microbiology research

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Promotion of Peanut (*Arachis hypogaea* L.) Growth by Plant Growth-Promoting Microorganisms

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Abstract: Brazil is an important peanut producer, but despite its high production, there still needs to be an inoculant for the peanut crop. In addition, the use of microorganisms that promote plant growth (PGPM) is not common, and this crop is highly dependent on chemical fertilizers. An excellent alternative to reduce the use of fertilizers and chemical inputs in peanut crops while reducing the production cost and environmental impact is the use of PGPM. The objective of this study was to evaluate the effects of *Azospirillum brasilense*, *Bacillus subtilis*, *Bradyrhizobium japonicum*, and *Trichoderma harzianum* as single inoculants and co-inoculants on the growth promotion and productivity of peanuts in greenhouse and field conditions. In the greenhouse, the experiment was conducted with 12 treatments with six repetitions. In the field conditions, the experiment was conducted with five treatments with four repetitions. Both experiments were conducted in randomized blocks. In general, all the microorganisms evaluated in the present study promoted increases in root dry mass, shoot dry mass, phosphorus concentrations, and plant height in the greenhouse and under field conditions compared with the control. Interestingly, the mixtures of microorganisms inoculated in peanut plants did not promote greater plant growth and development compared with inoculations of the microorganisms separately. Specifically, in the field, the highest productivity was found for the inoculation of *B. japonicum* alone. The PGPM evaluated in the present study for peanut crops generally promoted some increases in productivity in greenhouse and field conditions.

Keywords: *Arachis hypogaea*; biological nitrogen fixation; acid phosphatase; phosphorus solubilization; urease

Citation: Frezarin, E.T.; Santos, C.H.B.; Sales, L.R.; dos Santos, R.M.; de Carvalho, L.A.L.; Rigobelo, E.C. Promotion of Peanut (*Arachis hypogaea* L.) Growth by Plant Growth-Promoting Microorganisms. *Microbiol. Res.* **2023**, *14*, 316–332. https://doi.org/10.3390/microbiolres14010025

Academic Editor: Giacomo Zara

Received: 19 January 2023
Revised: 10 February 2023
Accepted: 22 February 2023
Published: 24 February 2023

1. Introduction

The peanut (*Arachis hypogaea* L.) is an annual cycle herb native to South America [1,2] and belongs to the family Fabaceae, order Fabales. It is mainly used for human consumption, either by direct consumption or oil production. Its grains and bagasse in the form of cake generated in oil extraction are also used in animal feed [3].

Peanut crops have great economic and social importance in Brazil. According to data from the National Supply Company [4], it is estimated that the area planted with peanuts in Brazil, including the first and second crops 2022/23, will reach 192.3 thousand hectares, with a production expectation of 725.5 thousand tons. Of this total, the state of São Paulo contributes more than 90% of all national production [4]. In Ribeirão Preto, peanut is particularly important because, due to its short cycle, it is the main alternative crop in the crop rotation in sugarcane fields [5].

Although peanut is a profitable agricultural crop, there are many economic losses related to decreased productivity caused by biotic and abiotic stresses. The biotic factors that most affect peanut crops are insect pests or diseases caused by pathogenic microorganisms. Abiotic factors involve stresses caused by drought, salinity, extreme temperatures, or flooding [6].

Peanut crops require large amounts of fertilizers and pesticides for production. The consumption of some foods grown in fertilized fields treated with agrochemicals has been associated with human diseases [7]. Additionally, recent reports have shown that 52% of all fertile food-producing soils globally are now classified as degraded, which poses new challenges for growing crops. Global food production is expected to decline by 12% over the next 25 years [8].

Because peanut is a legume species, it is characterized by its association with nitrogen-fixing bacteria, which enables a more efficient absorption process for this nutrient [9]. Biological nitrogen fixation is promoted by microorganisms that possess an enzyme called nitrogenase. This enzyme is responsible for converting atmospheric nitrogen (N_2) into ammonia (NH_3), a form which the plant can absorb. Most of these microorganisms are free-living, while others form symbiotic associations with plants, providing fixed nitrogen in exchange for other nutrients and carbohydrates of the host plant [10].

Nitrogen is the element that most limits plant growth, followed by phosphorus [11].

Nitrogen actively participates in metabolic functions and is present in different plant compounds [12]. To meet its demand, urea has been the most commonly used chemical fertilizer in agriculture in recent years [13]. After its application, the urease present in the soil catalyzes the hydrolysis of urea, transforming it into CO_2 and NH_3 [14]. Soil ammonia is converted into ammonium ions and thus can be absorbed by plants, nitrified, or lost by volatilization [15].

Phosphorus is an essential macronutrient found in soil in organic and inorganic forms. It plays a crucial role in agricultural production; however, it has low availability in tropical soils [16]. Fertilization with this nutrient is a common practice in agricultural soils; however, much of the applied phosphorus becomes unavailable to plants because it is rapidly immobilized by iron and aluminum ions in acidic soils and calcium ions in alkaline soils [17]. Using phosphate-solubilizing microorganisms is an alternative approach to improve the availability of this nutrient to plants. These microorganisms have the ability to extract or solubilize phosphorus from insoluble fractions in the soil and poorly soluble natural inorganic phosphates (solubilization) and mineralize the organic matter, releasing the phosphorus content (mineralization) [18].

In general, PGPM is a group of microorganisms with several abilities related to plant growth. These abilities are phosphorus solubilization, phytohormone production, induced systemic resistance, nitrogen fixation, biocontrol, etc. These abilities can be offered to plants, promoting increased growth and yields. In this way, these microorganisms can reduce the amounts of fertilizers and pesticides in crop production. Using PGPM is a promising alternative with a low environmental impact that increases the efficiency of chemical fertilizers and increases productivity with reduced cost-benefit ratios [19,20]. These microorganisms promote growth through direct and indirect mechanisms [21]. The genera *Azospirillum*, *Bacillus*, *Bradyrhizobium*, and *Trichoderma* have several abilities related to plant growth [22–24].

The genus *Bradyrhizobium* comprises the so-called slow-growing rhizobia, which are microorganisms that have the ability to nodulate and fix atmospheric nitrogen in symbiosis with legumes [25] and in association with nonlegumes such as rice, maize, and pine [26–28].

Bradyrhizobium japonicum is a Gram-negative, rod-shaped, nitrogen-fixing symbiont. *Bradyrhizobium* has been widely used for molecular genetics, physiology, and ecology studies because of its superior symbiotic nitrogen fixation activity. This symbiotic relationship provides a safe environmental niche for bacteria with a constant carbon source provided by the plant [29].

