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Rates of application of *Azospirillum brasilense* in tomato crop

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ABSTRACT

The use of plant growth-promoting bacteria (PGPB) may be a promising agronomic practice to improve the growth and productivity of vegetables. The objective of this work was to evaluate the effect of inoculation of *Azospirillum brasilense* on plant growth and tomato fruit production. Two experiments were carried out: the first one evaluating the growth of the plants in a greenhouse and the second one evaluating the production of fruits under field conditions. The experimental design used in two experiments was the completely randomized 2 x 5 factorial scheme, with four replications. The treatments resulted from the combination of two tomato cultivars (Gaúcho Melhorado and San Marzano) and four inoculant doses (0; 2; 4; 6 e 8 mL kg^{-1} of seed) containing the Ab-V5 and Ab-V6 strains of *A. Brasilense*. The application of inoculant containing *A. brasilense* improved the growth and dry matter production of tomato cultivars Gaúcho Melhorado and San Marzano. The highest values of plant height, stem diameter, root length, root volume, relative chlorophyll content and dry matter of shoots and roots are obtained with the dose of 4 to 6 mL kg^{-1} of inoculant, allowing to infer that the maximization of plant growth and the production of tomato fruits with the application of inoculant containing *Azospirillum brasilense* can be obtained with the use of approximately 5.0 mL kg^{-1} of seeds for tomato cultivars.

Keywords: interaction, development, production, beneficial.

Doses de aplicação de *Azospirillum brasilense* na cultura do tomate

RESUMO

O uso de bactérias promotoras de crescimento de plantas (BPCP) pode ser uma prática agronômica promissora para melhorar o crescimento e a produtividade das hortaliças. Este trabalho teve por objetivo avaliar o efeito da inoculação de *Azospirillum brasilense* no crescimento das plantas e na produção de frutos do tomateiro. Dois experimentos foram conduzidos: o primeiro avaliando o crescimento das plantas em casa de vegetação e, o segundo, a produção de frutos em condições de campo. O delineamento experimental utilizado em ambos os experimentos foi o inteiramente casualizado em esquema fatorial 2 x 5, com quatro repetições. Os tratamentos resultaram da combinação de duas cultivares de tomate (Gaúcho Melhorado e San Marzano) e da aplicação de quatro doses de inoculante (0; 2; 4; 6 e 8 mL kg^{-1} de semente) contendo as estirpes Ab-V5 e Ab-V6 de *A. brasilense*. A aplicação de inoculante contendo *A. brasilense* melhorou o crescimento e a produção de matéria seca das cultivares tomate Gaúcho Melhorado e San Marzano. Os maiores valores de altura de planta, diâmetro de caule, comprimento da raiz, volume radicular, índice relativo de clorofila e matéria seca da parte aérea e das raízes são obtidos com a dose de 4 a 6 mL/kg de inoculante, permitindo inferir que a maximização do crescimento da planta e da produção de frutos de tomate com a aplicação de inoculante contendo *Azospirillum brasilense* pode ser obtida com o uso de, aproximadamente, 5,0 mL kg^{-1} de sementes para as cultivares de tomate.

Palavras-chave: interação, desenvolvimento, produção, benéfica.

1. Introduction

Tomato production in Brazil in 2017 was 4,373,047 million tons. The largest producer is the state of Goiás with a production of 1,262,701 tons, followed by São Paulo with 938,800 tons, the state of Paraná is in the fifth position with 260,643 tons (IBGE, 2018). In the present scenario of horticulture, tomato cultivation has great economic and social relevance, since this activity produces food, generates employment and income, strengthens family agriculture, reduces rural exodus, and promotes regional development (Carvalho et al., 2014). Tomato cultivation shows high employment in the industry for the formation of sauces, juices, dehydrated pulp (Filgueira, 2008), besides being consumed "*in natura*". It has high levels of vitamins A, C and lycopene (Carvalho and Pagliuca, 2007).

