

Biofertilization and nutrition in the development of serrano pepper seedlings

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Abstract

The production of quality seedlings is essential for optimal growth and good crop yields during transplantation; therefore, the objective of the present investigation was to determine the effect of bacteria that promote plant growth and the management of nutrition in the physiology and growth of serrano pepper seedlings. Under greenhouse conditions, an experiment was carried out with serrano pepper var. Tampiqueña 74, under a completely randomized design with a factorial arrangement of $2 \times 2 \times 2$. The factors were: inoculation (with or without *Pseudomonas tolaasii*), substrate (with and without sterilization), and nutrient solution (Steiner and vermicompost tea) with a total of eight treatments with four repetitions each. The results indicate that the effect of *P. tolaasii* is highly significant in non-sterilized substrate and with Steiner solution, where the seedlings presented higher height, stem diameter, leaf area, and the dry biomass of the aerial part. In addition, the NO₃ concentration in sap increased. With the vermicompost tea the inoculation only impacted on the radical length and volume, while with the Steiner solution it only had an effect on the nutritional content of N and K⁺ and growth rates. The inoculation of *P. tolaasii* in serrano pepper seedlings favors the physiology and nutritional concentration as long as the substrate is not sterilized. The least effect on seedlings occurs if only vermicompost tea is supplied.

Keywords: plant nutrition, rhizobacteria, seedlings.

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Introduction

Seedling quality is the combination of characteristics such as height, stem diameter, root length and nutrient concentration (Aramendiz-Tatis *et al.*, 2013), number of leaves and leaf area (Vidigal *et al.*, 2011), that affect their vigor. A good development, free of pests and diseases, allows greater adaptability to transplantation, with a high capacity for absorbing water and nutrients (Salusso *et al.*, 2015). Sarduy *et al.* (2016) mentioned that producing quality seedlings of pepper (*Capsicum annuum* L.) from almacigo, guarantees excellent greenhouse production.

The generation of healthy and vigorous seedlings is essential to obtain optimal growth and high yields after transplanting, both in the field and in the greenhouse (Souri and Sooraki, 2019) and where factors such as the substrate, the nutrient solution and inoculants influence. The substrates are important for seedling production since they directly affect their physiology and quality (Richmond, 2010; López-Baltazar *et al.*, 2013).

In addition to nutrition, it plays a vital role in seedling germination, physiology and quality (Marschner, 2012; Santos *et al.*, 2014) and it is possible to control their growth by manipulating the concentrations and proportions of nutritional solutions (Souri and Sooraki, 2019). Vermicompost tea is defined by Tortosa (2017), as an organic aqueous liquid, obtained from mature vermicompost where microorganisms with beneficial and protective characteristics for plants are found and reproduce, in addition to possessing nutritional properties contains enough nutrients for the growth and development of horticultural species, therefore it is recommended as another alternative for conventional or hydroponic production (González *et al.*, 2013).

The inoculation of plant growth promoting rhizobacteria (RPCV) that can be a single strain or in a consortium (Ahemad and Khan, 2012), influence the biochemical, physiological and morphological changes of plants (Bhattacharyya and Jha, 2012) through mechanisms such as the production of phytohormones (Glick, 2012) and participate in the antagonism against pathogenic microorganisms (Carreón *et al.*, 2013). Their role as biofertilizers favors the nutritional status of crops (Mohamed *et al.*, 2019); through biological fixation and nitrogen availability and phosphorus solubilization (Aloo *et al.*, 2019; Syed and Tollamadugu, 2019).

The use of RPCV strengthens the biochemical and physiological processes of the seedlings, which generates greater resistance to post-transplant stress (Sapre *et al.*, 2018). The genus *Pseudomonas* encompasses numerous ecological niches, where some are promoters of plant growth (Riveros-Rosas *et al.*, 2019), function as a biofertilizer and can be useful in the biological control of diseases (Widnyana and Javandira, 2016).

There are few reports of *P. tolaasii* indicating its potencies as RPCV in pepper, this isolated strain, characterized by specific biochemical properties and growth promoter (Quiroz-Sarmiento *et al.*, 2019), is being evaluated in several crops. The hypothesis that is presented in the present study is that the inoculation of *P. tolaasii* combined with vermicompost tea favors the agronomic and nutritional development of serrano pepper seedlings.

