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# Seed Microbiolization Associate With Nitrogen Doses Increase the Nutrition of Tomato Fruits

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**Abstract**

Endophytic bacteria can promote growth and improve the quality of plant production. The objective of this work was to evaluate the efficiency of inoculation of a mix of non-host endophytic bacteria isolates in the nutrition of tomato fruits cultivated, fertilized with rock powder and different doses of nitrogen. The experimental design was in randomized blocks, in a factorial arrangement (2 × 6 + 3), with four replications. The treatments consisted of two methods of inoculation of the mix of endophytic bacteria: seed microbiolization and post-emergence inoculation; six doses of nitrogen fertilization: 0, 129, 258, 387, 516 and 645 kg $ha^{-1}$; and 3 controls (without inoculation of the bacterial mix). The average export of macro and micronutrients in tomato fruits was, in descending order: K > N > P > Ca > Mg > S (averages of 1.33; 0.46; 0.07; 0.06; 0.05 and 0.05 g $plant^{-1}$, respectively) and Mn > Cu > Fe > Zn (means of 2.67; 1.98; 1.71 and 0.79 mg $plant^{-1}$, respectively). The inoculation method by seed microbiolization associated with nitrogen doses promoted significant increment in the dry mass of the fruits and in the content of the nutrients P, Ca, Zn, Fe and Mn.

## 1. Introduction

Tomato is one of the most important vegetable species in the world, both from an economic and social point of view, due to the value of production and the generation of direct and indirect jobs; and health, due to the nutraceutical benefits. World tomato production in the year 2019 was 181 million tons. Brazil, which is the 10th largest producer, represents about 2.2% of total production (FAO, 2020).

It is currently the most consumed vegetable in the country (IBGE, 2020), and also one of the most demanding in nutritional terms. About 91% of the minerals accumulated in tomato fruits are made up of potassium (K), nitrogen (N) and phosphorus (P). In highly weathered soils, N and P are the main limiting nutrients for increasing the productivity and nutritional quality of crops (Baldotto et al., 2012).

It is currently the most consumed vegetable in the country and also one of the most demanding in nutritional terms. About 91% of the minerals accumulated in tomato fruits are made up of potassium (K), nitrogen (N) and phosphorus (P) (Purquerio et al., 2016). In highly weathered soils, N and P are the main limiting nutrients for increasing the productivity and nutritional quality of crops (Baldotto et al., 2012).

In recent years, the use of bioinoculants capable of promoting growth and increasing agricultural quality and production has been described by several authors. Among the microorganisms that promote plant growth, endophytic bacteria have shown enormous potential in improving nutritional quality, including tomato fruits (Tommonaro et al., 2021).

Endophytic bacteria are beneficial organisms that colonize the interior of healthy plant tissues, without causing symptoms or apparent damage to them. They act in the promotion of plant growth through the production of growth regulators, antagonistic effect to pathogens with the production of antibiotics and in the increment of mineral nutrition, through the fixation, solubilization and availability of nutrients (Rosenblueth & Martínez-Romero, 2006).

Despite the many known benefits, there is still much to be studied, mainly in agricultural crops under stressful climatic conditions, in the use of bacteria selected from another species, as well as on the most efficient inoculation methods and their interaction with conventional fertilization. Therefore, the objective of this work was to evaluate the inoculation efficiency of a mix of non-host endophytic bacteria isolates in the nutrition of tomato fruits, fertilized with rock powder and different doses of nitrogen.

## 2. Method

The work was carried out in a greenhouse at the State University of Montes Claros (UNIMONTES), Janaúba, MG, from August 2014 to February 2015. Located at the geographic coordinates: (-15°48′09″S, -43°18′32″W, 533 m altitude).

The experimental design was in randomized blocks, in a factorial arrangement (2 × 6 + 3), with four replications and one plant per plot. The treatments consisted of two methods of inoculation of the mix of endophytic bacteria (IMB): seed microbiolization (SM) and post-emergence inoculation (PEI), six doses of nitrogen fertilization (DN): 0, 129, 258, 387 , 516 and 645 kg ha$^{-1}$, and three controls (without inoculation of the bacteria mix): (1) absence of nitrogen fertilization and application of rock dust—Natural phosphate of sedimentary origin—Itafós; (2) 100% nitrogen fertilization and application of rock dust; and (3) 100% nitrogen fertilization and application of simple superphosphate (conventional control, based on the current model of chemical fertilization). Among all treatments, only the third control did not receive rock dust as a phosphorus source.

The hybrid tomato 'Dominador F1', salad type, from the Persimmon group, with indeterminate growth, high vigor, good leafing, firm fruits with excellent color and shape, high resistance to TYLCV (Geminivirus), cycle 120 days was used. The soil, classified as eutroferric red latosol, was collected at a depth of 0-20 cm and sterilized in an autoclave. Fertilization was performed according to soil analysis and crop recommendations (Ribeiro et al., 1999).