The use of plant growth-promoting bacteria (PGPB) may be an alternative to reduce the use of chemical fertilizer inputs (Adesemoye et al., 2009). Thus, using beneficial microorganisms that promote efficient growth from seedling to plant is relevant in the tomato production system, especially in regard to the organic system, which are dependent on alternative inputs (Zecchin et al., 2015).

Regarding the microorganisms associated with PGPB, these are notable for helping to develop plants due to their ability to provide nutrients and hormones directly and / or indirectly, due to processes and biological cycles (Bashan and De Basan, 2010). In this context, the species *Azospirillum brasilense* has shown agronomic relevance because it presents a series of benefits, in order to: influence significantly the biological nitrogen fixation capacity; increase the activity of nitrate reductase, when cultivated endophytically in plants; promote the production of hormones such as auxins (favors root development), gibberellins; assist phosphate solubility; favoring beneficial mycorrhizal-plant associations and may act indirectly on the growth of fungi or phytopathogenic bacteria, protecting the plant (Reis et al., 2008; Hungria, 2011).

Although the using of *Azospirillum brasilense* is a promising alternative studied by several authors (Díaz-Zoritta and Fernandez Canigia, 2008; Mazzuchelli et al., 2014; Lipório et al., 2015; Hungria et al., 2010), the studies referring to its use are very scarce in solanaceous, exceptionally on tomatoes. The objective of the present work was to evaluate the effects of inoculation of *Azospirillum brasilense* on plant growth and fruit production of tomato.

2. Material and Methods

In this study two experiments were developed: the first experiment had the purpose of evaluating the growth of tomato plants under greenhouse conditions; while the second experiment was carried out with the purpose of evaluating the production of tomato fruits under field conditions. Both experiments were performed between October and December 2017. The climate of the region is classified as subtropical humid mesothermal (Cfb), described by Köppen, mean annual temperature of 18° C, and an annual average rainfall of 1,800 mm (IAPAR, 2018).

The experimental design adopted in both experiments was the completely randomized 2 × 5 factorial scheme, with four replications. The treatments consisted of the combination of two tomato cultivars [Gaúcho Melhorado and San Marzano] and application of four inoculant rates (0; 2; 4; 6 e 8 mL kg^{-1} of seeds). The inoculant used contained *Azospirillum brasilense* AbV5 and AbV6 strains in the concentration of 2.0 x 10^8 viable cells mL^{-1}.

At 35 days after sowing, when the seedlings presented two pairs of expanded leaves, they were transplanted to polypropylene pots containing 8 dm^3 of soil (Experiment 1) or under field conditions (Experiment 2).

Experiment 1: Growth of tomato plants

This experiment was carried out under greenhouse conditions, at Universidade Federal da Fronteira Sul (UFFS), in Laranjeiras do Sul, PR, Brazil (25º26′41″ S and 52º26′19″ W). Plastic pots were filled with 8.0 dm^3 of a Red Latosol (Santos et al., 2013), with the following characteristics: pH in $CaCl_2$ = 4.93; P (Mehlich-1) = 6.20 mg dm^{-3}; K = 0.32 $cmol_c$ dm^{-3}, Ca = 3.28 $cmol_c$ dm^{-3}, Mg = 1.83 $cmol_c$ dm^{-3} e H + Al^{3+} = 4.78 $cmol_c$ dm^{-3}, organic matter = 40.1 g dm^{-3}; CEC = 10.21 $cmol_c$ dm^{-3}; soil base saturation = 53.2%; clay = 650 g kg^{-1}; silt = 150 g kg^{-1}; and, sand = 200 g kg^{-1}. The soil was collected in the layer of 0.0-0.20 m depth, dewatering, drying in air, sieved in mesh of 8.0 mm and packed in the pots. The poultry manure was used as nutrient source, the amount applied in transplanting being calculated by soil analysis, based on the recommendations proposed by the Guide of Fertilization and Liming for the state of Paraná (SBCS, 2017).

Two tomato seedlings were transplanted in the pots, and at 15 days after transplanting (DAT) were thinned, leaving only one plant per pot. The plants were tutored with a simple 1.70 m high stake. Scrubbing was done once a week.