The objective of the present investigation was to quantify the effect of the inoculation of *Pseudomonas tolaasii* on the physiology and development of serrano pepper (*Capsicum annuum* L.) seedlings using different nutritional solutions.

Materials and methods

Used materials

The experiment was installed in a tunnel-type greenhouse in the Plant Nutrition Area of the Postgraduate College, from May to July 2018. Serrano pepper (*Capsicum annuum* L.) variety Tampiqueña 74 (Caloro[®]), with a germination rate of 85%, was used as plant material. 25 round cavities (85.6 cm³ cavity⁻¹), black flexible polyethylene germination trays were used, disinfected with chlorine at a concentration of 200 ppm and washed with water.

Two seeds were sown per cavity to guarantee germination. The substrate was based on a mixture of peat moss (Premier[®]), perlite (Agrolita[®]) and vermiculite (Termita[®]) in a ratio of 1:1:1 (v/v/v), half of the mixture prepared it was sterilized with a pressure cooker (1.3 kg cm⁻²), the other half was used without sterilization according to the established treatments.

Establishment and experimental design

The experiment was established under a completely randomized design (DCA) with a factorial arrangement of $2 \times 2 \times 2$, where the study factors were: with or without inoculation with *P. tolaasii* (P), the sterilized substrate (SE) and substrate non-sterile (SNE) and the nutrient solution with Steiner (St) and vermicompost tea (Te).

A total of eight treatments with four repetitions each were evaluated. The experimental unit was a seedling tray with 25 serrano pepper seedlings, which were watered with sterilized water two to three times per day. Seedling emergence occurred after 12 days and they were thinned four days later, leaving one seedling per cavity.

Microbiological material

The *P. tolaasii* bacterial strain P61, belonging to the collection of the Soil Microbiology Laboratory of the Postgraduate Edaphology, was used as inoculum. The bacterium was inoculated six days after emergence, applying 1 mL of inoculum with a concentration of 1×10^9 CFU mL⁻¹ at the base of each seedling.

Nutritive solution

On the third day after inoculation, seedling nutrition was started, using the Steiner 25% universal nutrient solution (Steiner, 1984). As a second source of nutrition, vermicompost tea prepared according to the method of González-Solano *et al.* (2014). Both solutions were adjusted to pH 5.5 and 24 days later their concentration was increased to 50% in both solutions until transplantation. The concentration was calculated based on the electrical conductivity of the Steiner solution.

Variables evaluated

56 days after planting, a destructive harvest was carried out. The variables evaluated were: stem diameter (mm), seedling height (cm), number of leaves, leaf area (cm²) with an LI-COR[®] area measuring equipment, length (cm) and root volume (cm³) were determined with the water displacement method with a 25 mL cylinder.

In addition, NO₃⁻, K⁺ and Ca²⁺ were quantified in petiole (sap) cell extract with Laquatwin[®] ionometers. The seedlings were oven dried at 55 °C for 72 h and weighed to obtain the dry biomass of the aerial and root part. With the obtained values, the growth rates were calculated according to Hunt (2017) (Table 1).

Table 1. Growth rates according to description Hunt (2017).

Growth indicator	Formula	Units
Leaf area ratio	RAF= AF/BS	(cm ² g ⁻¹ of BS)
Specific leaf area	AFE= AF/BSAF	(cm ² g ⁻¹)

AF= leaf area of the plant; BS= total dry biomass; BSA= dry biomass of the leaf area.

Nutritional analysis

In the dry plant material, total nitrogen (N) was determined by the microKjeldahl method, phosphorus (P) and potassium (K) by means of wet digestion and plasma induction atomic emission spectroscopy using the methodology proposed by Alcántar and Sandoval (1999).

Statistical analysis

The data obtained from the evaluated variables were submitted to the Shapiro-Wilk normality test ($\alpha= 0.05$) and the Levene test for variance homogeneity. The variables of radical length, radical dry biomass, dry biomass of the aerial part, AFE, RAF, NO₃, K⁺, Ca²⁺ in cell extract and nutrient concentration (N, P, K), which met both tests, were applied analyzes of variance (Anova) and the Tukey mean comparison test ($\alpha= 0.05$).