There are many growth promotion strategies among bacteria of the genus *Bacillus*. They can control and produce phytohormones [30] and other metabolites that stimulate plant development. In addition, they enhance plant tolerance to biotic and abiotic stresses and prevent and inhibit infections caused by pathogens [31]. Microorganisms in the genus *Azospirillum* promote growth through different mechanisms, including the biosynthesis of phytohormones and biological nitrogen fixation [32], and are called facultative endophytic diazotrophs because they colonize both the interior and the surface of the roots [20], favoring root growth, which consequently increases the absorption of water and nutrients by the plant [21]. *Trichoderma* is a genus of filamentous fungi found both in association with roots and within plant tissues [33], which is widely used in agriculture due to its potential as a biocontrol agent, acting by antibiosis and mycoparasitism or by competition with nematodes and phytopathogens [34]. *T. harzianum* can produce auxins and other compounds that benefit the growth and development of plants [35]. However, there are few studies on inoculation, especially coinoculation in peanuts, and their effects on growth promotion in the crop [36].

The objective of this study was to evaluate the effects of *Azospirillum brasilense*, *Bacillus subtilis*, *Bradyrhizobium japonicum*, and *Trichoderma harzianum*, as single inoculants and co-inoculants on the growth promotion and productivity of peanuts in greenhouse and field conditions.

2. Materials and Methods

The experiment was initially conducted in a greenhouse at Universidade Estadual Paulista-UNESP–Campus de Jaboticabal under controlled water and temperature conditions. The cultivar used was IAC-503, developed by Instituto Agronômico de Campinas (IAC). This plant has an erect bearing with a cycle of 130 days and bushy leaves. In all, the experiment continued for 45 days, beginning with the sowing of the pots and ending at the beginning of the crop's flowering. The climate of the Jaboticabal region is Cwa, according to the Köppen classification, with humid summers and dry winters. The UNESP unit is located in the northwest region of São Paulo State (21°15′22″ S, 48°18′58″ W, and altitude 605 m). Subsequently, the study was also conducted under field conditions, with the treatments that showed better results in the first stage of the study (greenhouse conditions) in terms of height and dry matter. The agricultural area belongs to the municipality of Taiuva-SP (21°18′52″ S, 48°37′63″ W, and altitude 608 m), and the climate of this region is also Cwa type. For this trial, the cultivar IAC-OL3 was used, a variety developed by the Instituto Agronômico de Campinas (IAC) with creeping plants and bushy leaves, and the crops were grown in the field over a 120-day period.

2.1. Preparation of the Pots in the Greenhouse

Pots of 5 dm^3 filled with the soil of the latosol type were used. This soil was sieved and fertilized based on a previously performed soil chemical analysis. Following the recommendations of [37], however, with a 20% reduction in the fertilization required for the crop, the macronutrients calcium, sulfur, phosphorus, magnesium, and nitrogen were supplied, as well as the micronutrients boron, copper, molybdenum and zinc. After filling the pots with the fertilized soil, the peanuts were sown at a depth of 5 cm.

2.2. Experimental Design in the Field

Based on the data obtained in the greenhouse regarding shoot and root dry matter and plant height, five of the treatments mentioned above were selected and evaluated under field conditions. The plots were divided into randomized blocks with four replicates.

Plots of 15 m^2 (5 m $\times$ 3 m) with spacing between plants of 0.5 m $\times$ 0.2 m were determined. The property's soil is also Oxisol type, and the peanut sowing depth was the same as in the experiment in pots (5 cm). Micronutrients and primary macronutrients (N, P, and K) were supplied by mineral fertilization according to soil chemical analysis, but with a 20% reduction. The soil characteristics were as follows: pH 6.5; organic matter, 11 g dm^3; phosphorus, 20 mg dm^3; nitrogen 12 mg dm^3; potassium, 0.7 mmolc dm^3; magnesium, 17 mmolc dm^3; and the sum of bases, 24.4 mmolc dm^3. The secondary macronutrients (Ca = 60 g, Mg = 15 g, and S = 15 g) were supplied by liming and gypsum according to the recommendations of [38].

2.3. Inoculum Preparation and Applications

The strains used in the present study are from the collection of microorganisms of the Laboratory of Soil Microbiology (LSM) of the Faculdade de Ciências Agrárias e Veterinárias FCAV—Universidade Estadual Paulista—UNESP in Jaboticabal. The bacterium *B. subtilis* was isolated from maize plants (roots), identified through automatic sequencing and deposited in GenBank with the accession number MZ133755. The bacterium *A. brasilense* is from Embrapa *A. brasiliensis* strain F111 (accession number MZ133758) [39]. *B. japonicum* and *T. harzianum* were acquired from Embrapa, with the classifications NC 230 (SEMIA 6439) and CEN199, respectively [40,41].

The inoculants were prepared as follows. *B. subtilis* was prepared in nutrient broth, the fungus *T. harzianum* in malt extract, *A. brasilense* in DIG medium, and *B. japonicum* in YM medium. All the strains were grown in a microbiological chamber for 72 h and were applied at a final concentration of 1×10^8 colony-forming units (CFU) mL^{-1}. Except for *B. japonicum*, which was used only in the seed treatment (as recommended by Agriculture Brazilian Agency), the microorganisms were applied directly to the soil in a total of four applications in the greenhouse and a single application in the field. In the greenhouse, the first application was performed on the seventh day after sowing and subsequently every ten days; in the field, the application was performed on the thirtieth day after sowing. All the microorganisms were prepared separately. Each vase received 10 mL of inoculum when inoculating with a single microorganism. When the treatments received two microorganisms, each container received 5 mL of each microorganism. When the treatment received four microorganisms, each container received 2.5 mL from each microorganism.

2.4. Experimental Design in the Greenhouse

In the greenhouse, the pots were arranged in randomized blocks, with twelve treatments and six replicates. The treatments are listed in Table 1 below.

Table 1. Microbiological inoculants, including the control group used in the greenhouse. There were a total of twelve treatments, with six replicates each.

Treatments	Inoculants
1	Control (Without inoculant)
2	*Azospirillum brasilense*
3	*Bacillus subtilis*
4	*Bradyrhizobium japonicum*
5	*Trichoderma harzianum*
6	*A. brasilense* + *B. subtilis*
7	*A. brasilense* + *B. japonicum*
8	*A. brasilense* + *T. harzianum*
9	*B. subtilis* + *B. japonicum*
10	*B. subtilis* + *T. harzianum*
11	*B. japonicum* + *T. harzianum*
12	Consortium (*A. brasilense* + *B. subtilis* + *B. japonicum* + *T. harzianum*)

In the greenhouse conditions, the microorganisms were applied directly to the soil surface near the plant with a pipette. Under field conditions, the microorganisms were applied with an automatic sprayer. Table 1 shows the description of treatments.

2.5. Plant Height

Plant height (cm) was determined by measuring from the base of the plant to its apex using a graduated ruler. In the greenhouse, the height of all plants was determined; in the field, five plants were randomly chosen from each plot.