In the preparation of the mix, five isolates of endophytic bacteria selected from the banana crop were used: EB:40 (*Bacillus* sp.—GenBank no.: GQ340516.1), EB:51 (*Bacillus pumilus*—GenBank no.: HQ218993.1), EB:53 (*Lysinibacillus* sp.—GenBank no.: JN215512.1), EB:144 (*Paenibacillus* sp.—GenBank no.: EF178460.1) and EB:194 (*Bacillus* sp.—GenBank no.: FJ405377.1) (Andrade et al., 2014). Each isolate was cultivated in liquid Tryptic Soy Broth (TSB) synthetic aerobic culture medium, from the inoculation of 100 µL of bacterial suspension in erlenmeyers containing 100 mL of the medium, under constant agitation at 120 rpm for 48 hours at 27 °C. Suspensions were prepared in 0.85% saline. The isolates were calibrated at 0.4 absorbance and diluted to $10^6$ to adjust to $1 \times 10^9$ CFU mL$^{-1}$.

The seeds were disinfected and dried in a laminar flow chamber on sterile filter paper for two hours. At SM, the disinfected seeds were immersed in bacterial suspensions and kept in an automatic shaker for one hour, at 150 rpm, 27 °C. After microbiolization, the seeds were kept for two hours in trays with filter paper and then sown in polystyrene trays, with 128 cells (40 cm$^3$), containing commercial substrate (Bioplant-HT®), sterilized in an autoclave.

In PEI, the disinfected seeds were immersed in sterile water and kept in an automatic shaker for one hour, at 150 rpm, 27 °C. Subsequently, they were kept for two hours to dry on filter paper and seeded according to the methodology described above. Seven inoculations were performed: the first, 10 days after seedling emergence and the following at 15, 30, 45, 60, 75 and 90 days after transplanting. In the first inoculation, 2.6 mL of the mix was applied per cell of the tray, while in the inoculations carried out after transplanting, 100 mL of the mix was applied per pot.

Transplanting into a plastic pot with 8 $dm^3$ of previously sterilized soil was carried out 40 days after sowing. The harvest started at 61 days after transplanting, and lasted 71 days, being carried out from the physiological maturity, greenish color with pink tip.

The data of temperature and relative humidity of the air were monitored daily in Thermo-Hygro-Anemometer-Luxmeter Portable Digital Model THAL-300. The dry mass of the fruits and the nutrient content of the fruits were evaluated. This being estimated based on the contents of the elements and the production of dry mass. To obtain the contents, the samples were digested wet, using nitro-perchloric digestion (Sarruge & Haag, 1974). The P content was determined by spectrophotometry by the ammonium vanado-molybdate method, and the K, Ca, Mg, Fe, Mn, Zn and Cu contents by atomic absorption spectrophotometry. N contents were determined by Micro-Kjeldahl (Sarruge & Haag, 1974).

The data were submitted to analysis of variance and, when the "F" test was significant, the N doses were submitted to the regression study, and the inoculation methods were compared by the F test, excluding the controls. In order to compare the controls, in relation to each nitrogen dose and inoculation method, the Dunnet test ($p < 0.05$) was used, using the SAS GLM procedure (SAS Institute Inc., 2004).

## 3. Results and Discussion

The use of a mix of endophytic, non-host (exotic) bacteria showed a positive association with the tomato crop Dominator F1, fertilized with rock powder and different doses of nitrogen. A significant interaction was observed between Inoculation Methods (IM) and Nitrogen Doses (DN) for fruit dry mass (FDM) (Figure 1) and the nutritional contents of Ca, Zn, Fe and Mn (Figures 2, 3, 4 and 5). In isolation, the IM showed a significant difference only for the phosphorus content in the fruits (Table 1). DN promoted linear increases in the nutritional contents of N, P, K, Mg, S and Cu (Figures 6, 7 and 8).