The variables height, stem diameter, number of leaves and leaf area that did not comply with the tests of homogeneity of variance or normality, were transformed to natural logarithm (ln) until normality and homoscedasticity were observed, later the Anova was calculated and the Tukey mean comparison ($\alpha= 0.05$).

Data on root volume, dry leaf biomass did not meet both tests, so a Kruskal-Wallis non-parametric analysis and Wilcoxon's rank summation were performed, according to Siegel (2015) methodology. For all cases, we worked with the statistical program Statistical Analysis System (SAS) Version 9.4 (SAS, 2014).

Results and discussion

In the evaluated experiment, the analyzes of variance of the agronomic variables and those of nutritional analysis (Table 2) were highly significant ($p \leq 0.001$) between treatments and the low values of the coefficient of variation gave reliability of the analyzes.

Table 2. Statistical significance of treatments ($p < 0.05$) in agronomic variables and nutritional analysis of inoculated serrano pepper seedlings and different nutrient solution.

FV	LR	BSR	BSA	AFE	RAF	NO ₃	K	Ca	N	P	K
TREAT	**	**	**	**	**	**	**	**	**	*	**
CV (%)	19.38	46.86	27.75	14.52	15.5	17.97	8.73	22.72	9.98	9.02	37.85
R ²	0.167	0.106	0.483	0.261	0.275	0.838	0.861	0.808	0.729	0.951	0.584
MEAN	16.04	0.15	0.25	200.87	151.38	4278.13	367.8	40.56	45654.8	2911.1	23074

FV= source of variation; TREAT= treatment; CV= coefficient of variation; LR= radical length; BSR= radical dry biomass; BSA= total aerial dry biomass; AFE= specific leaf area; RAF= leaf area ratio; NO₃= nitrates in sap; K= potassium in sap; Ca= calcium in sap; N= nitrogen in seedling; P= phosphorus in seedling; K= potassium in seedling; * = significant ($p > 0.001 - p \leq 0.05$); ** = highly significant ($p \leq 0.001$).

Agronomic variables

The length and radical dry biomass, the dry biomass of the aerial part, AFE and RAF presented statistical differences between the treatments according to Tukey ($p < 0.05$) (Figure 1). Seedlings that developed in non-sterile substrate had a slight increase in root length, unlike those that grew in sterile substrate, however, in dry root biomass there are no differences between them.

Regarding the nutrition source, when tea is applied as a solution, there is an increase in the length and dry biomass, although the Tukey test indicates in some cases that there are no differences (Figure 1a). It is interesting to see that the height and root biomass in seedlings without sterile substrate and with tea have the highest values of length and dry radical biomass.

Favorable responses to organic sources have been recorded by David-Santoya *et al.* (2018) for habanero pepper (*Capsicum chinense* Jacquin) and with inoculants to increase the radical length of *C. annuum* with *Rhizobium etli*, and with *Trichoderma viride* by Vazallo *et al.* (2013). A greater root length in seedlings allows it to efficiently cope with transplantation and achieve rapid adaptation and absorption of water and nutrients (Vazallo *et al.*, 2013).

In the analysis of the aerial part, Figure 1b shows that the PSNESt and PSESt treatments registered the highest dry biomass (stem + leaves) of the seedlings, reaching 0.34 g and 0.33 g plant⁻¹, respectively. This means that in the aerial part of the seedling there is a response to the inoculation of *Pseudomonas tolaasii* and the application of Steiner nutrient solution regardless of the sterilization of the substrate.

