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Biological Strategies to Minimize Fertilizer Use in Maize: Efficacy of *Trichoderma harzianum* and *Bacillus subtilis*

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Abstract: The present study investigated the efficacy of *Trichoderma harzianum* and *Bacillus subtilis* in minimizing phosphorus fertilizer use in maize cultivation. Maize plants, cultivar Bm207, were subjected to 10 treatments with varying levels of phosphorus fertilization (0, 50, and 100%) and inoculation with *B. subtilis*, *T. harzianum*, or both. The plant growth parameters, including the height, stem diameter, shoot, and root dry weight, root volume, phosphorus content in the soil and plant tissues, and chlorophyll and carotenoid content, were evaluated. Treatments without mineral fertilization showed the lowest values for most parameters, despite the microbial inoculation. The combination of 100% mineral fertilizers with microbes did not improve the plant growth compared with the controls. However, the treatments with 50% mineral fertilization along with microbial inoculation generally maintained parameter values similar to those of the 100% fertilized control, suggesting the potential for reducing fertilizer doses by 50% without compromising plant development. Inoculation with *B. subtilis* and *T. harzianum* coupled with the use of mineral fertilizers improved the soil phosphorus availability compared to fertilizer application alone. This study highlights the potential of these microorganisms to enhance soil fertility and plant growth while reducing chemical fertilizer use in maize cultivation, although further field research is necessary to verify the long-term sustainability of this approach.

Keywords: reduction costs; environmental impact; mineral fertilizers dose

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1. Introduction

Maize is a significant crop with immense importance in agriculture and food systems worldwide. It ranks as the third most important crop globally based on harvested area [1] and is set to become the most widely grown and traded crop in the coming decade [2]. Maize production has surged in recent decades, driven by rising demand and technological advances. In 2019, the global maize production reached 1.15 billion tons [3]. Its versatility as a multi-purpose crop contributes to its widespread cultivation, serving primarily as animal feed but also as a crucial food source, especially in sub-Saharan Africa and Latin America [2]. Maize is used in diverse industrial products and as an alternative crop for biogas production [4].

Interestingly, although maize production is vital for global food security, it also contributes to environmental pressure. The cultivation of irrigated maize, along with other major crops, has driven water scarcity worldwide [5]. However, maize performs better than rice and wheat in combined land/water assessments on a global scale [5]. This highlights the need for the sustainable intensification of maize production within planetary boundaries [2].

Phosphorous fertilizers play a crucial role in maize production and significantly affect its yield and quality. Phosphorous is an essential macronutrient for crop growth and its deficiency can severely hinder plant development [6]. Studies have shown that phosphorus application can increase maize grain yield, dry matter stover yield, and crude protein content compared to controls without P fertilizer [7]. The efficiency of phosphorus fertilizers can vary depending on the type and application method used. For instance, rock phosphate and triple superphosphate have shown different levels of effectiveness in increasing corn plant dry matter and p uptake, and their performance is influenced by the soil characteristics [8]. Advanced fertilizers, such as those with enhanced nutrient use efficiency and targeted nutrient delivery, offer promising prospects for sustainable maize farming [9].

Interestingly, while phosphorus fertilizers are crucial, their misuse can lead to environmental problems such as greenhouse gas emissions. Recent studies in China have shown that it is possible to optimize fertilizer application rates to stabilize or increase maize yields while reducing the environmental impact of fertilizer use [10]. Mineral phosphorus fertilization provides numerous benefits for maize production. However, there are several negative aspects associated with phosphorus mineral fertilization. The excessive and continuous use of phosphorous fertilizers has led to significant soil contamination, turning agricultural lands into "chemical time bombs" [11]. This overuse has resulted in phosphorus accumulation in soil, making it susceptible to mobilization through erosion, surface runoff, and subsurface leaching [12]. The runoff of phosphorus into water bodies has contributed to eutrophication and hypoxia in surface waters globally, leading to dense blooms of toxic, odor-causing phytoplankton that deteriorate water quality [12]. This is particularly problematic in regions such as the Midwestern Corn Belt of the U.S., where phosphorous fertilization has resulted in a net positive soil phosphorous balance [12].

Phosphate rocks, the source of mineral phosphorus fertilizers, contain hazardous chemical elements such as Cd, Pb, Hg, U, Cr, and As. These toxic elements contaminate agricultural soil through fertilizer use and can enter the food chain, potentially causing various diseases including gastrointestinal, pulmonary, and kidney ailments [11]. For instance, cadmium has been identified as one of the most dangerous elements found in phosphate fertilizers and has been linked to the "Itai-Itai" disease [11].