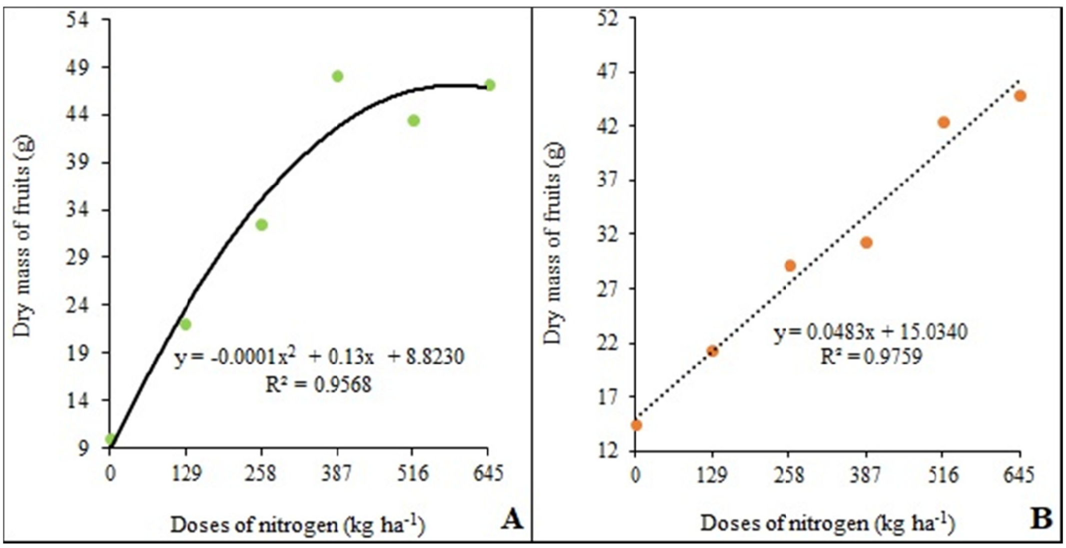


Figure 1. Dry mass of "Dominador F1" tomato fruits as a function of different doses of nitrogen (DN) in a greenhouse. (A) microbiolized seeds; (B) post emergence inoculation

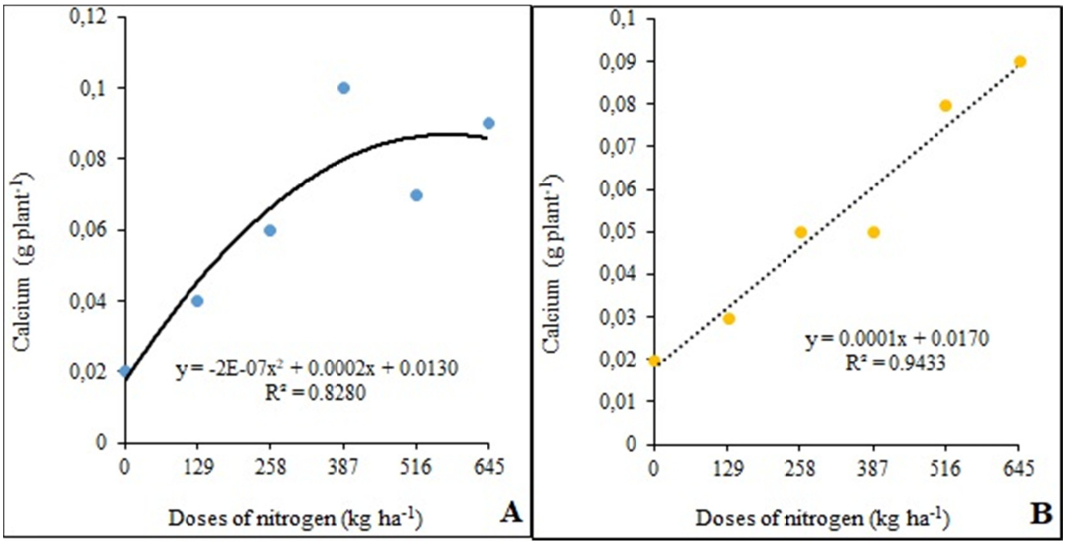


Figure 2. Nutritional calcium content in “Dominador F1” tomato fruits, as a function of different nitrogen doses, in a greenhouse. (A) microbiolized seeds; (B) post emergence inoculation

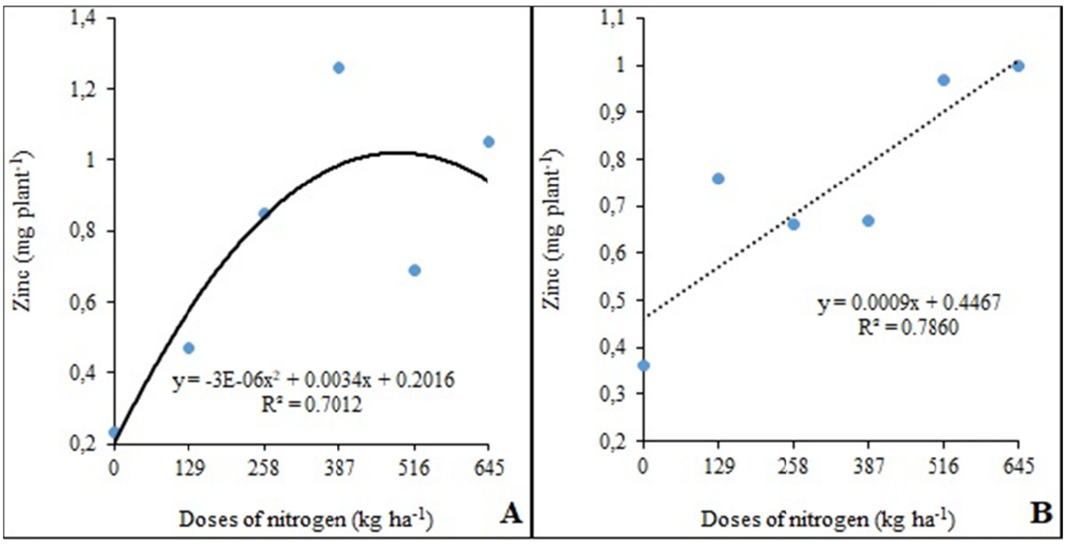


Figure 3. Nutritional zinc content in “Dominador F1” tomato fruits, as a function of different nitrogen doses, in a greenhouse. (A) microbiolized seeds; (B) post emergence inoculation