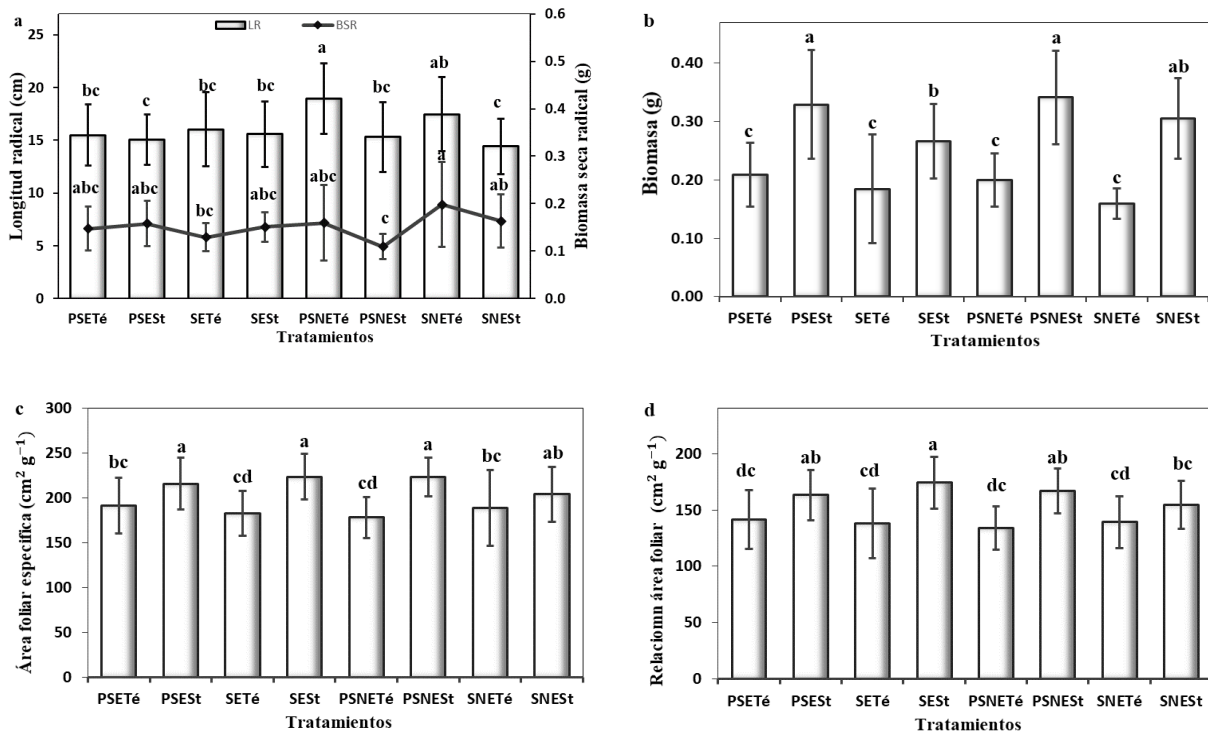


Figure 1. a) radical length and radical dry biomass; b) dry biomass of the aerial part; c) specific leaf area; and d) ratio of leaf area of serrano pepper seedlings inoculated with *P. tolaasii* and different nutritional solution. Bars with different letters are statistically different according to Tukey's mean test ($p < 0.05$). P= *Pseudomonas tolaasii*; S= substrate; E= sterilized; NE= Not sterilized; St= Steiner; Te= vermicompost tea.

This is attributed to the fact that *P. tolaasii* benefited from the absorption of available nutrients incorporated with Steiner solution (Angulo-Castro *et al.*, 2018) and to the improvement in root development when inoculated with rhizobacteria (Natarajan *et al.*, 2012). The inoculation of *P. tolaasii* to serrano pepper plants favors the development of the plant (increase in leaf area and fresh weight of foliage), although it is not reflected in an increase in fruit production (Ortiz *et al.*, 2016).

The biomass of the aerial part of the seedlings grown in non-sterile substrate (Figure 1b) and watered with Steiner solution was very similar to those inoculated with *Pseudomonas*; however, the root length and biomass were reduced (Figure 1a) and this has a lot to do with the adaptation of seedlings to transplantation. In the AFE (Figure 1c) and RAF (Figure 1d) indices, the incorporation of Steiner solution without inoculation increased the values, in sterilized substrates.

Similar results have been recorded in pepper seedlings (Luna *et al.*, 2013) and in jalapeño peppers (Angulo-Castro *et al.*, 2018), when they were inoculated with *Pseudomonas*. Amanullah *et al.*, 2007, indicate that high AFE values represent greater sheet thickness and a better balance between the potential capacity of photosynthesis and the cost of respiration.

