replications, using a 2 x 4 factorial scheme. The first factor was two types of fertilizers; NPK (nitrogen, phosphorus and potassium) at formulation 9-45-11 (in percentage of weight of N, P_2O_5 and K_2O) and, a commercial biostimulant composed of NPK 9-45-11 + 400 mg kg^{-1} of indole acetic acid (auxin). The second factor was the rates of the fertilizers; 50; 100; 150 and 200% of the recommended rate of the commercial biostimulant for tomato experiment and 50; 75; 100 and 125% of the recommended rate for the Collard Greens experiment. The rate was the same for both fertilizers at each treatment, once that the main objective was to seek for differences due to the presence of auxin in the commercial product. Each experimental plot was composed of 25 plants, being the 9 central plants considered for analysis and the rest considered as border.

The recommended rate of the commercial biostimulant was 10 g L^{-1} in the irrigation water. It was adopted the common irrigation volume of 400 mL of water per tray. This way, each cell received after application 3.1 mL of solution.

Fertilizers were weighed on a precision digital scale to apply on the cells. The treatments were applied 21 days after sowing, when the plants had three to four leaves. The treatments were dissolved in distilled water and applied using a 10 mL shot, applying 3.1 mL per cell of solution.

The harvest was done 33 days after sowing. Nine plants per plot were taken, in which the following measurements were made: stem length, root length, stem diameter, total chlorophyll content, root dry mass and shoot dry mass. The plants were removed from the cells with substrate on their roots, being those washed later to remove the substrate.

In order to evaluate the total chlorophyll content on the leaves, an equipment to measure chlorophyll (Falker, model ClorofiLOG-CFL1030) was used, which shows the Falker Chlorophyll Index (ICF) as measurement unit. The stem and root length were measured with a tape-measure and the stem diameter with a digital pachymeter. In order to obtain dry mass, the plants were split in root and aerial part (stem and leaves) and put in paper bags to dry in an oven at 65 °C for 72 hours. After, they were weighed in digital scale with precision of three digits.

The variables were submitted to analysis of variance using the statistical software SISVAR and the means were compared using Tukey's test.

Results and Discussion

There was interaction between the fertilizers type and rates of fertilizers applied on the stem length of tomato plants (Table 1). It was observed that at all rates tested, the seedlings that received NPK fertilizer had higher heights than those treated with biostimulant (NPK + auxin). It didn't have differences between rates of NPK fertilizer, with height varying from 20.4 to 21.65 cm. With the biostimulant, the highest stem length was observed with 50% of the recommended rate, while the double of the recommended rate produced 39% reduction in the plants height (Table 1).

Leal et al. (2007) and Rodrigues et al. (2010) found higher vigor seedlings when their heights were 6.9 and 10.3 cm, respectively, using the evaluation period of 33 days after emergency (DAE). It was observed that foliar NPK induced the stretching of the plants, what didn't favor their vigor, since stretched plants tend to be less resistant to environmental stresses diseases attack, especially after transplanting (Seleguini et al., 2013).

The tomato root length wasn't influenced by fertilizer sources and ranged from 5.88 to 8.76 cm (Table 2). Opposite to this result, Cruz et al. (2015) observed that auxin interfered in the development of corn. According to the authors, there was modulation of genes and proteins of the cell cycle in the root.

Although it didn't influence the length, the auxin biostimulant favored root dry matter accumulation (RDM), being 39% higher than NPK fertilizers only (Table 2). The stem diameter was lower (1.9 mm) in plants that received auxin, but the value found was close to that found in high vigor seedlings by Rodrigues et al. (2010).

Table 1. Stem length (cm) of tomato plants, after treatment with two fertilizers and rates.

Fertilizer	rate (% of recommended rate)			
	50	100	150	200
NPK	20.49aA	20.68aA	20.89aA	21.65aA
Biostimulant	11.58bA	8.33bB	7.09bB	7.03bB
CV (%)	9.51			

Means followed by different letters, lowercase in the column and uppercase in the row, differ between each other according to Tukey's test, at 0.05 of significance. CV – Coefficient of variation.

Table 2. Root length (RL), stem diameter (SD) and root dry mass (RDM) of tomato plants after treatment with two fertilizers (values represents an average of all four rates).