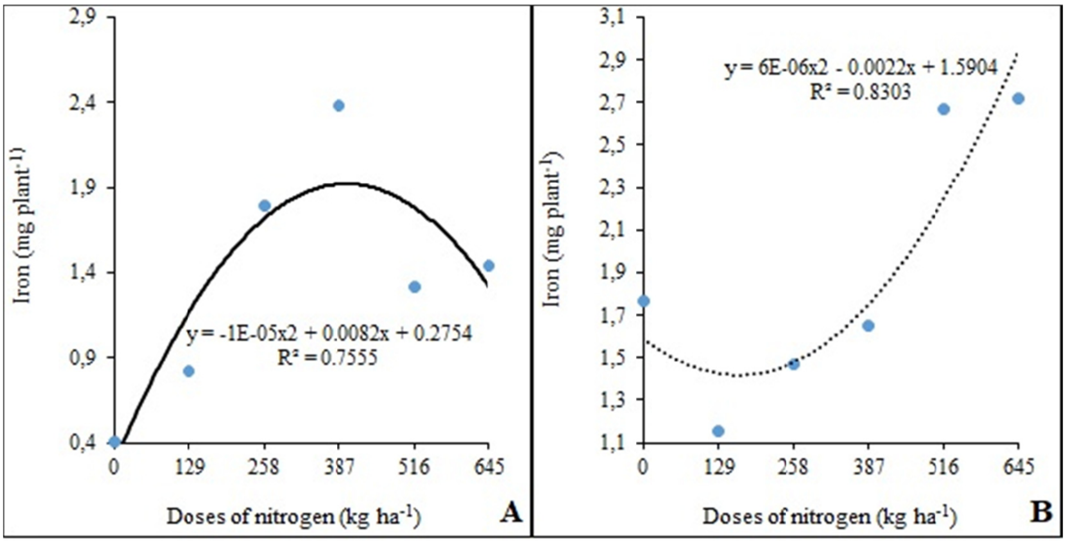


Figure 4. Nutritional iron content in "Dominador F1" tomato fruits, as a function of different nitrogen doses, in a greenhouse. (A) microbiolized seeds; (B) post emergence inoculation

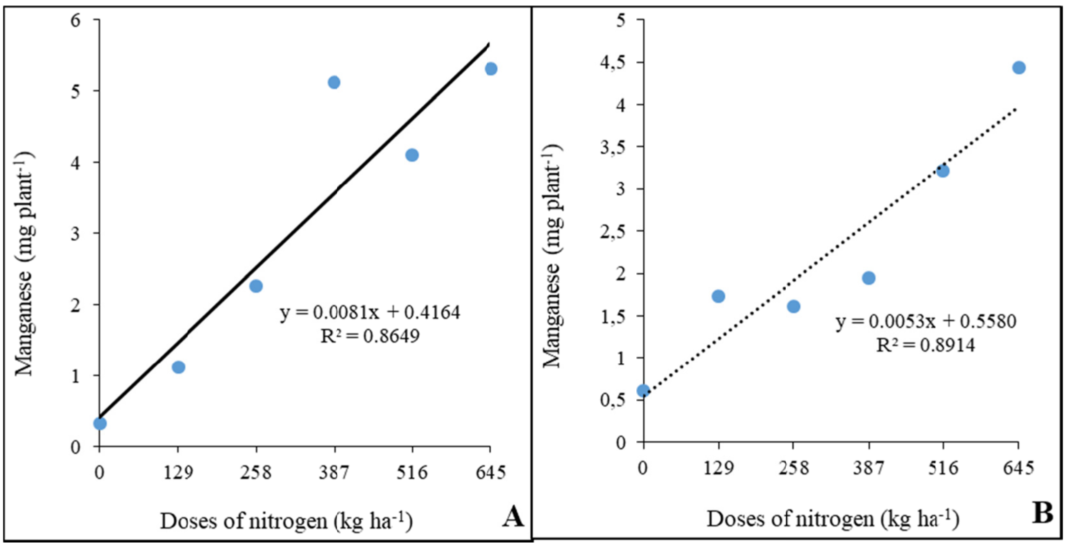


Figure 5. Nutritional manganese content in "Dominador F1" tomato fruits, as a function of different nitrogen doses, in a greenhouse. (A) microbiolized seeds; (B) post emergence inoculation