These values were reached in seedlings where there was a sufficient supply of inorganic nutrients (St), which generates greater vigor of the plant, determined by the RAF, as well as a greater leaf thickness regulated by the AFE (Hunt, 2017), index that allows explaining up to 80% of the variation in growth rates (Villar *et al.*, 2004).

In the Figure 2a shows the means of the variables of seedling height and stem diameter, where inoculation generated higher height (20% more) and stem diameter (between 13 and 5% more) between the PSNESt and PSESt treatments, regarding their controls (SNESt and SESt). Tukey's test shows highly significant differences between these two treatments and the others. Similar results were obtained by Luna *et al.* (2013), with a significant increase in height and stem diameter of pepper seedlings when inoculating *Bacillus subtilis* up to 37%, compared to the control.

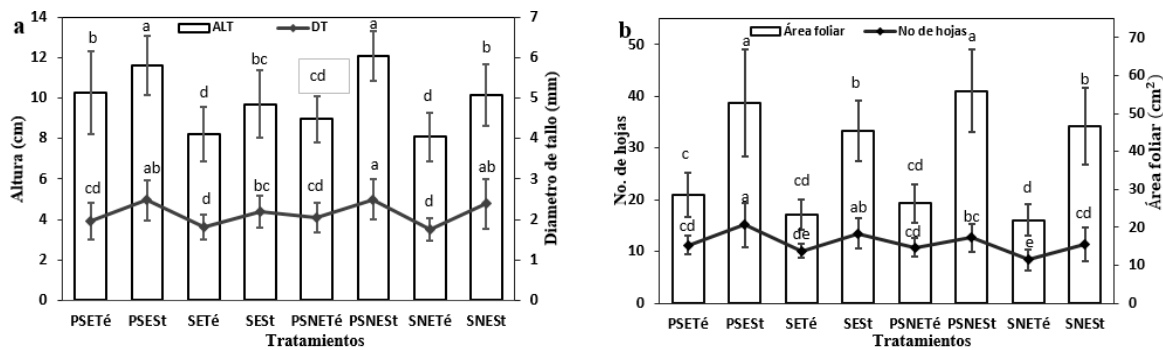


Figure 2. a) seedling height and stem diameter; and b) number of leaves and leaf area of inoculated serrano pepper seedlings and different nutrient solution. P= *Pseudomonas tolaasii*; S= substrate; E= sterilized; NE= not sterile; St= Steiner; Te= vermicompost tea.

Indolacetic acid (AIA) produced by the inoculated strain is the main phytohormone that induces plant growth, by increasing cell division and tissue differentiation (Bhattacharyya and Jha, 2012; Glick, 2012). In the case of poblano pepper, the increase in growth was 35% compared to the control due to the effect of inoculation with growth promoting bacteria, including *P. tolaasi* (Quiroz-Sarmiento *et al.*, 2019).

In the number of leaves and leaf area per seedling, the treatment inoculated with *P. tolaasii* and watered with Steiner nutrient solution in sterile substrate (PSESt) was the one that registered the highest number of leaves, up to 12% more than the control (SESt), as shown in Figure 2 b. In contrast, for the variable leaf area, the greatest leaf surface was observed (up to 20% more than the control), with the PSNESt treatment. The results of this variable indicate that the inoculation and the nutrient solution of Steiner at 25% supplies the balanced nutrients that are reflected in the increase in the number of leaves in all the seedlings that were watered with Steiner. Since rhizobacteria possibly provide growth benefits to plants when low amounts of chemical fertilizer are added to the soil that do not inhibit growth as established by Bhattacharyya and Jha (2012); Glick (2012).

Statistically significant differences in root volume were found according to the Kruskal-Wallis test, with the PSNETe treatment registering the highest volume with an average Wilcoxon range of 180.18 (data not shown), while the highest dry leaf biomass, was registered with the PSNESt treatment, with an average Wilcoxon range of 224.75 and the PSESt treatment with 216.72. Figure 3a shows the radical volume means, where it can be seen that the PSNETe treatment presented 3 cm³, surpassing 31% of its control (SNESt) and the SESt treatment obtained 2.2 cm³.