Fertilizer	RL (cm)	SD (mm)	RDM (g)
NPK	8.76a	3.12a	0.38b
Biostimulant	5.88a	1.91b	0.53a
CV (%)	11,03	12.22	24.76

Means followed by different letters, lowercase in the column, differ between each other according to Tukey's test, at 0.05 of significance. CV – Coefficient of variation.

Collard Greens seedlings also showed a better development of stem length (height) with NPK application (Table 3). However, the plants didn't stretch like tomato plants. However, the characteristics root length, stem diameter, chlorophyll and root dry mass were not influenced by fertilizers source.

Table 3. Stem length (SL), root length (RL), stem diameter (SD), total chlorophyll - Falker Chlorophyll Index (FCI), root dry mass (RDM) of Collard Greens plants after treatment with two fertilizers (values represents an average of all four rates).

Fertilizer	SL (cm)	RL (cm)	SD (mm)	FCI	RDM (g)
NPK	6.21a	8.61a	1.40a	43.59a	0.28a
Biostimulant	5.77b	8.25a	1.26a	44.25a	0.24a
CV (%)	7.0	8.42	12.34	8.10	36.82

Means followed by different letters, lowercase in the column, differ between each other according to Tukey's test, at 0.05 of significance. CV – Coefficient of variation.

The literature shows that the presence of auxin may favor the root system development, being the concentration and type of auxin significantly affect the rooting process (Aina et al., 2015). On this study, the rates of the fertilizers used (with or without auxin) didn't show effect on stem length, stem diameter and root dry mass both on tomato (Table 4) and Collard Greens (Table 5) seedlings.

The absence of response of both tomato and Collard Greens plants to the application of rates of exogenous auxin can be explained by the complex mechanism of root system growth and formation. Other hormones, genes, nutrients, environmental conditions and managements applied to the plants interact with each other and with the rhizosphere and determine the root development (Rogers and Benfey, 2015).

Table 4. Root length (RL), stem diameter (SD) and root dry mass (RDM) of tomato plants after treatment with rates of fertilizer (values represent an average of the two fertilizers).

Rate (%)	RL (cm)	SD (mm)	RDM (g)
50	7.26a	2.59a	0.51a
100	7.36a	2.53a	0.49a
150	7.2a	2.50a	0.45a
200	7.46a	2.45a	0.38a
CV (%)	11.03	12.22	24.76

Means followed by different letters, lowercase in the column, differ between each other according to Tukey's test, at 0.05 of significance. CV – Coefficient of variation.

Similarly to this study Oliveira et al. (2011) concluded that the use of algae extracts at the rates 0.0, 2.0, 4.0, 6.0 and 8.0 mL L^{-1} didn't produce significant differences in root length and root dry mass in yellow passion fruit seedlings.

High rates of biostimulant fertilizer didn't favor total chlorophyll content in Collard Greens plants (Table 5). The synergistic or antagonistic effects of biostimulants vary according to the specie of plant. According to Ferraz et al. (2014), a 12 mg kg^{-1} rate of Stimulate promoted an increase in the chlorophyll content of the plants when applied to the seeds of passion fruit "Kenyan Roe". The same was observed on citric rootstock "Cleopatra" (Souza et al., 2013).

Table 5. Stem length (SL), root length (RL), stem diameter (SD), total chlorophyll - Falker Chlorophyll Index (FCI) and root dry mass (RDM) of Collard Greens plants after treatment with rates of fertilizer (values represent an average of the two fertilizers).

Rate (%)	SL (cm)	RL (cm)	SD (mm)	FCI	RDM (g)
50	5.93a	8.26a	1.29a	41.33a	0.29a
75	5.87a	8.21a	1.4a	42.43ab	0.24a
100	5.86a	8.23a	1.27a	44.56ab	0.26a
125	6.29a	8.61a	1.35a	47.36b	0.26a
CV (%)	7.0	8.42	12.34	8.10	36.82

Means followed by different letters, lowercase in the column, differ between each other according to Tukey's test, at 0.05 of significance. CV – Coefficient of variation.

Table 6. Stem dry mass (SDM) of plants of tomato and Collard Greens after treatment with two fertilizers and rates.