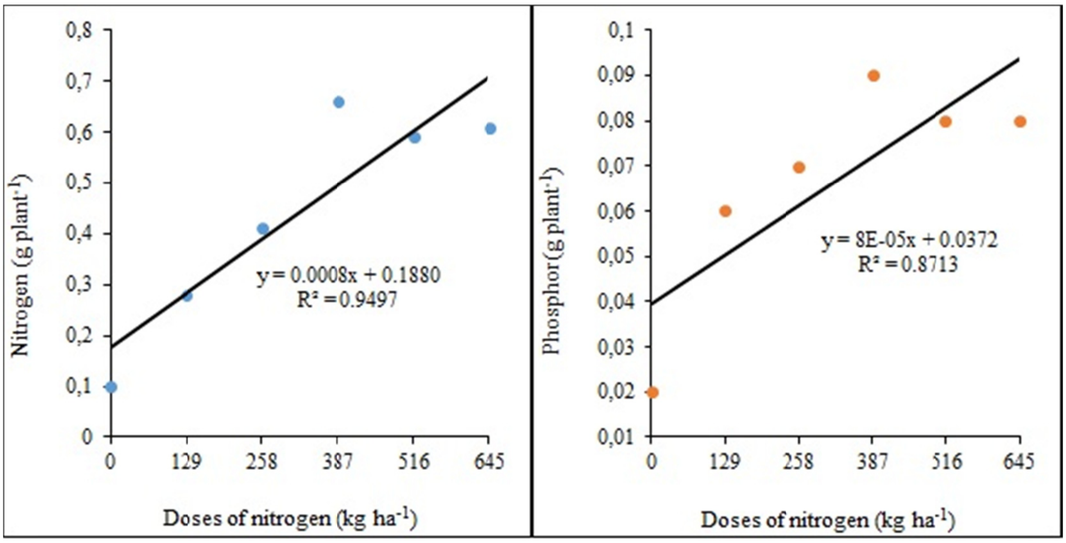


Figure 6. Nutritional content of nitrogen and phosphorus in "Dominador F1" tomato fruits, as a function of different nitrogen doses, in a greenhouse

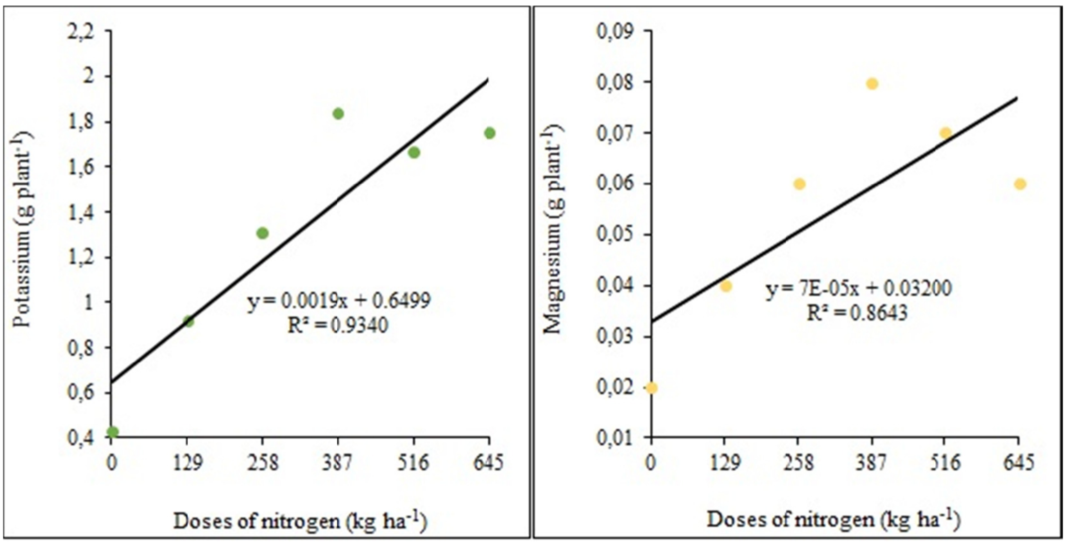


Figure 7. Nutritional content of potassium and magnesium in "Dominador F1" tomato fruits, as a function of different nitrogen doses, in a greenhouse

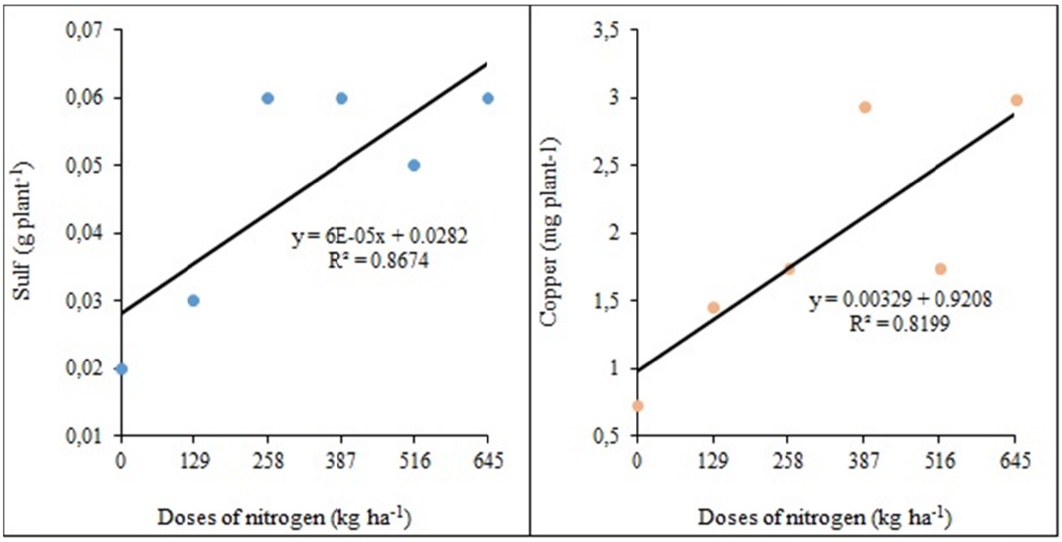


Figure 8. Nutritional content of sulfur and copper in "Dominador F1" tomato fruits, as a function of different nitrogen doses, in a greenhouse