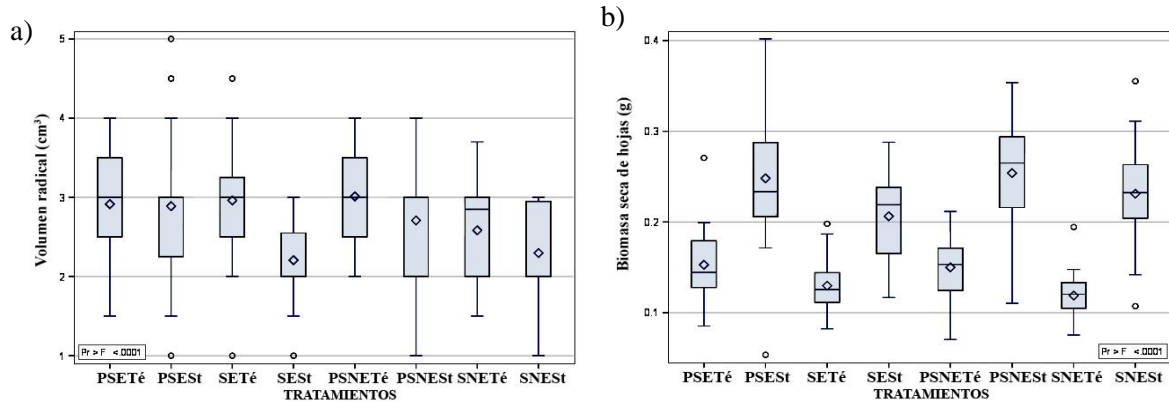


Figure 3. Box diagram contrasting: a) radical volume; and b) dry biomass of inoculated serrano pepper seedlings and different nutrient solution. P= *Pseudomonas tolaasii*; S= substrate; E= sterilized; NE= not sterile; St= Steiner; Te= vermicompost tea (Kruskal-Wallis non-parametric analysis).

Pérez *et al.* (2015) obtained increases in root volume of up to 64% in blackberry seedlings (*Rubus glaucus* L.) inoculated with *Pseudomonas migulae* compared to the treatment without inoculation. According to Amara *et al.* (2015) *Pseudomonas* is a genus of bacteria capable of producing and excreting auxins that are used by seedlings; the most investigated is indole-3-acetic acid, which is a phytohormone involved in stimulating radical growth (Glick, 2012).

Figure 3b shows that the highest dry biomass was presented in the PSNESt treatments with an average of 0.253 g plant⁻¹ and PSESt up to 0.248 g plant⁻¹. Parra-Terraza (2016) identified that the supply of NO₃⁻ in the nutrient solution increased the dry biomass of leaves; however, with the participation of *P. tolaasii* this agronomic variable is 20% and 9% higher than its SNESt and SESt controls, respectively.

Nutritional variables

Statistical differences were found in the concentration of nitrates, potassium and calcium in the cellular petiole extract of the chili seedlings ($p < 0.05$). Figure 4a shows that the seedlings grown on non-sterile substrate, inoculated and watered with tea, they presented the lowest nitrate values (1 450 ppm) The seedlings grown with sterile substrate did not show significant statistical differences between them.

Potassium in the cell extract (Figure 4b) presented the highest values in seedlings grown on non-sterile substrate and only in the sterile control (SESt), whereas Ca²⁺, increased its content in seedlings with non-sterile substrate and the inoculation of *P. tolaasii* regardless of the nutrient solution. Below these and statistically equal was the content in the non-sterile and sol-watered substrate plants, Steiner (Figure 4c).

The importance of knowing the chemical composition lies in the quality of the plant material of the seedlings, and the reserve of mineral nutrients, high concentrations of nitrogen and potassium, can influence the growth and survival of the seedlings (Villar *et al.*, 2004). Shabayev (2012) reported that inoculation with *Pseudomonas*, increases the absorption of nutrients by plants,

growth, nutritional absorption and excretion of the root system associated with the secretion of phenolic compounds that has, among other things, the mission of promoting microniche of the rhizobacterium.