At 30 and 60 days after transplantation (DAT) the following characteristics were evaluated: plant height (cm), measuring the stem length from the base of the plant to the apical meristem with the aid of a ruler graduated in centimeters; stem diameter (mm), reading made 3.0 cm from the base of the plant with the aid of a digital caliper; and relative chlorophyll index (RCI) was determined using a portable chlorophyllometer model SPAD-502 (Soil and Plant Analysis Development) from Minolta Co., Osaka, Japan.

At 60 DAT the following variables were also evaluated: root length (cm), measuring the lengths of the longest roots with the help of a graduated ruler; (cm³), determined by the mass displacement method, using a 100 mL beaker graduated in milliliters (mL) and containing distilled water and 70% alcohol in the ratio 4:1 (v:v). For the determination of shoot dry matter (SDM) and roots (RDM), the plants were separated in shoots and roots, dried in an oven at 65 ºC for 72 h and weighed in an analytical balance with an accuracy of 0.0001 g, the results being expressed in g plant^{-1}.

Experiment 2: Production of tomato fruits

This experiment was carried out at the field in Quedas do Iguaçu, PR (25º31′14″ S, 52º48′37″ W and altitude of 710 m). The soil was classified as Red Latosol (Santos et al., 2013), and had the following physical-chemical characteristics: clay = 640 g kg^{-1}; silt = 150 g kg^{-1}; sand = 210 g kg^{-1}; organic matter = 40.11 g dm^{-3}; pH in $CaCl_2$ = 5.0; P (Mehlich-1) = 5.76 mg dm^{-3}; K = 0.24 cmol$_c$ dm^{-3}, Ca = 3.98 cmol$_c$ dm^{-3}, Mg = 1.39 cmol$_c$ dm^{-3}, H + Al^{3+} = 4.64 cmol$_c$ dm^{-3}; CEC = 10.53 cmol$_c$ dm^{-3}; and soil base saturation of 54.7%.

The planting grooves were prepared 25 cm deep, 20x20 cm wide in length. The seedlings were transplanted at 35 days when they had four to six expanded leaves. The planting spacing was 60 cm between plant and 100 cm between row. The plants were guarded by vertical system with stakes of 1.7 m height. In all plants, the apical pruning was performed above the third leaf of the seventh cluster, in order to standardize the height and number of clusters per plant. Scrubbing was performed weekly.

At 30 and 60 days after the transplant (DAT) of the seedlings were evaluated the height of the plant. Up to 125 DAT the mature fruits were manually collected, identified, weighed, then the tomato production per plant was determined (kg plant^{-1}).

The data of the two experiments were submitted to analysis of variance (ANOVA) using statistical software SISVAR® version 5.6 for Windows (Ferreira, 2015). For the inoculant doses containing *A. brasilense*, the polynomial regression analysis was used and the significant equations (F test, $p \leq 0.05$) with the highest determination coefficients (R^2) were adjusted.

3. Results and Discussion

Experiment 1: Growth of tomato plants

The inoculation of tomato seeds with *Azospirillum brasilense* had no significant effect (p>0.05) at the time of the two cultivars at 30 DAT (days after transplantation). There was interaction (p<0.05) between inoculant doses containing *A. brasilense* and tomato cultivar at plant height at 60 DAT (Figure 1). This significant effect for the interaction between the factors studied indicates the tomato cultivars have distinct responses to the application of *A. brasilense*. In the cultivar Gaúcho Melhorado, the inoculant doses promoted higher growth, and the maximum plant height (91.3 cm) was obtained with the use of 5.1 mL kg^{-1} of seed. In the cultivar San Marzano the maximum height obtained was of 83.5 cm, with the application of 4.7 mL kg^{-1}. In both cultivars there was an increase in the height of tomato plants when the seeds were inoculated with *Azospirillum brasilense*. These results suggest that there is a symbiotic relationship between this species of plant and bacteria of the genus *Azospirillum*, which favored the greater growth of the plants. According to Vieira (2017), bacteria of the genus *Azospirillum* contribute positively for N_2 fixation, increased nutrient and water absorption, plant hormone production and increased phosphate solubilization, which potentiates plant growth.