Trichoderma harzianum can be effectively used to reduce phosphorus fertilization in maize crops through its ability to solubilize phosphate and enhance the nutrient uptake of plants. *Trichoderma harzianum* acts as a biofertilizer by producing volatile compounds and solubilizing phosphate, making it more suitable for plants than other uses [13]. This fungus can increase the availability and uptake of nutrients, including phosphorus, by plants [14]. In maize, inoculation with *T. harzianum* in the rhizosphere during the vegetative growth phase has been shown to alter the plant's metabolism and affect the arthropod community associated with maize foliage [15]. Interestingly, the effectiveness of *T. harzianum* in enhancing nutrient uptake and plant growth can vary depending on the type of soil and its properties. A study on *Brassica rapa* showed that *T. harzianum* strains were particularly effective in enhancing the fertility and productivity of nutrient-poor soils even without NPK fertilization [16]. This suggests that *T. harzianum* could potentially reduce the need for phosphorous fertilization in maize grown in nutrient-deficient soils.

Bacillus subtilis has shown potential in reducing the phosphorus fertilization requirements in maize crops, although the results vary across studies. Some studies have indicated that *B. subtilis* can enhance the phosphorus availability and uptake in maize. For instance, when applied as a bioorganic fertilizer, *B. subtilis* improves available potassium and pH of soil, while also enriching beneficial soil microbes that may aid in nutrient cycling [17]. Additionally, *B. subtilis* has been found to increase the maize grain yield by up to 15.6% in certain cases [18]. However, contradictory findings have been reported in the literature. One study found that the application of *B. subtilis,* either alone or in combination with phosphate fertilization, did not significantly influence the nutritional, biometric, or productive parameters of maize in soils with a naturally low p content [19]. Another study reported adverse effects when rock phosphate fertilization was combined with bacterial inoculation, suggesting potential negative interactions that require further investigation [20].

Bacillus subtilis shows promise in reducing the phosphorus fertilization needs in maize, although its effectiveness appears to be context-dependent. Factors such as the soil type, pH, bacterial concentration, and application method can influence the outcomes [21]. Further research is required to optimize the application of *B. subtilis* for consistent benefits in maize production under various environmental conditions.

Trichoderma harzianum and *B. subtilis* solubilize phosphorus through several mechanisms. They secrete organic acids such as citric acid, gluconic acid, and oxalic acid. These acids lower the pH of the rhizosphere, which helps solubilize inorganic phosphate compounds. These microorganisms also produce phosphatase enzymes that can break down organic phosphorus compounds and release inorganic phosphate that plants can absorb. The ability to produce siderophores enables them to chelate iron and other metals. This process can indirectly increase the phosphorus availability by preventing the formation of insoluble metal–phosphate complexes. Another two abilities of these microorganisms are the capability to stimulate root growth and branching, increasing the plant's ability to explore the soil and access phosphorus. In addition, these microorganisms may interact with other soil microorganisms, enhancing their collective ability to solubilize phosphorus [15,22].

The objective of the present study was to evaluate whether inoculation with *B. subtilis*, a bacterium known to be an effective phosphate-solubilizing bacterium (PSB), or *T. harzianum*, a fungus that enhances plant metabolism, either individually or in combination, would permit fertilizer reduction in the growing of maize plants and improve the plant's growth.

2. Materials and Methods

2.1. Microorganisms

The *B. subtilis* strain used in this study was obtained from the collection of the Agricultural Microbiology Laboratory at the UNESP campus in Jaboticabal, Brazil. This bacterium, initially isolated from a maize plant, was identified through sequencing, with its sequence available under GenBank accession number MZ133755. Concurrently, *T. harzianum* was isolated by Dr. Noemi Carla Baron Consentino during her doctoral research. This strain was sourced from soil on a rural property in Taquaritinga, São Paulo, Brazil. For inoculum development, *B. subtilis* was cultured in nutrient broth for 48 h at 28 °C, whereas *T. harzianum* was cultured in potato dextrose broth for 14 d at 28 °C. After the incubation period, the concentration was evaluated using the serial dilution method and standardized to 1×10^9 colony-forming units (CFU) mL^{-1}.