2.6. Dry Matter

Shoots and roots were collected at the end of both the greenhouse and field experiments (5 plants/plot chosen randomly). It was necessary to wash the roots in running water to remove the soil still present. All material was placed in an oven with forced air circulation at 65 °C for 72 h. After this period, the material was weighed on a semianalytical scale to determine root dry matter (RDM) and shoot dry matter (SDM).

2.7. Nitrogen and Phosphorus Concentration

Roots and shoots, after drying, were ground in a Wiley mill. The nitrogen concentration was determined by sulfuric digestion of the material and subsequent titration. To determine the phosphorus concentration, nitric-perchloric digestion of the material was performed, followed by spectrophotometry. Both analyses were performed according to [42].

2.8. Urease and Acid Phosphatase Enzymatic Activity

The enzymatic activity of acid phosphatase was determined according to [43]. The urease activity was determined according to [44]. Both experiments were performed using soil samples collected from the greenhouse experiment.

2.9. Productivity

At the end of the field experiment, the peanuts were harvested and weighed in their pods on a semianalytical scale to determine the total pod yield. The productivity in kg ha^{-1} relating to the production per unit area was also determined.

2.10. Analysis of Results

The data obtained were subjected to variance analysis with an F test using the program AgroEstat [45]. When there was significance, a comparison of means was performed using the Scott Knott test at 10% probability.

3. Results

Table 2 shows the selected inoculants previously evaluated in the greenhouse.

Table 2. Selected microbiological inoculants, including the control group used in the field. There were a total of five treatments, with four replicates each.

Treatment	Inoculant
1	Control-No inoculant
2	*Azospirillum brasilense*
3	*Bradyrhizobium japonicum*
4	*A. brasilense* + *B. japonicum*
5	*A. brasilense* + *Trichoderma harzianum*

3.1. Dry Matter of Plants

In the greenhouse, peanut plants treated with *Azospirillum brasilense* + *Bradyrhizobium japonicum* (T7) showed the highest average root dry matter (0.225 g), which was 32.44% higher than that obtained in the control group (0.152 g) (T1). The plants treated with *T. harzianum* (0.15 g) and *A. brasilense* (0.152 g) presented lower root development than treatment 7 (*A. brasilense* + *B. japonicum*), similar to all other treatments ($p > 0.05$) (Figure 1).

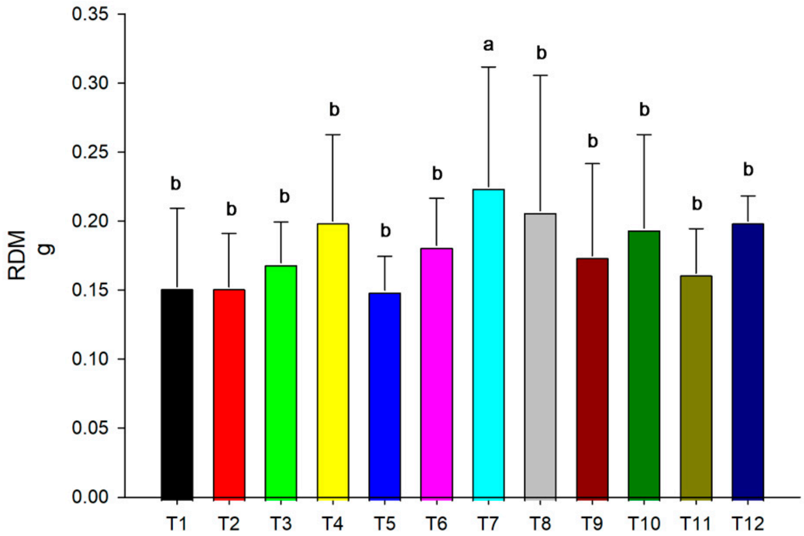

Figure 1. Average root dry matter (RDM) with standard deviation of peanut plants treated with different bacterial and fungal inoculants under greenhouse conditions. T1 = control; T2 = *Azospirillum brazilense*; T3 = *Bacillus subtilis*; T4 = *Bradyrhizobium japonicum*; T5 = *Trichoderma harzianum*; T6 = *A. brasilense* + *B. subtilis*; T7 = *A. brasilense* + *B. japonicum*; T8 = *A. brasilense* + *T. harzianum*; T9 = *B. subtilis* + *B. japonicum*; T10 = *B. subtilis* + *T. harzianum*; T11 = *B. japonicum* + *T. harzianum*; T12 = consortium (*A. brasilense* + *B. subtilis* + *B. japonicum* + *T. harzianum*). The means followed by the same letters do not differ by the Scott Knott test at 10% probability.

For the shoot dry matter, the treatment with *A. brasilense* + *B. japonicum* (T7) also presented the highest mean (2.57 g) ($p < 0.05$), a value 24.94% higher than that found in the control group (1.93 g). The other treatments did not differ from each other ($p < 0.05$) (Figure 2).

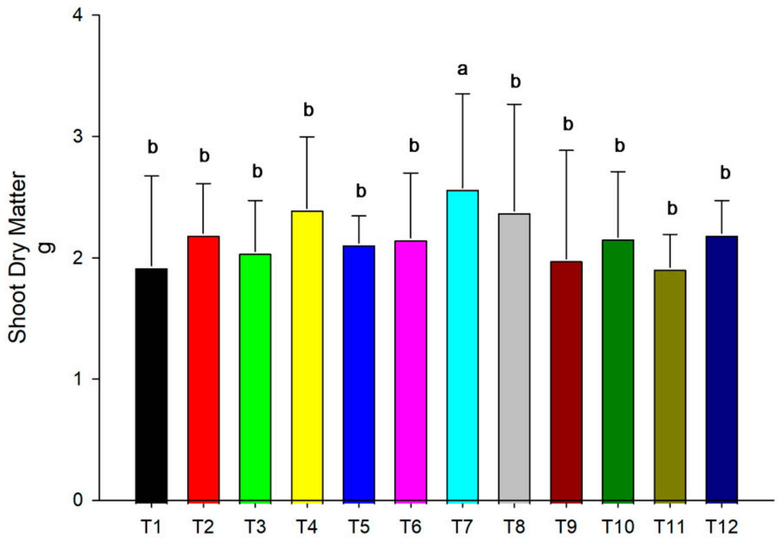

Figure 2. Average shoot dry matter (SDM) with a standard deviation in grams of peanut plants treated with different bacterial and fungal inoculants in a greenhouse. T1 = control; T2 = *Azospirillum brazilense*; T3 = *Bacillus subtilis*; T4 = *Bradyrhizobium japonicum*; T5 = *Trichoderma harzianum*; T6 = *A. brasilense* + *B. subtilis*; T7 = *A. brasilense* + *B. japonicum*; T8 = *A. brasilense* + *T. harzianum*; T9 = *B. subtilis* + *B. japonicum*; T10 = *B. subtilis* + *T. harzianum*; T11 = *B. japonicum* + *T. harzianum*; T12 = consortium (*A. brasilense* + *B. subtilis* + *B. japonicum* + *T. harzianum*). The means followed by the same letters do not differ by the Scott Knott test at 10% probability.