Table 1. Analysis of the unfolding of inoculation method (IM) within each nitrogen dose level (DN) for fruit dry matter (FDM) and nutritional contents of calcium (Ca), zinc (Zn), iron (Fe) and manganese (Mn), and mean values of phosphorus (P) content submitted to different inoculation methods in "Dominador F1" tomato fruits

| Variable | MI | Nitrogen doses | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 129 | 258 | 387 | 516 | 645 |
| | | kg ha$^{-1}$ | | | | | |
| FDM (g) | SM | 9.97 a | 22.13 a | 32.56 a | 48.18 a | 43.39 a | 47.15 a |
| | PEI | 14.42 a | 21.35 a | 29.15 a | 31.31 b | 42.44 a | 44.92 a |
| Ca (g) | SM | 0.02 a | 0.04 a | 0.06 a | 0.10 a | 0.07 a | 0.10 a |
| | PEI | 0.02 a | 0.03 a | 0.05 a | 0.05 b | 0.08 a | 0.09 a |
| Zn (mg) | SM | 0.23 a | 0.47 a | 0.66 a | 1.26 a | 0.69 a | 1.05 a |
| | PEI | 0.36 a | 0.76 a | 0.85 a | 0.67 b | 0.97 a | 1.06 a |
| Fe (mg) | SM | 0.41 b | 0.82 a | 1.79 a | 2.38 a | 1.32 b | 1.44 b |
| | PEI | 1.77 a | 1.15 a | 1.46 a | 1.65 b | 2.67 a | 2.72 a |
| Mn (mg) | SM | 0.32 a | 1.12 a | 2.26 a | 5.12 a | 4.10 a | 5.31 a |
| | PEI | 0.61 a | 1.74 a | 1.62 a | 1.95 b | 3.22 a | 4.43 a |
| P (g) | SM | 0.07 a | | | | | |
| | PEI | 0.06 b | | | | | |

*Note*. Considering the same cause of variation, different letters in the column differ from each other by the F test at 5% significance. IM: inoculation methods; DN: nitrogen doses; SM: seed microbiolization; PEI: post-emergence inoculation ands: standard error.

The relationship between host and endophyte is a complex and interdependent process, that is, both need to act in the process of recognition and interaction. The root system of plants has the ability to produce secondary metabolites that are secreted by the roots and are known as root exudates. They act as messengers that communicate and initiate biological and physical interactions between roots and soil organisms, and may play symbiotic or defensive roles (Walker et al., 2003). In turn, microorganisms also secrete biomolecules to recognize and stimulate the process of interaction with the host (Ahemad & Kibret, 2014).

The application of endophytic bacteria in non-host plants is important because it allows the use of several isolates with the potential to produce phytohormones and provide nutrients, present in another species, on crops of agronomic interest (Souza et al., 2017). Therefore, it is an alternative for the development of sustainable agriculture, reducing the use of pesticides and chemical fertilizers (Souza et al., 2017).

Increases in DN resulted in gains in FDM in both IM (Figure 1). However, the highest averages were observed for seed microbiolization (SM), which presented a quadratic effect for this variable, with a maximum point at 51.1 g, estimated at a dose of 650 kg $ha^{-1}$ of N. linear effect with an increment of 0.0483 g for each kilogram of N added. Halo et al. (2020) when evaluating the use of an endophytic, non-host fungus obtained from a plant in the desert observed, among multiple advantages, gains of 27% in the increase in weight of tomato fruit grown under water stress.

SM also showed a quadratic effect for Ca, Zn and Fe contents, as a function of DN. The estimated doses as a function of the maximum points for these nutrients were 500; 486 and 410 kg $ha^{-1}$ of N, respectively. Only the Mn content showed linear adjustment for both inoculation methods. Even so, the increments from the SM were higher than those from the PEI. For each kilogram of N added to the soil, there was an increment of 0.0081 and 0.0053 mg of Mn for these methods, respectively. These results suggest that SM may result in higher yield and nutritional quality of tomato fruits. According to Joe et al. (2012) and Souza et al. (2017) the inoculation of endophytic bacteria by the seed microbilization method is the most efficient, besides being economical, safe, reliable and fast (Hallmann et al., 1997).