2.2. Experimental Design of Greenhouse Experiment

Three plants of the maize variety Bm207, brand Biomatrix, were initially planted in each 5 L pot, which were subsequently thinned to two plants per pot after initial growth. The pots were filled up to 90% of their capacity with eutrophic red latosol soil, characterized by its chemical properties, including a pH of 6.9, 10% organic matter, 23 mg/dm^3 available phosphorus, 0.7 $mmolc/dm^3$ available potassium, 79 $mmolc/dm^3$ calcium, 13 $mmolc/dm^3$ magnesium, and 11 $mmolc/dm^3$ hydrogen. Controlled environmental conditions were maintained in the greenhouse at a temperature of 24 ± 2 °C, $50 \pm 2\%$ relative humidity, and a light cycle of 16:8 h of light to dark. The Aw climate of the region was classified by Köppen and Geiger. The soil was sieved and fertilized according to a previously performed soil chemical analysis. The soil was fertilized with 80 milligrams per cubic decimeter ($mg\ dm^{-3}$) of nitrogen in the form of urea, 200 milligrams per cubic decimeter ($mg\ dm^{-3}$) of phosphorus as simple superphosphate, and 180 milligrams per cubic decimeter ($mg\ dm^{-3}$) of potassium as simple superphosphate of potassium in the form of potassium chloride. In addition to these nutrients, the following micronutrients were added: 0.5 milligrams per cubic decimeter ($mg\ dm^{-3}$) of boron (boric acid), 1.5 milligrams per cubic decimeter ($mg\ dm^{-3}$) of copper (copper sulfate), 3.0 milligrams per cubic decimeter ($mg\ dm^{-3}$) of manganese (manganese), and 3.0 milligrams per cubic decimeter ($mg\ dm^{-3}$) of iron (iron sulfate), as recommended by [23]. The specified quantity of fertilizer was applied at a rate of 100% in the treatment, whereas the remaining treatments received 0%, 50%, or 100% of the same quantity of fertilizer. The experimental setup was a completely randomized design within a greenhouse located in Jaboticabal, SP, Brazil (21°15′17″ S, 48°19′20″ W). This study aimed to evaluate the effects of microbial inoculation on the growth of maize plants of the variety Bm207, brand Biomatrix, using 10 different treatments. Treatments were carried out as follows: **T1** = 100% mineral fertilization without inoculant; **T2** = 0% mineral fertilization + *B. subtilis*; **T3** = 50% mineral fertilization + *B. subtilis*; **T4** = 100% mineral fertilization + *B. subtilis*; **T5** = 0% mineral fertilization + *T. harzianum*; **T6** = 50% mineral fertilization + *T. harzianum*; **T7** = 100% mineral fertilization + *T. harzianum*; **T8** = 0% mineral fertilization + *B. subtilis* + *T. harzianum*; **T9** = 50% mineral fertilization + *B. subtilis* + *T. harzianum*; and **T10** = 100% mineral fertilization + *B. subtilis* + *T. harzianum*. The microorganisms were applied in three different ways. First, for the treatments that received microbial inoculation, all seeds received microorganisms. After sowing, microorganisms were applied to the soil. Then, for five weeks, the microorganisms were applied via foliar application. Application via seed consisted of applying 200 mL of each or both microorganisms to 50 kg of seed, both at a concentration of 1×10^9 colony-forming units (CFU) mL^{-1}. For the treatments that received both microorganisms, there was a 20 min drying interval between the application of the bacterium and the fungus. For application via soil, the inoculum, at a concentration of 1×10^9 CFU mL^{-1}, was applied once directly to the soil at a volume of 10 mL per pot. In the treatments that received both microorganisms, each pot received 10 mL of each microorganism at the same volume and concentration, as mentioned above. During the 50-day study period, each treatment group received five weekly microbial reinoculations through foliar application, consisting of the same concentration and volume as the initial inoculation.

2.3. Plant Height, Stem Diameter, Shoot Dry Weight (SDW) and Root Dry Weight (RDW)

In this study, maize plants were assessed using several growth parameters. The plant height was measured from the apex to the base of the plant. The biomass was then processed by splitting the shoots from the roots, which were dried in a forced ventilation oven at 65 °C for 72–96 h and subsequently weighed on a semi-analytical scale. Stem diameter was determined using a packmeter FORTG, Jaboticabal, Brazil.

2.4. Determination of Root Volume

Washed roots were placed in a graduated cylinder containing a known volume of water. The displacement of the water volume comes from the volume of the root; thus, the difference expressed in mL was converted to cm^3 using unit equivalence [24].

2.5. Determination of Nitrogen and Phosphorus in Shoots and Roots

For nitrogen analysis, 500 g each of dried and ground plant samples were weighed and placed into 50 mL digestion tubes, and were then left to decouple at room temperature for 1.5 h. The tubes were then positioned in a digestion block and heated to 80 °C for 20 min before the temperature was increased to 160 °C. The tubes were monitored and removed once the material ascended the tube walls and most of the HNO_3 evaporated, leaving a clear solution. After cooling, 1.3 mL of the concentrated $HClO_4$ was added to each tube. The tubes were returned to the block and the temperature was increased to 210 °C, and digestion was deemed complete when the solution became colorless with dense white vapors of $HClO_4$ and H_2O formed above the dissolved material. The tubes were cooled and the contents were diluted to 25 mL with water in a snap-cap glass [25]. For phosphorus analysis, 1 mL of the digested sample was transferred to a test tube to which 4 mL of water and 2 mL of reagent mix (comprising equal parts of 5% ammonium molybdate and 0.25% vanadate) were added. The mixture was allowed to rest for 15 min before absorbance was measured at 420 nm using a UVVIS spectrophotometer Mettle, Toledo, Brazil [26].