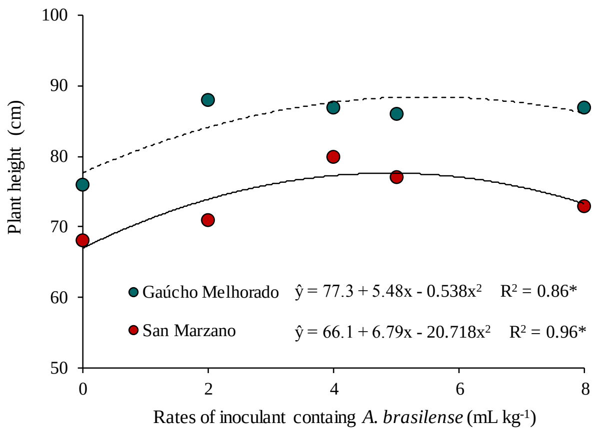

Figure 1. Effect of the rates of inoculant containing *Azospirillum brasilense* at plant height of the two tomato cultivars at 60 DAT (days after transplant), when grown under greenhouse conditions. Laranjeiras do Sul, PR, 2018. * and ** significant 5% and 1% probability, respectively.

The use of *A. brasilense* provided an increase in stem diameter (p<0.01) at 30 DAT and 60 DAT (Figure 2a and 2b), so that the maximum dose was obtained at the concentration of 7.695 mL kg^{-1} and 4.994 mL kg^{-1} for respective periods. Terry et al. (2000) evaluating the interaction between the inoculation of *Azospirillum brasilense* and nitrogen application in the tomato crop, verified that the diameter of the stem varied from 13.1 to 13.3 mm. These values are similar to those reported in this study. This increase in stem diameter may be related to the greater need for translocation of water and nutrients, absorbed by a well-developed root system.
According to Vieira (2017), phytohormones have a great impact on root growth. Milléo and Cristófoli

(2016) also reported that maize seeds inoculated with *Azospirillum brasilense* resulted in similar root growth (dry mass) when compared to nitrogen fertilizer application. In both cases, the results were different from the control treatment without inoculation of *Azospirillum* and application of nitrogen. Lipório et al. (2015) also concluded that the use of *Azospirillum brasilense* reduced the need for nitrogen fertilization of corn in 50% of the recommended dose for the crop.

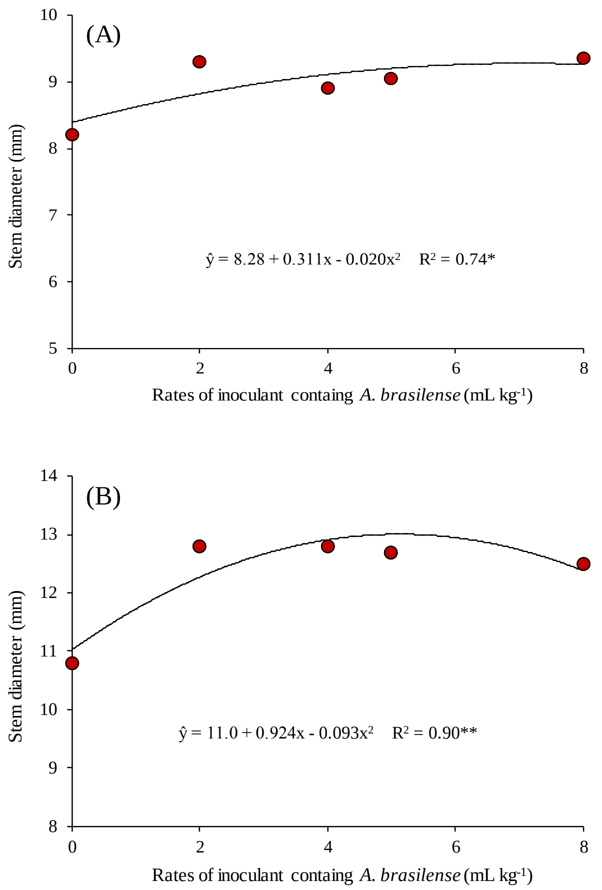

Figure 2. Stem diameter (mm) of the cultivars Gaúcho Melhorado and San Marzano with inoculation *Azospirillum brasilense* at 30 (A) and at 60 days after transplant (B). Laranjeiras do Sul, PR, 2018. * and ** significant 5% and 1% probability respectively.