Among the treatments applied under field conditions, the mixture of *A. brasilense* + *T. harzianum* (T5) obtained the highest average shoot dry matter (30.34 g) ($p < 0.05$), which was 60.0% higher than that found in the control group (18.03 g). The other treatments did not differ from each other (Figure 3).

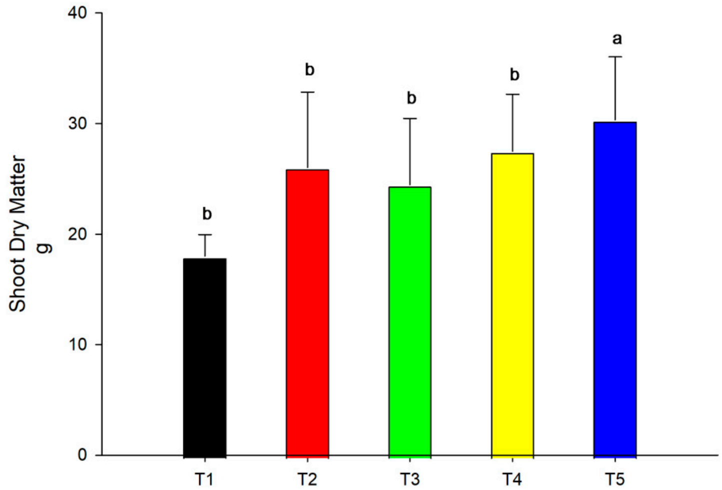

Figure 3. Average with standard deviation of shoot dry matter in grams of peanut plants treated with different bacterial and fungal inoculants under field conditions. T1 = control; T2 = *Azospirillum brazilense*; T3 = *Bradyrhizobium japonicum*; T4 = *A. brasilense* + *B. japonicum* and T5 = *A. brasilense* + *T. harzianum*. Means followed by the same letters did not differ by Duncan's test at 10% probability.

3.2. Height

Treatment 7 (*A. brasilense* + *B. japonicum*) had the highest average height among the treatments (46 cm) ($p < 0.05$); this value was 9.78% higher than that found in the plants of the control group (41.5 cm). There was no significant difference regarding the height of the peanut plants in other treatments (Figure 4).

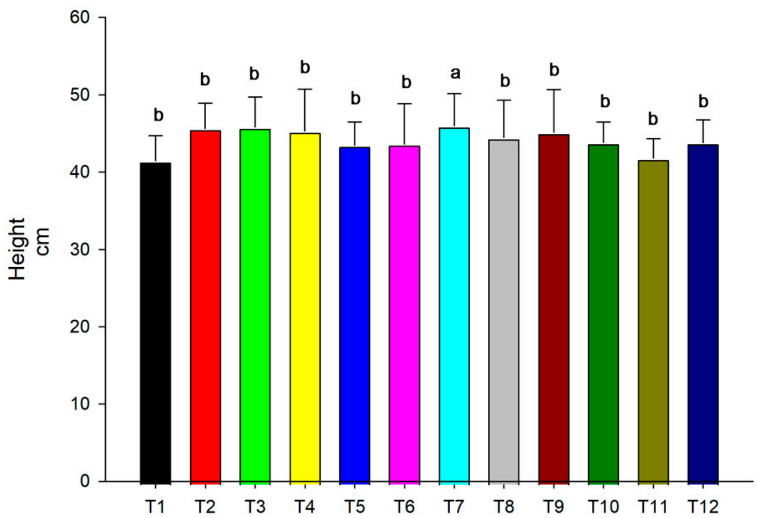

Figure 4. Average with standard deviation of height in centimeters of peanut plants treated with different bacterial and fungal inoculants in a greenhouse. T1 = control; T2 = *Azospirillum brazilense*; T3 = *Bacillus subtilis*; T4 = *Bradyrhizobium japonicum*; T5 = *Trichoderma harzianum*; T6 = *A. brasilense* + *B. subtilis*; T7 = *A. brasilense* + *B. japonicum*; T8= *A. brasilense* + *T. harzianum*; T9 = *B. subtilis* + *B. japonicum*; T10 = *B. subtilis* + *T. harzianum*; T11 = *B. japonicum* + *T. harzianum*; T12 = consortium (*A. brasilense* + *B. subtilis* + *B. japonicum* + *T. harzianum*). The means followed by the same letters do not differ by the Scott Knott test at 10% probability.

Under field conditions, peanut plants treated with *Azospirillum brasilense* + *Bradyrhizobium japonicum* (T4) showed the highest mean height (68.42 cm) ($p < 0.05$), which was 9.38% higher than that obtained by the control group (62 cm). The other treatments did not differ from each other ($p > 0.05$) (Figure 5).

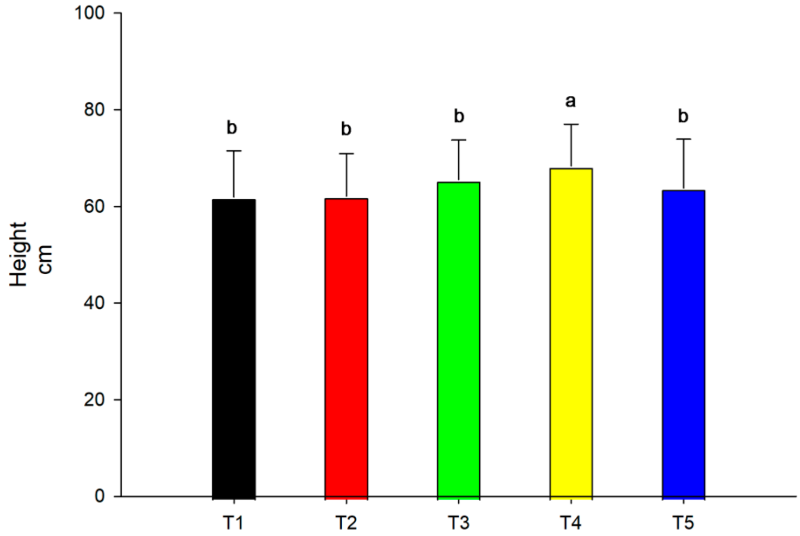

Figure 5. Average with standard deviation of height in centimeters of peanut plants treated with different bacterial and fungal inoculants in the field. T1 = control; T2 = *Azospirillum brazilense*; T3 = *Bradyrhizobium japonicum*; T4 = *A. brasilense* + *B. japonicum* and T5 = *A. brasilense* + *T. harzianum*. Means followed by the same letters did not differ by Duncan's test at 10% probability.