In the study of the IM splitting within each DN level it can be observed that at the dose of 387 kg $ha^{-1}$ of N, regardless of the variable, the SM presented higher, significant means, in comparison with the PEI. The gains were of the order of 65; 50; 47; 31 and 62 % for FDM and for the nutritional contents of Ca, Zn, Fe and Mn, respectively. The higher nutritional contents may be related to the release of siderophores, responsible for the chelation of Fe and Mn, and compounds that solubilize phosphate and zinc by the bacterial mix, thus improving the absorption of these nutrients by plants (Firdous et al., 2019; Zecchin & Mógor, 2017). Other studies, in tomato cultivation, also emphasize the efficiency of endophytic bacteria in the supply of macro and micronutrients (Barretti et al., 2008) and in improving the quality of the vegetable in a greenhouse (Mena-Violante & Olalde-Portugal, 2007).

The IM, independently of the DN, influenced the phosphorus content of the tomato fruit. Plants resulting from SM showed fruits with higher P content compared to those that received PEI. This result suggests that the mix of endophytic bacteria has the potential to solubilize low-solubility phosphate forms. This is probably due to the production of organic acids that reduce soil pH by increasing phosphate solubilization (He et al., 1996).

In the comparison between treatments and controls (control treatments) (Table 2). The conventional control (SI/100%N/SS), fertilized with simple superphosphate, presented, for all variables, means statistically superior to the other treatments inoculated with the mix of bacteria and fertilized with rock dust. Possibly, the solubilization of phosphorus by the bacteria occurred at insufficient levels for adequate plant nutrition. Needing, therefore, a nutritional complementation of this nutrient. For, according to Turan et al. (2007), the use of phosphate solubilizing bacteria, *Bacillus* FS-3, can convert approximately 20% of unavailable phosphorus into labile forms during the tomato production cycle.

Table 2. Fruit dry matter (FDM) and nutrient content in tomato plants submitted to different methods of inoculation with a mix of endophytic bacteria and nitrogen doses in a greenhouse

| Trataments | MSFR | N | P | K | Ca | Mg | S | Zn | Cu | Fe | Mn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | g plant$^{-1}$ | | | | | | | mg plant$^{-1}$ | | | |
| SM+0%N+PR | 9.97$^{BC}$ | 0.10$^{BC}$ | 0.02$^{BC}$ | 0.43$^{BC}$ | 0.02$^{BC}$ | 0.02$^{BC}$ | 0.02$^{BC}$ | 0.23$^{BC}$ | 0.73$^{C}$ | 0.41$^{BC}$ | 0.32$^{BC}$ |
| SM+20%N+PR | 22.1$^{BC}$ | 0.28$^{BC}$ | 0.06$^{AC}$ | 0.92$^{BC}$ | 0.04$^{BC}$ | 0.04$^{BC}$ | 0.03$^{C}$ | 0.47$^{BC}$ | 1.46$^{C}$ | 0.82$^{BC}$ | 1.12$^{BC}$ |
| SM+40%N+PR | 32.6$^{AC}$ | 0.41$^{AC}$ | 0.07$^{AC}$ | 1.31$^{AC}$ | 0.06$^{ABC}$ | 0.06$^{AC}$ | 0.06$^{AC}$ | 0.85$^{AC}$ | 1.74$^{AC}$ | 1.79$^{C}$ | 2.26 |
| SM+60%N+PR | 48.2$^{AC}$ | 0.66$^{AC}$ | 0.09$^{AC}$ | 1.84$^{AC}$ | 0.10$^{AC}$ | 0.08$^{A}$ | 0.06$^{AC}$ | 1.26$^{A}$ | 2.94$^{AC}$ | 2.38$^{A}$ | 5.12$^{A}$ |
| SM+80%N+PR | 43.4$^{AC}$ | 0.59$^{AC}$ | 0.08$^{AC}$ | 1.67$^{AC}$ | 0.07$^{AC}$ | 0.07$^{A}$ | 0.05$^{AC}$ | 0.69$^{C}$ | 1.74$^{AC}$ | 1.32$^{C}$ | 4.10$^{A}$ |
| SM+100%N+PR | 47.2$^{AC}$ | 0.61$^{AC}$ | 0.08$^{AC}$ | 1.75$^{AC}$ | 0.09$^{AC}$ | 0.06$^{AC}$ | 0.06$^{AC}$ | 1.05$^{A}$ | 2.99$^{ABC}$ | 1.44$^{C}$ | 5.31$^{A}$ |
| PEI+0%N+PR | 14.4$^{BC}$ | 0.18$^{BC}$ | 0.03$^{BC}$ | 0.59$^{BC}$ | 0.02$^{BC}$ | 0.03$^{BC}$ | 0.02$^{BC}$ | 0.36$^{BC}$ | 0.45$^{BC}$ | 1.77$^{C}$ | 0.61$^{BC}$ |
| PEI+20%N+PR | 21.4$^{BC}$ | 0.32$^{C}$ | 0.05$^{BC}$ | 0.92$^{BC}$ | 0.03$^{BC}$ | 0.04$^{BC}$ | 0.04$^{C}$ | 0.76$^{AC}$ | 1.36$^{C}$ | 1.15$^{BC}$ | 1.74$^{BC}$ |
| PEI+40%N+PR | 29.2$^{ABC}$ | 0.40$^{AC}$ | 0.05$^{BC}$ | 1.21$^{AC}$ | 0.05$^{BC}$ | 0.05$^{C}$ | 0.04$^{C}$ | 0.66$^{BC}$ | 1.90$^{AC}$ | 1.46$^{C}$ | 1.62$^{BC}$ |
| PEI+60%N+PR | 31.3$^{ABC}$ | 0.44$^{AC}$ | 0.06$^{AC}$ | 1.27$^{AC}$ | 0.05$^{BC}$ | 0.05$^{AC}$ | 0.05$^{C}$ | 0.67$^{BC}$ | 2.17$^{AC}$ | 1.65$^{C}$ | 1.95$^{C}$ |
| PEI+80%N+PR | 42.4$^{AC}$ | 0.64$^{AC}$ | 0.07$^{AC}$ | 1.62$^{AC}$ | 0.08$^{AC}$ | 0.07$^{A}$ | 0.06$^{AC}$ | 0.97$^{AC}$ | 2.46$^{AC}$ | 2.67$^{A}$ | 3.22$^{A}$ |
| PEI+100%N+PR | 44.9$^{AC}$ | 0.67$^{AC}$ | 0.08$^{AC}$ | 1.77$^{AC}$ | 0.09$^{AC}$ | 0.07$^{A}$ | 0.06$^{AC}$ | 1.06$^{A}$ | 2.30$^{AC}$ | 2.72$^{A}$ | 4.43$^{A}$ |
| *Controls* | | | | | | | | | | | |
| 1-SI+0%N+PR | 12.5$^{BC}$ | 0.14$^{BC}$ | 0.03$^{BC}$ | 0.50$^{BC}$ | 0.02$^{BC}$ | 0.02$^{BC}$ | 0.02$^{BC}$ | 0.27$^{BC}$ | 0.43$^{BC}$ | 1.20$^{C}$ | 0.66$^{BC}$ |
| 2-SI+100%N+PR | 46.4$^{AC}$ | 0.55$^{AC}$ | 0.08$^{AC}$ | 1.73$^{AC}$ | 0.09$^{AC}$ | 0.07$^{A}$ | 0.06$^{AC}$ | 1.14$^{A}$ | 1.78$^{AC}$ | 2.15 | 3.58$^{A}$ |
| 3-SI+100%N+SS | 65.6$^{AB}$ | 0.94$^{AB}$ | 0.24$^{AB}$ | 2. 49$^{AB}$ | 0.14$^{AB}$ | 0.09$^{A}$ | 0.11$^{AB}$ | 1.48$^{A}$ | 5.19$^{AB}$ | 2.80$^{A}$ | 4.00$^{A}$ |
| CV (%) | 20.4 | 23.5 | 16.9 | 19.7 | 23.3 | 25.7 | 25.7 | 27.5 | 29.2 | 27.7 | 31.4 |
| Means | 34.1 | 0.46 | 0.07 | 1.33 | 0.06 | 0.05 | 0.05 | 0.79 | 1.98 | 1.71 | 2.67 |