The relative chlorophyll index (RCI) at 30 and 60 DAT was significantly influenced ($p < 0.01$) with the application of inoculant doses containing *Azospirillum brasilense* (Table 1). It was verified that the maximum RIC at 30 DAT was in the dose 4.8 mL kg^{-1} for the cultivar Gaúcho Melhorado and of 4.8 mL kg^{-1} for the cultivar San Marzano. The maximum point observed in RCI at 60 DAT was 4.83 and 4.90 mL kg^{-1} of inoculant for the cultivars Gaúcho Melhorado and San Marzano, respectively. It was possible to observe that there was a significant interaction ($p<0.01$) between the use of *Azospirillum brasilense* and the tomato cultivars studied in relation to RCI. This result indicates that the tomato cultivars respond differently to the application of *A. brasilense*. This result confirms those reported by Quadros et al. (2014), that tested hybrids of corn also verified interaction between the hybrids and inoculation of *Azospirillum brasilense* for plant height, RCI and mass of a thousand grains.

This positive relation with the use of *Azospirillum brasiliense* in the increase of the RIC was observed by Jordão et al. (2010) in corn plants, which attributed this increase in the RIC to the higher nitrogen concentration of the leaves. Bacteria of the genus *Azospirillum*, besides the ability to fix N, help in the availability of hormones, among them auxins, which is related to root development. In addition, when plants have their nutritional needs met, it increases their allocation of resources to the shoot. This may explain the increase in N concentration in the leaves, a sum of hormonal factors and N fixation by the bacteria (Taiz and Zeiger, 2013).

The maximum root length (55.37 cm), root volume (137 cm^3) were obtained with the application of 5.1 mL kg^{-1} (Figure 3a and 3b). Terry et al. (2000) showed that the inoculation of *Azospirillum brasilense* associated with N application in cover resulted in the higher growth of the roots of tomato plants when compared to the treatment without inoculation.

Tabela 1. Regression equations for the stem diameter (SD), relative index of chlorophyll (RIC), shoot dry matter (SDM), root dry matter (RDM) and plant height (PH) for the cultivars Gaúcho Melhorado and San marzano

Cultivar	Evaluation Period	Variable	Equation	R^2	MP (mL kg^{-1})
Gaúcho Melhorado	30 DAT	RCI	$y = -0.8643x^2 + 8.252x + 20.63$	0.77**	4.8
	60 DAT	RCI	$y = -0.3357x^2 + 3.245x + 24.99$	0.74**	4.8
		PH	$y = -0.7179x^2 + 6.765x + 74.20$	0.81	4.7
San Marzano	30 DAT	RIC	$y = -0.3425x^2 + 3.283x + 24.98$	0.75	4.8
	60 DAT	RIC	$y = -0.8286x^2 + 8.066x + 20.71$	0.76**	4.9
		PH	$y = -0.4271x^2 + 4.559x + 34.77$	0.96**	5.3

DAT (days after transplantation); MP (maximum point). * and ** significant 5% and 1% probability.