2.6. Determination of Chlorophyll Contents and Carotenoids

Chlorophyll content was measured using a spectrophotometer. Leaf squares were cut (1 cm^2) for this analysis. Each leaf square was then cut into smaller pieces and placed in an Eppendorf tube containing 5 mL of dimethylformamide. The tubes were stored at 8 °C for 72 h in the dark. An aliquot of 3 mL of the liquid extract was then collected for reading in a spectrophotometer at 470, 647, and 664 nm. The chlorophyll content was determined according to the equation proposed by [27].

2.7. Statistical Analysis

A completely randomized design was used for the experiments, and the data were analyzed by analysis of variance (ANOVA). Duncan's test at 5% was used to separate the means. The analyses were performed at a level of significance of $p \leq 0.05$, and all analyses were performed using the statistical software 2.0 T-Statistic [28].

3. Results

Treatments T2, T5 and T8 exhibited the lowest plant heights compared with T1 ($p > 0.01$) (control). The remaining treatments showed no significant differences compared with the T1 control. The treatment without phosphate mineral fertilization showed the lowest height values, and the addition of microorganisms did not make a difference (Figure 1).

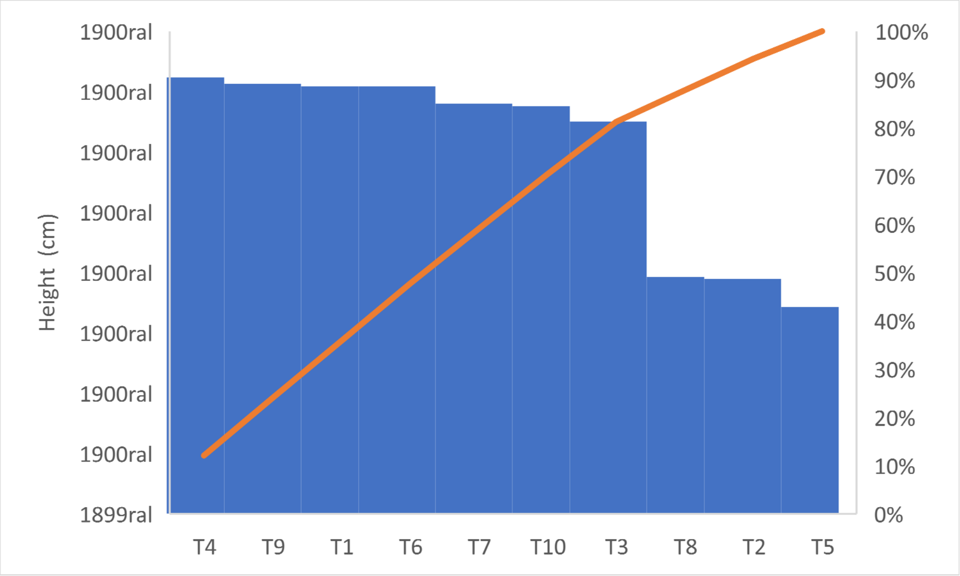

Figure 1. Mean height of maize plants in response to varying phosphate mineral fertilizer concentrations and the presence or absence of *B. subtilis* and *T. harzianum* inoculation. **T1** = 100% mineral fertilization without inoculant; **T2** = 0% mineral fertilization + *B. subtilis*; **T3** = 50% mineral fertilization + *B. subtilis*; **T4** = 100% mineral fertilization + *B. subtilis*; **T5** = 0% mineral fertilization + *T. harzianum*; **T6** = 50% mineral fertilization + *T. harzianum*; **T7** = 100% mineral fertilization + *T. harzianum*; **T8** = 0% mineral fertilization + *B. subtilis* + *T. harzianum*; **T9** = 50% mineral fertilization + *B. subtilis* + *T. harzianum*; and **T10** = 100% mineral fertilization + *B. subtilis* + *T. harzianum*.

The smallest stem diameter was observed in T5, followed by T8 and T2, compared to T1 ($p > 0.01$) (control). The other treatments did not show a significant difference compared with the control (Figure 2).