*Note*. Means followed by the letter (A) (B) and (C) differ from the controls 1-(SI/0%N/PR), 2-(SI/100%N/PR) and 3-(SI/100%N/SS), respectively, at a 5% significance level by Dunnett's test. SM: seed microbiolization; PEI: post-emergence inoculation; SI: without inoculation of the endophytic bacteria mix; PR: rock dust; SS: single superphosphate.

In the average nutrient export by tomato, the fruits accumulated, preferentially, in descending order: K > N > P > Ca > Mg > S (averages of 1.33; 0.46; 0.07; 0.06; 0.05 and 0.05 g plant-$^{1}$, respectively) and Mn > Cu > Fe > Zn (with means of 2.67; 1.98; 1.71 and 0.79 mg plant$^{-1}$, respectively) (Table 2). Similar results of the order of preferential accumulation of macronutrients were observed by (Purquerio et al., 2016), with divergence only between Mg and S.

Research that addresses the use of endophytic, non-host bacteria and their different inoculation methods, associated with the use of fertilizers in agricultural crops, sensitive to stressful climatic conditions, is necessary to improve the use of resources and technologies in cropping systems, especially in cost reduction. The information obtained in this work can help researchers to understand the interactions between plant, endophyte and climate, where the best inoculation method and its relationship with the use of fertilizers was determined.

## 4. Conclusions

Endophytic, non-host bacteria present nutritional benefits in tomato crops even under heat stress conditions. The inoculation method by seed microbiolization associated with different doses of nitrogen in the tomato crop 'Dominador F1' promoted significant gains in the dry mass of the fruits and in the content of the nutrients P, Ca, Zn, Fe and Mn.

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**Authors Contributions**

MAS, SN, WFDM, JASN, GLOD, MCTP, AAX, MJM, MRM and CARM were responsible for study design and revising. IOS, LPS, NCN, TJPS, DSM, ZCS, JCF and JRV was responsible for data collection. MJM, MAS, RMA, GBO, HSNS, EAP, ALFSN, FCR, FCR and LKLM drafted the manuscript and MJM and MAS revised it. All authors read and approved the final manuscript.

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**Competing Interests**

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No additional data are available.