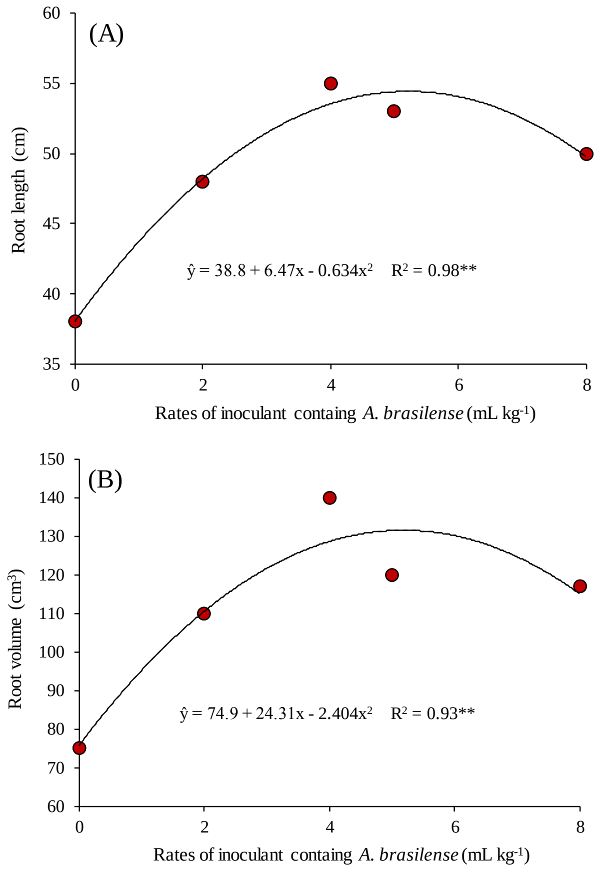

Figure 3. Effect of inoculant doses containing *Azospirillum brasilense* on root length (a) and root volume (b) of the two tomato cultivars at 60 days after transplant, when cultivated under greenhouse conditions. Laranjeiras do Sul, PR, 2018. * and ** significant 5% and 1% probability.

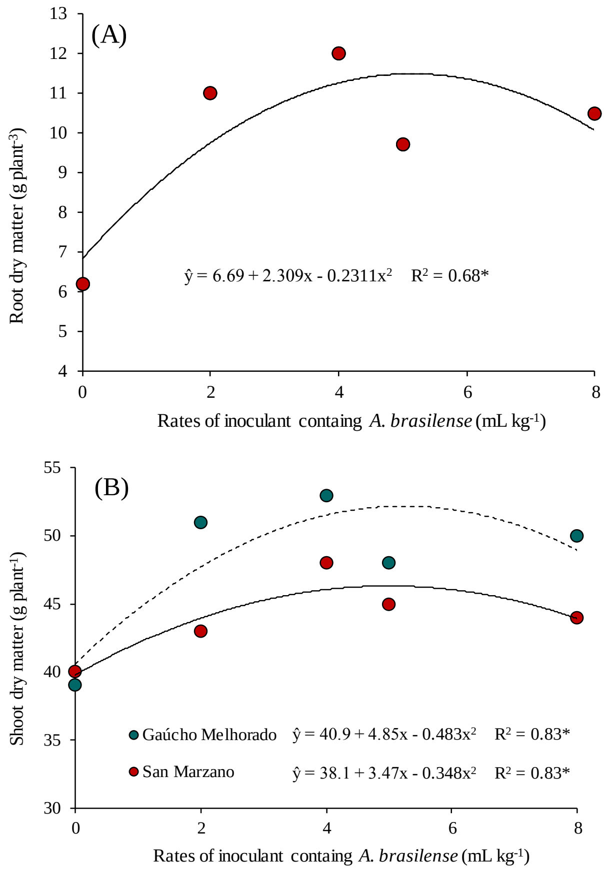

Figure 4. Shoot dry matter (a) and root dry matter (b) values of the Gaúcho Melhorado and San Marzano tomato cultivars, using different concentrations of *Azospirillum brasilense*. Laranjeiras do Sul, PR, 2018. * and ** significant 5% and 1% probability.

In general, the results obtained are satisfactory, since the use of inoculation increased the growth of the roots. The association of microorganisms such as *Azospirillum* provides a greater number of root hairs, increasing the root surface, consequently the plant can absorb more nutrients and water, thus aiding in greater plant growth and development (Vogel et al., 2013). Bashan et al. (1996) reports that the microorganism *Azospirillum* sp. is capable of producing hormones, which favor root growth, among them auxin, which aids in stretching and cell division.