Tomato				
Fertilizer	Rate (%)			
	50	100	150	200
NPK	2.34aB	2.62aA	2.95aA	2.90aA
Biostimulant	1.08bB	0.58bAB	0.44bA	0.36bA
CV (%)				

Collard Greens				
Fertilizer	Rate (%)			
	50	75	100	125
NPK	0.87aA	0.92aA	0.94aA	0.93aA
Biostimulant	0.84aA	0.68bAB	0.58bB	0.81aA
CV (%)	11.06			

Means followed by different letters, lowercase in the column and uppercase in the row, differ between each other according to Tukey's test, at 0.05 of significance. CV – Coefficient of variation. w

There was significant interaction between the fertilizer source and the rate of fertilizer for the characteristic stem dry mass, both for tomato (Table 6) and Collard Greens (Table 6).

The stem dry mass (aerial part) of tomato was inferior in plants that received biostimulant, in all tested rates. The same behavior was observed on Collard Greens plants at 75% and 100% of the recommended rate. Foliar applications of NPK and biostimulant didn't stimulate the accumulation of stem dry mass on tomato at 50% of the recommended rate, and the other rates didn't differ among themselves. Being so, it's possible to conclude that increasing rates from 100 to 200% doesn't promote dry mass accumulation in the leaves in any of the fertilizers tested.

The stem dry mass on Collard Greens showed no difference on the different rates of NPK. Plants that received auxin combined with NPK showed lower accumulation at 100% of the recommended rate (Table 6).

It's evident that each species of plant has a more adequate rate for its development. However, most part of studies obtained positive effects when using rates smaller or near to the lowest rate tested in these experiments, regardless the biostimulant used or its form of application, once it's applied in the initial stages of development.

Other studies with the biostimulant (Stimulate) shows that, the pre-soaking of sunflower seeds with the 3.3 mL L^{-1} rate promoted increase in seedling emergence under stress conditions of toxic aluminum (Couto et al., 2012). Treating soybean seeds in 6 mL kg^{-1} favored the initial development of seedlings (Binsfeld et al., 2014). In addition, maize and bean treated with 2 mL kg^{-1} and 0.75 mL kg^{-1} rates, respectively, showed higher grain yield (Dourado Neto et al., 2014).

Silva et al. (2008) tested three rates of three biostimulants; 5.0 mL kg^{-1} of Booster, a biostimulant composed of amino acids, auxin and cytokines; 10 mL kg^{-1} of Cellerate, composed of amino acids and gibberellin; 12.5 mL kg^{-1} Stimulate; and the mixture of the last two, to evaluate the effect on the physiological quality of maize seeds. The authors didn't observe improvement in seed quality. It's possible to conclude that different biostimulants composed of different growth regulators, couldn't produce positive effects, possible due to inadequate rates.

It is important to remember that the relationship between growth regulators and nutrients also interfere with plant responses. For example, rice seeds treatment with 4 mL kg^{-1} of Stimulate, associated with rates below 50 mg dm^{-3} of phosphorus at planting, contributed to the development of plants, but the use of Stimulate with high rates of phosphorus caused negative effects (Garcia et al., 2009).

Dong et al. (2020) found different response to the same biostimulants when applied in tomato and strawberry. The authors related the difference to root system architecture, tomato being more responsive due robust and long roots system, more effectively and efficiently to absorb biostimulants.

The application of higher rates, such as 100 mL L^{-1} of Stimulate, during the vegetative and reproductive development of tomato, promoted greater development and production of the plants when compared to the single or double application of growth regulators contained in the biostimulant (Cato et al., 2013). This shows that plants in more advanced stages can use higher rates of regulator in its metabolic processes without a phytotoxic effect. In addition, this work shows the importance of the hormonal interaction in the physiological responses of the plants, being the tomato well responsive to foliar applications.

Conclusions

The biostimulant fertilizer containing auxin favors the development of tomato seedlings, since it provides a greater accumulation of root dry mass and does not promote the stretching of the seedlings, as occurs in the application of only NPK fertilizers. On the other hand, the Collard Greens seedlings were not affected by the auxin in the biostimulant fertilizer and showed higher stem length with NPK application. Concentrations of 50% to 200% of the recommended rate of the fertilizers do not interfere in root length, diameter and MSR of tomato and Collard Greens seedlings.

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