3.3. Urease and Nitrogen Concentration

The values of urease activity varied from 124,043 µg NH_4^+/gram of dry soil to 231.175 µg NH_4^+/gram of dry soil, and there was no difference among the treatments ($p > 0.05$) (Figure 6).

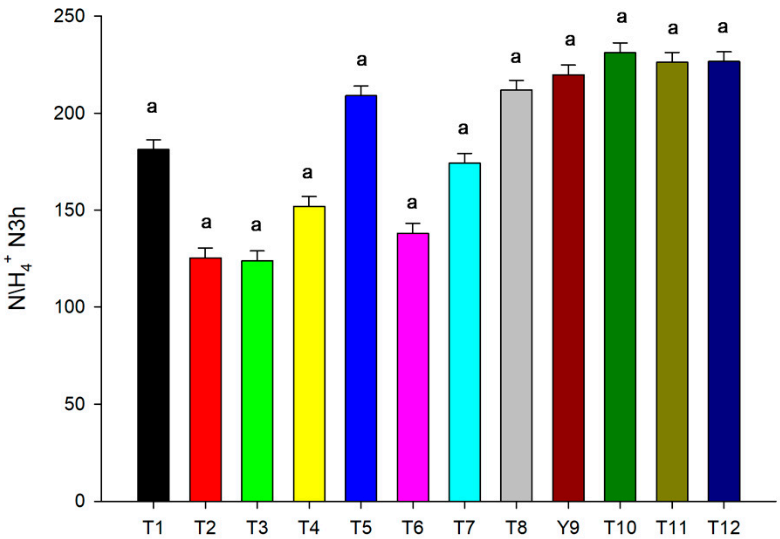

Figure 6. Average with standard deviation of urease in µg NH_4^+—N/3 h. g dry soil of peanut plants treated with different bacterial and fungal inoculants in a greenhouse. T1 = control; T2 = *Azospirillum brazilense*; T3 = *Bacillus subtilis*; T4 = *Bradyrhizobium japonicum*; T5 = *Trichoderma harzianum*; T6 = *A. brasilense* + *B. subtilis*; T7 = *A. brasilense* + *B. japonicum*; T8 = *A. brasilense* + *T. harzianum*; T9 = *B. subtilis* + *B. japonicum*; T10 = *B. subtilis* + *T. harzianum*; T11 = *B. japonicum* + *T. harzianum*; T12 = consortium (*A. brasilense* + *B. subtilis* + *B. japonicum* + *T. harzianum*). The means followed by the same letters do not differ by the Scott Knott test at 10% probability.

Under greenhouse conditions, the nitrogen content from roots varied from 18.96 g kg^{-1} to 23.63 g of nitrogen kg^{-1} root. There was no significant difference among the treatments ($p > 0.05$) (Figure 7). Regarding the nitrogen concentration in the shoots of the plants, *B. subtilis* (T3) was the inoculant that promoted the highest concentration among the other treatments, with 31.15 g of nitrogen kg^{-1} in the shoot, a value 15.46% higher than that found by the control group (26.33 g kg^{-1}). The other treatments did not differ from each other (Figure 7).

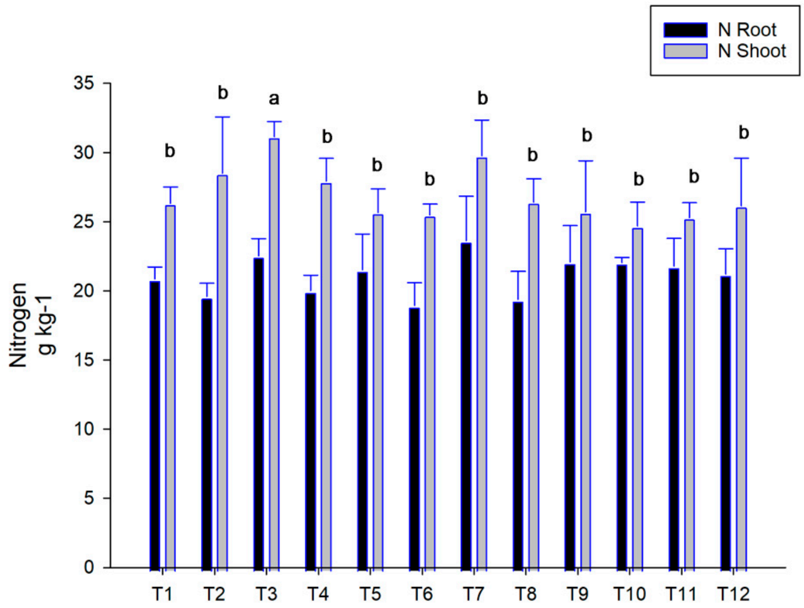

Figure 7. Average with standard deviation of nitrogen concentration in grams per kilogram of peanut plants treated with different bacterial and fungal inoculants in a greenhouse. T1 = control; T2 = *Azospirillum brazilense*; T3 = *Bacillus subtilis*; T4 = *Bradyrhizobium japonicum*; T5 = *Trichoderma harzianum*; T6 = *A. brasilense* + *B. subtilis*; T7 = *A. brasilense* + *B. japonicum*; T8 = *A. brasilense* + *T. harzianum*; T9 = *B. subtilis* + *B. japonicum*; T10 = *B. subtilis* + *T. harzianum*; T11 = *B. japonicum* + *T. harzianum*; T12 = consortium (*A. brasilense* + *B. subtilis* + *B. japonicum* + *T. harzianum*). The means followed by the same letters do not differ by the Scott Knott test at 10% probability.

In the field, there was no significant difference regarding the nitrogen concentration in the roots and shoots of the peanut plants, and all treatments were similar to the control (Figure 8).

3.4. Acid Phosphatase and Phosphorus Concentration

In all treatments that received inoculants, the activity of the acid phosphatase enzyme was significantly higher than that of the control group. The result in *Bradyrhizobium japonicum* + *Trichoderma harzianum*, for example, 1,449,365 µg pNF g^{-1} h^{-1}, was 33.93% higher than that found in the control group (957,586 µg pNF g^{-1} h^{-1}). Interestingly, the treatment with *A. brasilense* (T2) was higher than the control but lower than the other treatments (Figure 9).

Under greenhouse conditions, the highest phosphorus concentration in peanut roots was found in plants that received *A. brasilense* (T2), *B. subtilis* (T3), *A. brasilense* + *B. japonicum* (T7), *B. subtilis* + *B. japonicum* (T9), *B. subtilis* + *T. harzianum* (T10), and consortium (T12). The other treatments did not differ from each other, nor did they differ from the control group (Figure 10).