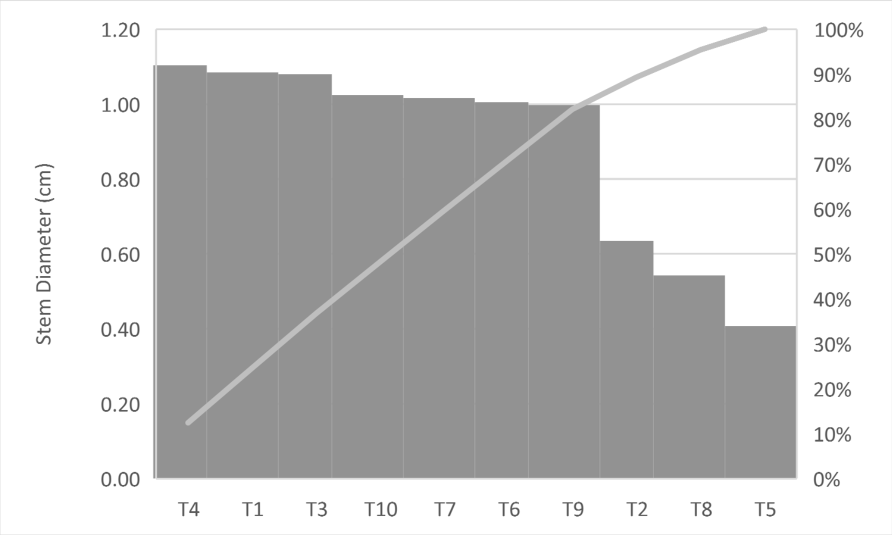

Figure 2. Mean stem diameter of maize plants in response to varying phosphate mineral fertilizer concentrations and the presence or absence of *B. subtilis* and *T. harzianum* inoculation. **T1** = 100% mineral fertilization without inoculant; **T2** = 0% mineral fertilization + *B. subtilis*; **T3** = 50% mineral fertilization + *B. subtilis*; **T4** = 100% mineral fertilization + *B. subtilis*; **T5** = 0% mineral fertilization + *T. harzianum*; **T6** = 50% mineral fertilization + *T. harzianum*; **T7** = 100% mineral fertilization + *T. harzianum*; **T8** = 0% mineral fertilization + *B. subtilis* + *T. harzianum*; **T9** = 50% mineral fertilization + *B. subtilis* + *T. harzianum*; and **T10** = 100% mineral fertilization + *B. subtilis* + *T. harzianum*.

For the shoot dry weight (SDW), T2 had the lowest value, followed by T5 and T8, compared with the control ($p > 0.01$). The other treatments showed no significant differences compared with the control ($p > 0.01$) (Figure 3).

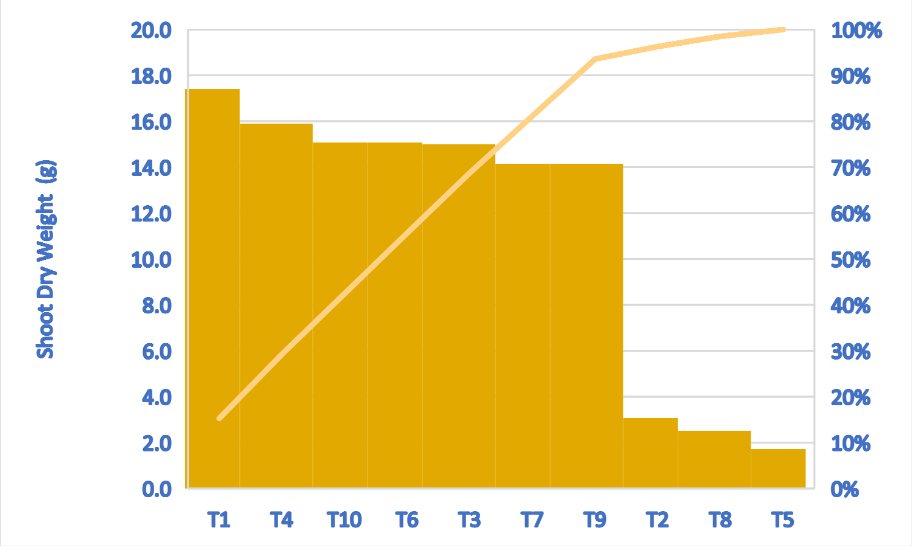

Figure 3. Mean shoot dry weight of maize plants in response to varying phosphate mineral fertilizer concentrations and the presence or absence of *B. subtilis* and *T. harzianum* inoculation. **T1** = 100% mineral fertilization without inoculant; **T2** = 0% mineral fertilization + *B. subtilis*; **T3** = 50% mineral fertilization + *B. subtilis*; **T4** = 100% mineral fertilization + *B. subtilis*; **T5** = 0% mineral fertilization + *T. harzianum*; **T6** = 50% mineral fertilization + *T. harzianum*; **T7** = 100% mineral fertilization + *T. harzianum*; **T8** = 0% mineral fertilization + *B. subtilis* + *T. harzianum*; **T9** = 50% mineral fertilization + *B. subtilis* + *T. harzianum*; and **T10** = 100% mineral fertilization + *B. subtilis* + *T. harzianum*.

The lowest root dry weight (RDW) was observed in T5, followed by T8 and T2, compared with the control ($p > 0.01$). The other treatments did not significantly differ from the control ($p > 0.01$) (Figure 4).