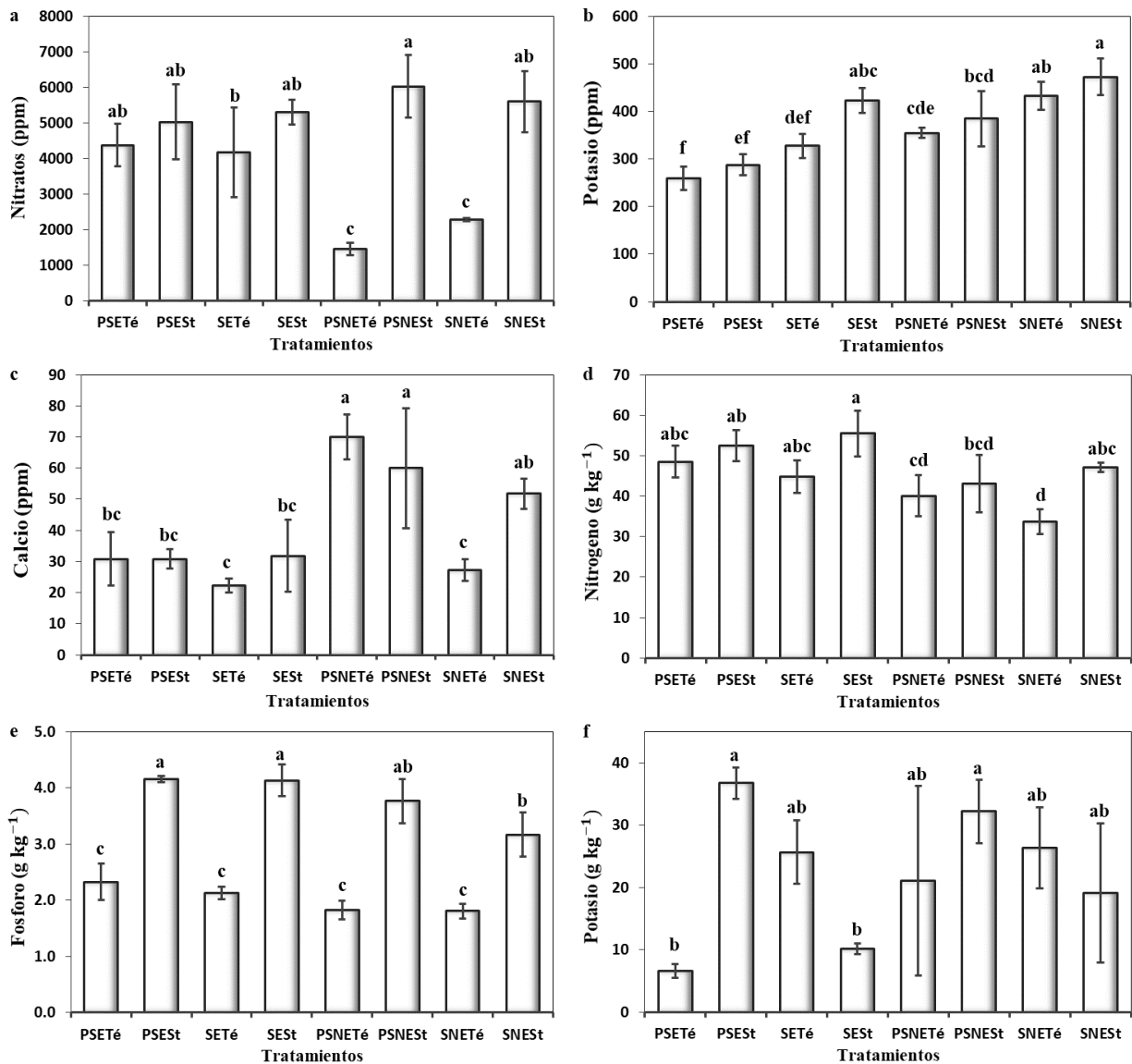


Figure 4. Nutritional variables: a) nitrates in sap; b) potassium in sap; c) calcium in sap; d) nitrogen; e) phosphorus; and f) potassium in inoculated serrano pepper seedlings and different nutrient solution. Columns with different letters are statistically different according to Tukey's mean test ($p < 0.05$). P= *Pseudomonas tolaasii*; S= substrate; NE= not sterile; St= Steiner; Te= vermicompost tea.