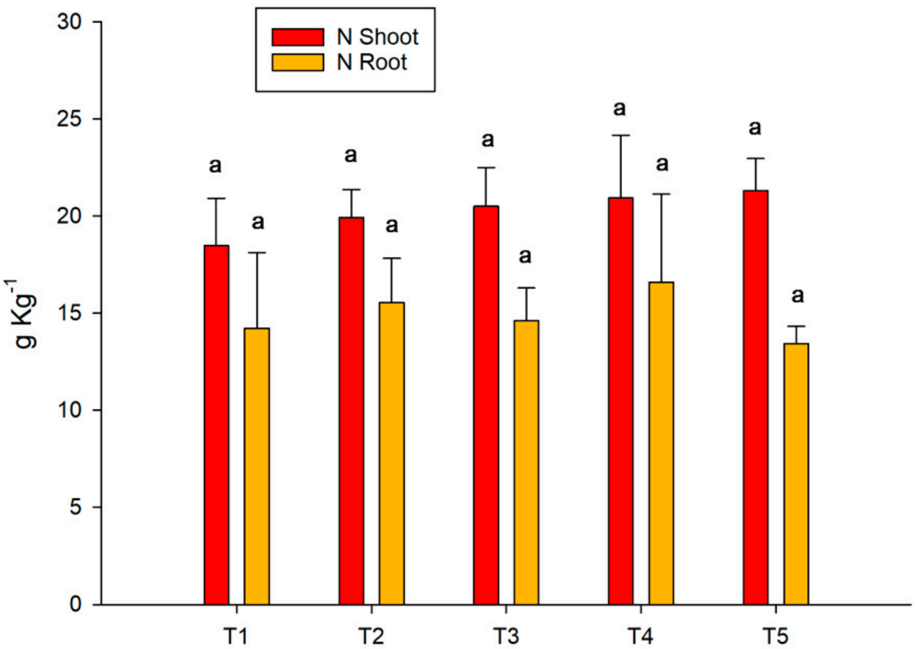

Figure 8. Average with standard deviation of nitrogen concentration in grams per kilogram of peanut plants treated with different bacterial and fungal inoculants in the field. T1 = control; T2 = *Azospirillum brazilense*; T3 = *Bradyrhizobium japonicum*; T4 = *A. brasilense* + *B. japonicum*; T5 = *A. brasilense* + *T. harzianum*. Means followed by the same letters did not differ by Duncan's test at 10% probability.

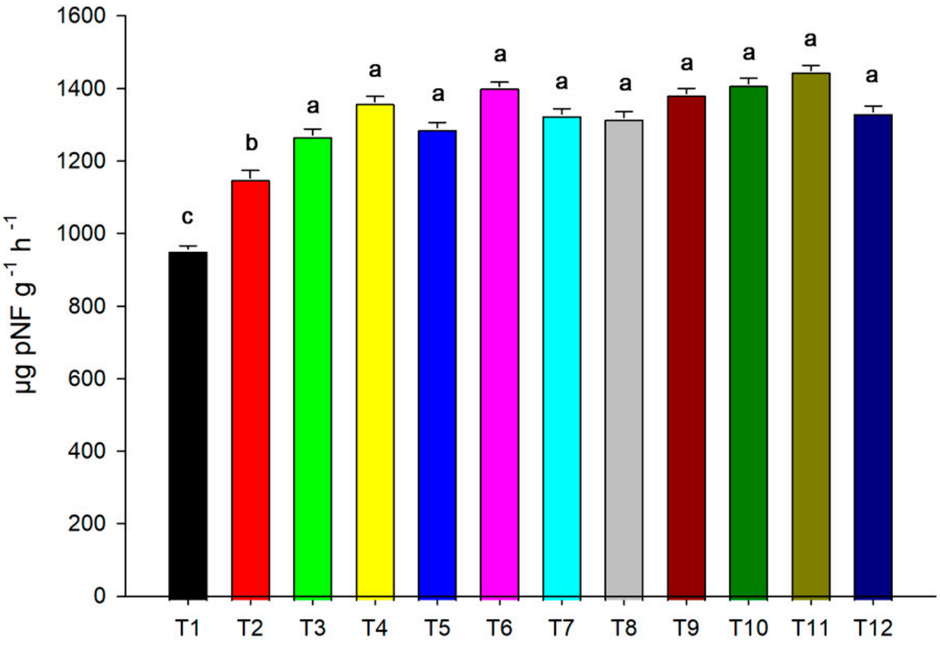

Figure 9. Average with a standard deviation of acid phosphatase in µg pNF g^{-1} h^{-1} of peanut plants treated with different bacterial and fungal inoculants in a greenhouse. T1 = control; T2 = *Azospirillum brazilense*; T3 = *Bacillus subtilis*; T4 = *Bradyrhizobium japonicum*; T5 = *Trichoderma harzianum*; T6 = *A. brasilense* + *B. subtilis*; T7 = *A. brasilense* + *B. japonicum*; T8= *A. brasilense* + *T. harzianum*; T9 = *B. subtilis* + *B. japonicum*; T10 = *B. subtilis* + *T. harzianum*; T11 = *B. japonicum* + *T. harzianum*; T12 = consortium (*A. brasilense* + *B. subtilis* + *B. japonicum* + *T. harzianum*). The means followed by the same letters do not differ by the Scott Knott test at 10% probability.

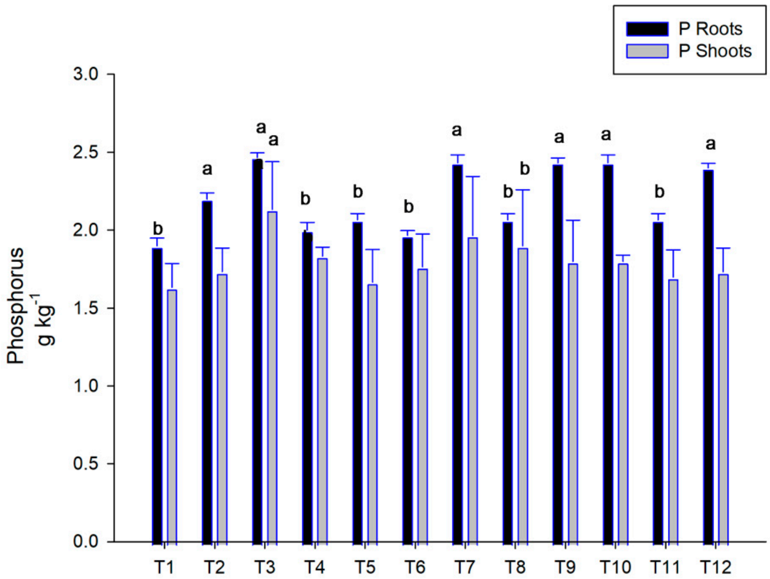

Figure 10. Average phosphorus concentration with standard deviation in grams per kilogram of peanut plants treated with different bacterial and fungal inoculants in a greenhouse. T1 = control; T2 = *Azospirillum brazilense*; T3 = *Bacillus subtilis*; T4 = *Bradyrhizobium japonicum*; T5 = *Trichoderma harzianum*; T6 = *A. brasilense* + *B. subtilis*; T7 = *A. brasilense* + *B. japonicum*; T8 = *A. brasilense* + *T. harzianum*; T9 = *B. subtilis* + *B. japonicum*; T10 = *B. subtilis* + *T. harzianum*; T11 = *B. japonicum* + *T. harzianum*; T12 = Consortium (*A. brasilense* + *B. subtilis* + *B. japonicum* + *T. harzianum*). The means followed by the same letters do not differ by the Scott Knott test at 10% probability.