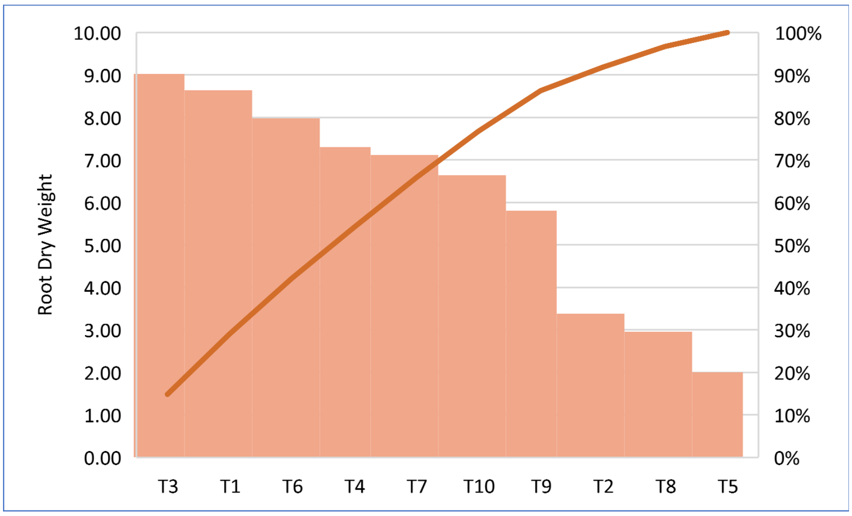

Figure 4. Mean root dry matter of maize plants in response to varying phosphate mineral fertilizer concentrations and the presence or absence of *B. subtilis* and *T. harzianum* inoculation. **T1** = 100% mineral fertilization without inoculant; **T2** = 0% mineral fertilization + *B. subtilis*; **T3** = 50% mineral fertilization + *B. subtilis*; **T4** = 100% mineral fertilization + *B. subtilis*; **T5** = 0% mineral fertilization + *T. harzianum*; **T6** = 50% mineral fertilization + *T. harzianum*; **T7** = 100% mineral fertilization + *T. harzianum*; **T8** = 0% mineral fertilization + *B. subtilis* + *T. harzianum*; **T9** = 50% mineral fertilization + *B. subtilis* + *T. harzianum*; and **T10** = 100% mineral fertilization + *B. subtilis* + *T. harzianum*.

The smallest root volume was observed in T5, followed by T8 and T2, compared to control ($p > 0.01$). The other treatments did not significantly differ from the control ($p < 0.05$) (Figure 5).

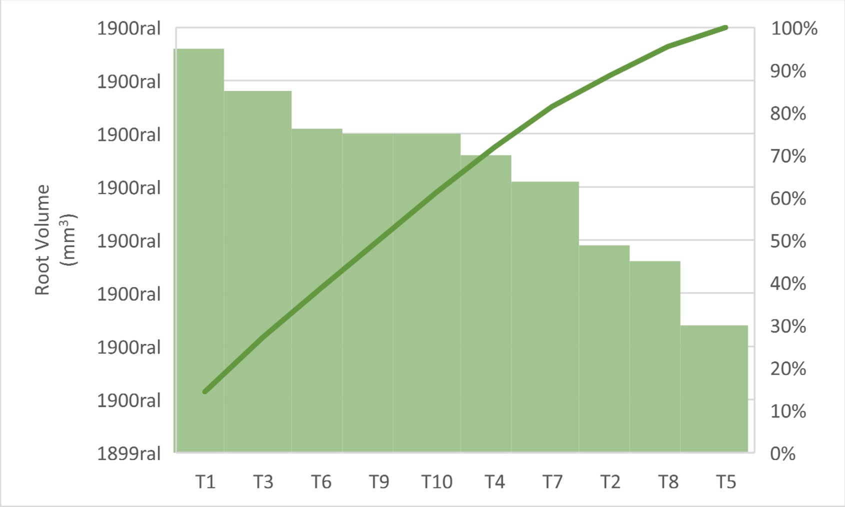

Figure 5. Mean root volume of maize plants in response to varying phosphate mineral fertilizer concentrations and the presence or absence of *B. subtilis* and *T. harzianum* inoculation. **T1** = 100% mineral fertilization without inoculant; **T2** = 0% mineral fertilization + *B. subtilis*; **T3** = 50% mineral fertilization + *B. subtilis*; **T4** = 100% mineral fertilization + *B. subtilis*; **T5** = 0% mineral fertilization + *T. harzianum*; **T6** = 50% mineral fertilization + *T. harzianum*; **T7** = 100% mineral fertilization + *T. harzianum*; **T8** = 0% mineral fertilization + *B. subtilis* + *T. harzianum*; **T9** = 50% mineral fertilization + *B. subtilis* + *T. harzianum*; and **T10** = 100% mineral fertilization + *B. subtilis* + *T. harzianum*.

The soil analysis revealed that treatment T10 had the highest phosphorus content ($p > 0.05$), followed closely by T7 and T3; treatments T4, T9, and T1 (control) exhibited lower phosphorus levels ($p > 0.05$); and the lowest phosphorus content was found in treatments T4, T5, and T8 (Figure 6).