Moreno *et al.* (2018) indicated that the RPCV participate in the gradual release of nutrients and contribute to the reserve of organic N and P from the soil, reducing the losses of N by leaching and the fixation of P (nutrient cycle). In addition, to supply micronutrients to crops (Glick, 2012). In Figures 4d, e, f, the concentration of N, P and K in dry seedling biomass is shown, with highly significant differences between treatments ($p < 0.05$).

In the SESt treatment, it presented the highest concentration of N with an average of 55.5 g kg⁻¹; while the SNETe treatment registered the lowest concentration (33.6 g kg⁻¹). The other treatments were statistically similar. Regarding the concentration of P, the sterile substrate treatments with Steiner solution (PSESt and its control SESt) were better, which registered 4.15 and 4.13 g kg⁻¹ respectively.

Inoculation combined with Steiner’s nutrient solution (PSESt and PSNESt), increased the K concentration in dry biomass, surpassing the controls (SESt and SNESt) by 150% to 68%, on the other hand, the PSETe treatment registered the lowest concentration of K (6.56 g kg⁻¹), being 36% less than its control (SESt). Jones (2014) indicated that fertilization with Steiner solution improved seedling quality for transplanting, due to greater availability of nutrients.

While in the present investigation the supply of Steiner solution benefited the nutritive variables in seedlings and some agronomic variables. The proper balance and utilization of ions in the nutrient solution was achieved through the use of their universal nutrient solution formulas that improve the concentration of nutrients in plant matter (Steiner 1984).

In the Table 3 summarizes the variables under study on the effect of inoculation with *Pseudomonas tolaasii* that when combined with Steiner nutrient solution in non-sterile substrates had better results in agronomic variables such as height, stem diameter, leaf area and dry aerial biomass, noting that the combination of inoculation with vermicompost tea in non-sterile substrate influences with increasing length and radical volume.

Table 3. Response of serrano pepper seedlings to the inoculation of *Pseudomonas tolaasii* on the agronomic and nutritional variables.

<i>Pseudomonas tolaasii</i>		Substratum	Nutritive solution	Dry biomass																
				Height	Diameter stem	Longitude radical	Number leaves	Leaf area	Radical vol	Leaf	Area	Radical	NO ₃	K ⁺	Ca ⁺²	N	P	K	Leaf area ratio	Specific leaf area
With	Not sterile	Steiner	*	*			*	*	*	*	*	*	*			*		*		
		Tea			*		*						*					*		
	Sterile	Steiner	*	*		*		*	*							*	*	*		
		Tea																		
Without	Sterile	Steiner												*	*		*	*		
		Tea																		
	Not sterile	Steiner											*							
		Tea								*										

*= best factor response.

Not so when only vermicompost tea is applied, since it does not present any positive response, which means that the inoculation of *P. tolaasii* probably has positive effects when it interacts with other native or coinoculated microorganisms with different promoter capacity (substrate and tea) (Ahemad and Khan, 2012; Rojas *et al.*, 2001).

However, when only Steiner solution is applied, eliminating all the microorganisms, only the nutritional concentration benefits and influences the growth rates because the nutrition applied to the plants can affect the structure of the bacterial communities in the rhizosphere (Glick, 2012; Shabayev, 2012). And the colonization process is a decisive step in the interaction between bacteria and plants, the latter secreting nutrient-rich exudates that are used by bacteria in the first stages of recognition (Abaid-Ullah *et al.*, 2015).

The hypothesis that is presented in the present study is that the inoculation of *P. tolaasii* combined with vermicompost tea favors the agronomic and nutritional development of serrano pepper seedlings. Therefore, the objective of the present investigation was to quantify the effect of the inoculation of *Pseudomonas tolaasii* on the physiology and development of serrano pepper (*Capsicum annuum* L.) seedlings using different nutritional solutions.

Conclusions

The production of quality seedlings of serrano pepper occurs with the inoculation of *Pseudomonas tolaasii* and the application of a 25% Steiner solution as a nutritional source. The use of non-sterile substrate appears to promote seedling development with or without inoculation of the growth promoting bacteria. Vermicompost tea, despite being a source of nutrients, is not sufficient for obtaining well-developed seedlings, despite promoting root development.

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