The treatments with doses of *Azospirillum brasilense* increased the shoot dry matter (SDM) and root dry matter (RDM) of tomato plants at 30 DAT (Figure 4a and 4b, respectively). Shoot dry matter production at maximum 30 DAT was obtained with the application of 5.0 mL kg^{-1} for both tomato cultivars. For the production of RDM, no interaction (p>0.05) between the cultivars and the application of *Azospirillum brasilense* was observed, and the 5.0 mL kg^{-1} rate resulted in maximum RDM production. This increase in root dry matter is consistent with the increase in root volume and root length observed in Figure 3. While SDM can also be a consequence of changes in root volume/length and stem diameter.

Hadas and Okon (1987) also verified that the root dry matter production was higher with the application of *Azospirillum* sp. compared to uninoculated control treatment.

Experiment 2: Production of tomato fruits

Plant height of the two tomato cultivars was significantly influenced (p<0.01) by inoculant doses containing *Azospirillum brasilense*. At 30 DAT, the maximum height was obtained with 5.3 mL kg^{-1}, while at 60 DAT the average of the two cultivars obtained the highest growth with 4.7 mL kg^{-1} (Table 1). The plant height response at 60 DAT confirms the results found by Terry et al. (2000), who report that *Azospirillum brasilense* increases growth and helps in the development of tomato plants.

The inoculation of tomato seeds with *Azospirillum brasilense* significantly affected (p<0.01) fruit yield per plant in both tomato cultivars (Figure 5). The cultivar Gaúcho Melhorado had the maximum yield of fruits per plant with the inoculant dose of 4.8 mL kg^{-1} while the cultivar San Marzano with 4.8 mL kg^{-1} of seeds. The maximum fruit yield for the cultivars Gaúcho Melhorado and San Marzano was 2,821 g plant^{-1} and 2,104 g plant^{-1}, respectively.

These results corroborate with those found by Bashan (1998), who showed that the inoculation of *Azospirillum* sp. is beneficial in several crops including tomato. Similarly, Terry et al. (2000), showed that treatments that received *Azospirillum brasilense* at sowing were the ones that obtained the biggest increase in production.

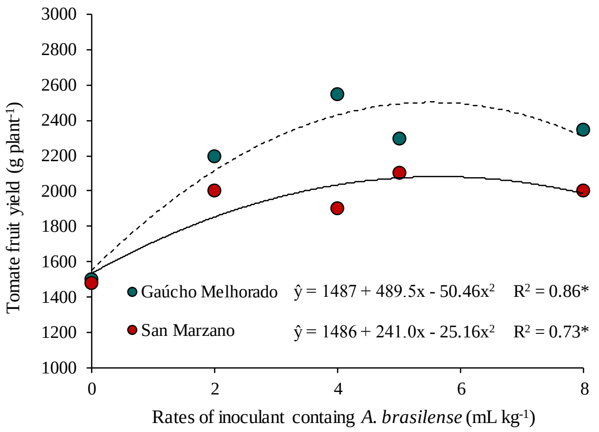

Figure 5. Effect of inoculant doses containing *Azospirillum brasilense* on fruit yield per plant for both tomato cultivars when grown in the field. Quedas do Iguaçu, PR, 2018. * and ** significant 5% and 1% probability.

Esquivel-Cote et al. (2017) obtained higher fruit yield (1,800 g plant^{-1}) of the Juanita tomato cultivar with the inoculation of *Azospirillum* sp. associated with application of 170 kg N ha^{-1}. The results obtained in this study demonstrate that it is possible to promote phytotechnical increments through inoculation with *Azospirillum brasilense* in the tomato varieties observed. Studies involving more varieties and different edaphoclimatic conditions are needed. Most of the literature that studies the interaction of this bacterium is made with corn and other poaceae.

4. Conclusions

The maximization of plant growth and tomato fruit production with the application of inoculant containing *Azospirillum brasilense* can be obtained with the use of approximately 5.0 mL kg^{-1} of seeds for the cultivars 'Gaúcho Melhorado' and 'San Marzano'.

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