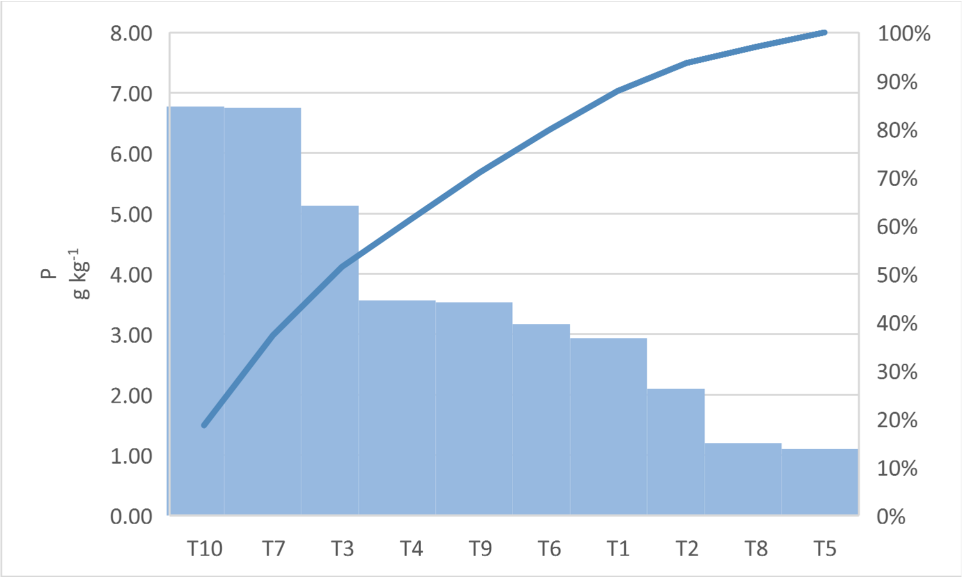

Figure 6. Mean phosphorus content in the soil in response to varying phosphate mineral fertilizer concentrations and the presence or absence of *B. subtilis* and *T. harzianum* inoculation. **T1** = 100% mineral fertilization without inoculant; **T2** = 0% mineral fertilization + *B. subtilis*; **T3** = 50% mineral fertilization + *B. subtilis*; **T4** = 100% mineral fertilization + *B. subtilis*; **T5** = 0% mineral fertilization + *T. harzianum*; **T6** = 50% mineral fertilization + *T. harzianum*; **T7** = 100% mineral fertilization + *T. harzianum*; **T8** = 0% mineral fertilization + *B. subtilis* + *T. harzianum*; **T9** = 50% mineral fertilization + *B. subtilis* + *T. harzianum*; and **T10** = 100% mineral fertilization + *B. subtilis* + *T. harzianum*.

Regarding the phosphorous content in the shoots, the highest values were found in treatments T10, T4, T9, T6, and T1. The lowest values were observed in the T2, T5, T8, and T7 treatments. For the roots, the highest values were found in T10, T3, T1 (control), and T4, and the lowest values were found in treatments T8, T5, T2, and T9 (Figure 7).

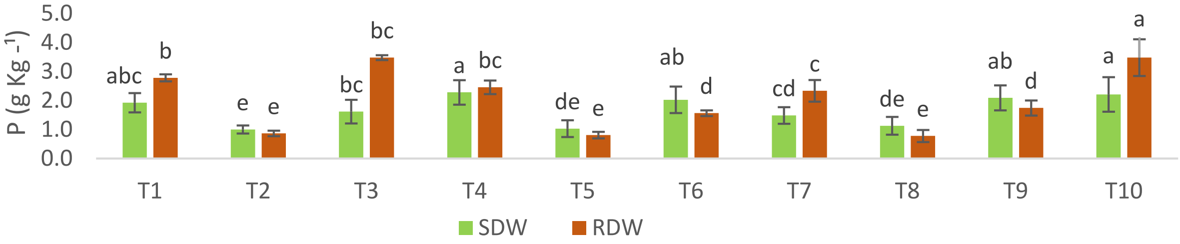

Figure 7. Mean phosphorus content in the shoots and roots of maize plants in response to varying phosphate mineral fertilizer concentrations and the presence or absence of *B. subtilis* and *T. harzianum* inoculation. SDW, shoot dry weight; RDW, root dry weight. Difference letters mean statistical differences according to Duncan 5%.

The highest values for the chlorophyll a, chlorophyll b, and carotenoid contents were found in treatments T4, T1, T3, T6, and T10. The lowest values were observed in T8, T2, T5, T9, and T7 (Figure 8).