Among the treatments selected and taken to the field, there was no significant difference regarding the phosphorus concentration in the shoot and roots of the peanut plants (Figure 11).

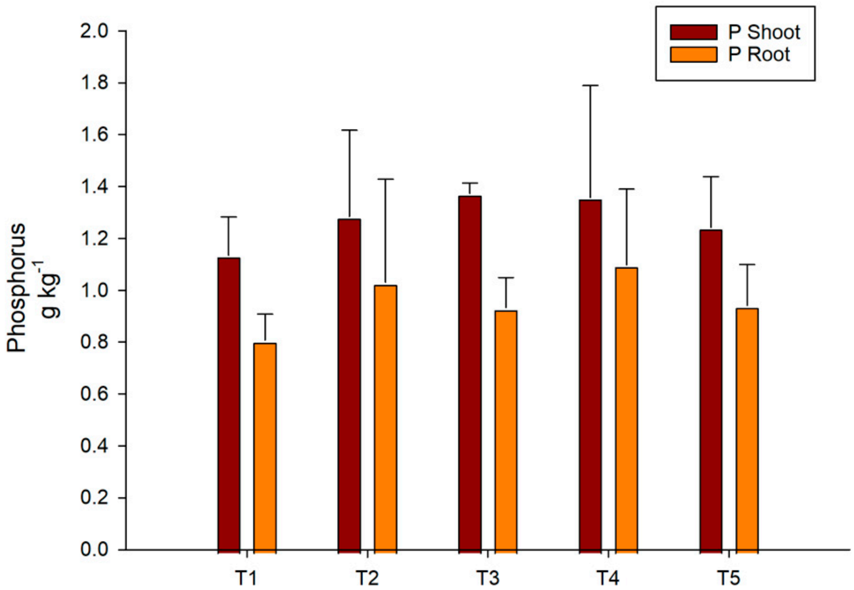

Figure 11. Average phosphorus concentration with standard deviation in grams per kilogram of peanuts treated with different bacterial and fungal inoculants in the field. T1 = control; T2 = *Azospirillum brazilense*; T3 = *Bradyrhizobium japonicum*; T4 = *A. brasilense* + *B. japonicum*; T5 = *A. brasilense* + *T. harzianum*. Means followed by the same letters did not differ by Duncan's test at 10% probability.

3.5. Productivity

B. japonicum was the only treatment that promoted the highest yield (12,623 kg ha^{-1}), a value 18.52% higher than that found for the control group (10,285 kg ha^{-1}). The other treatments did not differ from each other (Figure 12).

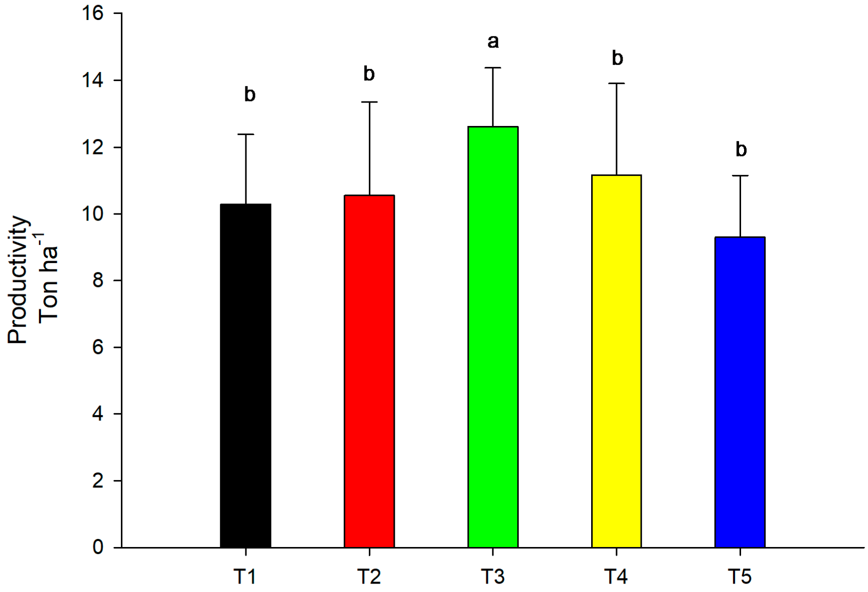

Figure 12. Yield in kilograms per hectare of peanut pods treated with different bacterial and fungal inoculants in the field. T1 = control; T2 = *Azospirillum brazilense*; T3 = *Bradyrhizobium japonicum*; T4 = *A. brasilense* + *B. japonicum*; T5 = *A. brasilense* + *T. harzianum*. Means followed by the same letters did not differ by Duncan's test at 10% probability.

4. Discussion

Studies of biological inoculants used in peanut cultivation worldwide are scarce, and specifically under Brazilian and regional conditions in Brazil, are at a very early stage [46]. On the other hand, much knowledge has been generated using plant growth-promoting microorganisms, which are microorganisms isolated from the rhizosphere or plant tissues that have different abilities to promote plant growth and development [19,47]. These abilities of microorganisms can affect the plant in a direct way. These include phyto-stimulating actions such as the synthesis of phytohormones that promote greater root development, increase water and nutrient absorption and the exploitation efficiency of plants in exploring a greater volume of soil [20]. Phytohormones also promote greater shoot development, thereby increasing photosynthetic efficiency [48].

These microorganisms, such as bacteria, also increase the supply and availability of soil nutrients to plants because they can fix nitrogen, transforming atmospheric nitrogen into ammonia, which is a form assimilated by plants [49]. On the other hand, fungi, although they cannot fix atmospheric nitrogen, can synthesize enzymes such as urease that are part of the nitrogen cycle and make nitrogen available from the mineralization of organic matter [50]. In addition, other microorganisms can produce various organic acids and enzymes, such as phytases and phosphatases, which solubilize inorganic phosphorus and phosphorus adsorbed in the soil, increasing its availability to plants and soil microorganisms [51].

The use of plant growth-promoting microorganisms is an excellent strategy that can be used to address the significant challenges of peanut cultivation, such as reducing production costs, the dose of chemical fertilizers, and environmental impacts, without detriment to productivity [47,52].

The results show that, in general, the microorganisms evaluated in the present study promoted increases in the various parameters analyzed (root dry mass, shoot dry mass, phosphorus concentrations, and plant height) in both greenhouse and field conditions compared with the control treatments that did not receive microbial inoculation. These results show that it is possible to obtain increases in peanut crop productivity with the inoculation of plant growth-promoting microorganisms, without the need for significant changes in the management of the crop.

Bradyrhizobium japonicum exerts a positive impact on biological nitrogen fixation and has been the focus in recent studies, as it is effective for increasing sustainable and environmentally friendly food production and long-term crop productivity. Plant improvement aims to increase the percentage of seed production. *B. japonicum* strains form a nitrogen-fixing root nodule symbiosis with some legume plants. For highly effective *B. japonicum* inoculation, it must survive and establish in the soil environment. Many studies have evaluated the effect of *B. japonicum* inoculation on soybean, but few studies have evaluated the effect on peanuts.

Yang [53] isolated five genotypes of *Bradyrhizobium* from peanuts forming small white nodules and showed that peanut plants are hosts of several genotypes of *Bradyrhizobium*, and there is no specificity for one genotype being classified as a promiscuous host.

Badawi [39] evaluated the coinoculation of *B. japonicum* with *Serratia marcescens* and *T. harzianum* in two field experiments with peanuts. The authors found increases in several plant parameters, as shown in the present study. However, the results were better when *B. japonicum* was inoculated with *S. marcescens* than when *B. japonicum* was inoculated alone. This result may be a consequence of the synergistic mode of action between these two microorganisms. Interestingly, the mixture of *B. japonicum* and *T. harzianum* did not benefit the peanut plants in this study. In another study, *Phomopsis liquidambari* coinoculated with *B. japonicum* increased root nodule number and shoot accumulated nitrogen by 28.25% and 29.71%, respectively. Nodulation dynamics analysis showed that *P. liquidambari* accelerated nodule initiation and subsequent nodule development. Meanwhile, *P. liquidambari* could colonize the peanut root as an endophyte. The dynamics of *P. liquidambari* and bradyrhizobial root colonization analysis showed that *P. liquidambari* inoculation significantly increased the rate of bradyrhizobial colonization [54].

The experiment evaluated under field conditions allowed a better evaluation of the potential of the tested microorganisms to promote plant growth and development. Some increments or beneficial effects that occurred under controlled conditions were not repeated under field conditions. Interestingly, although under greenhouse conditions, the treatment that received the *B. subtilis* + *T. harzianum* mixture showed a higher value for the urease enzyme, none of the evaluated microorganisms promoted increases in nitrogen concentration in the plants. On the other hand, all the microorganisms evaluated promoted increases in soil phosphatase levels compared with the control treatment. However, the phosphorus concentrations in the roots increased only in plants that received inoculation with the *B. subtilis* bacterium under greenhouse conditions. Under field conditions, surprisingly, only the bacterium *B. japonicum*, an excellent nitrogen-fixing bacterium, promoted increases in the concentration of phosphorus in the peanut roots compared with the control treatment that did not receive the inoculation of this microorganism. Biological nitrogen fixation requires the expenditure of ATP; therefore, phosphorus is needed at higher concentrations [55].

Regarding productivity, the main parameter considered by the peanut farmer, the best results were found with the application of *B. japonicum*, and no other treatment promoted an increase in this parameter compared with the control.

PGPM has several growth-promoting abilities, as mentioned above. However, some inoculations with these microorganisms failed in this purpose. This failure in growth promotion results from the interference of several factors, causing a variation in the intensity of plant growth promotion or even harming plant development [56,57]. The environmental temperature, soil pH, phenological state of the plant, and edaphoclimatic factors, among others, may affect these growth promotion effects. However, the plant genotype is the factor that most affects the interaction with microorganisms and, consequently, the success of the interaction and microbial action [58]. Therefore, a strategy to increase the chances of a positive interaction between the microorganism in the inoculant and the plant is to use formulations with more than one microorganism or even a microbial consortium. In addition, there is the possibility of synergism between the microorganisms of the formulation, leading to an increase in the plant growth-promoting effect [59].

The present study evaluated mixtures of microorganisms. Interestingly, the mixtures of microorganisms inoculated in peanut plants did not promote greater plant growth and development compared with inoculations of the same microorganisms separately. The use of formulated preparations consisting of a single microbial species or isolates as inoculants (i.e., a single antagonist against a single pathogen) has often resulted in inconsistent performance in agriculture and, consequently, has low representation in the world inoculant market. One of the reasons for such failures may be that a single microbial agent is unlikely to be active in all soil environments (in the presence of different biotic and abiotic stressors) or against all pathogens attacking the host plant. One way to overcome this problem is to include various species or strains of beneficial microbes in the same microbial formulation. The application of binary or multiple mixtures mimics the natural situation more closely and broadens the spectrum of biocontrol activity [56]. However, coinoculation may not promote effects when the strain interacts with the plant genotype. Specifically, in the field experiment in this study, the highest productivity was found for the inoculation of *B. japonicum* alone, and the mixture of microorganisms did not have the same effect. These results show that mixing microorganisms with different abilities does not necessarily guarantee an advantage for the peanut crop.

Of all the treatments applied in this study, inoculation with the bacterium *B. japonicum* was the most promising. Interestingly, this species is highly adapted to soybean and less adapted to common beans (both legumes), showing that the success of the *B. japonicum* plant interaction is dependent on specificity. Despite this specificity, *B. japonicum* promoted increases in peanut crop productivity.

5. Conclusions

The PGPM evaluated in the present study for peanut crops generally increased plant growth and development in greenhouse and field conditions. The best results were shown by the bacterium *B. japonicum*, which increased several parameters evaluated in the peanut plant, including productivity. Therefore, in the future, *B. japonicum* can be used in peanut crops as an inoculant.

Author Contributions: Conceptualization, E.C.R. and E.T.F.; methodology, E.T.F.; software, C.H.B.S. and L.A.L.d.C.; validation, L.R.S., R.M.d.S. and E.T.F.; formal analysis, E.T.F.; investigation, E.T.F.; resources, E.T.F.; data curation, E.T.F.; writing—original draft preparation, E.T.F.; writing—review and editing, E.C.R.; visualization, E.T.F.; supervision, E.C.R.; project administration, E.C.R.; funding acquisition, E.C.R. All authors have read and agreed to the published version of the manuscript.

Funding: The authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for financial support.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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