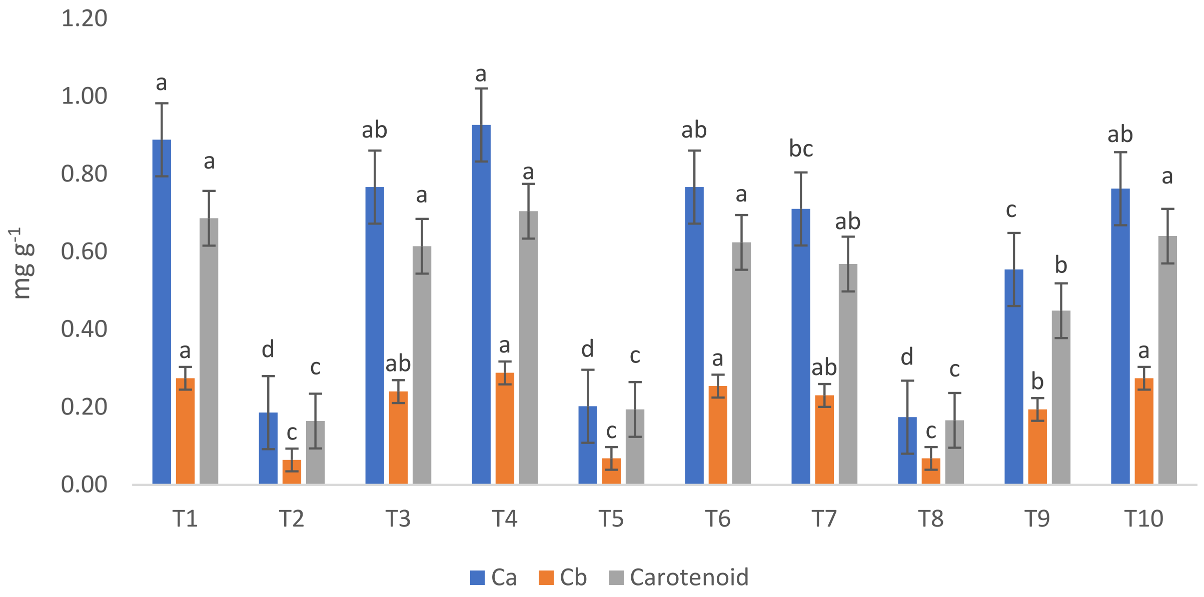

Figure 8. Content of chlorophyll a, chlorophyll b, and carotenoids from maize plants in response to varying phosphate mineral fertilizer concentrations, and the presence or absence of *B. subtilis* and *T. harzianum* inoculation. Ca—chlorophyll a; Cb—chlorophyll b. Difference letters mean statistical differences according to Duncan 5%.

4. Discussion

Overall, treatments T2, T5, and T8 had the lowest values for nearly all parameters. Despite being inoculated with *B. subtilis*, *T. harzianum*, or both, the plants that received these treatments did not receive mineral fertilizers. Inoculation alone, without mineral fertilizers, could not maintain parameter values similar to those found in the control that received mineral fertilizer. Except for the phosphorus content in the soil, the treatments (T4, T7, and T10) that received microbial inoculation with *B. subtilis*, *T. harzianum*, or both did not show higher values for the evaluated parameters than the respective controls that received mineral fertilizers without microbial inoculation. These results suggest that the combination of 100% mineral fertilizers and these microbes did not improve plant growth. However, some studies have demonstrated that it is possible to increase maize yield using *B. subtilis* along with a 100% fertilization dose. For instance, [29] reported that seed inoculation with *B. subtilis* combined with nitrogen fertilization improved the growth and increased the yield of maize. The highest grain yields were observed at nitrogen doses higher than 120 kg ha^{-1} along with *B. subtilis* inoculation. Similarly, [30] found that soil application with *B. subtilis* along with a 100% fertilization dose increased the maize yield by 5.5–13.4% compared to the control treatment.

Although the inoculation with the microorganisms did not show improvement in the parameters when they were applied with 100% mineral fertilizers, it is important to note that the treatments T3, T6, and T9 received 50% of the dose of mineral fertilization and, in general, the values of the parameters for these treatments were not lower than those found in the treatment control that received 100% of the dose. These results strongly suggest that the use of these microorganisms could reduce the mineral fertilizer dose to 50% without harming plant development. Studies have demonstrated that the application of *T. harzianum* as a biofertilizer can significantly improve maize growth, yield, and nutrient content, including phosphorus content [31].

The reduction in the fertilization dose, including mineral phosphate, is a consequence of the diverse mechanisms of action exhibited by *T. harzianum*. This fungus produces phytohormones that enhance the root development by more than 50% in maize crops and also increases the availability of phosphorus, thereby elevating phosphorus levels within the plant. These dual mechanisms of action typically allow for a 30% reduction in the phosphorus dose [32]. Regarding the bacterium, importantly, the use of *B. subtilis*-based biofertilizers can reduce the amount of the mineral fertilizers required for plant growth by up to 25% [33]. Bueno et al. [34] evaluated several mineral fertilizer doses along with different microbial inoculation concentrations of *B. subtilis* and observed that the presence of *B. subtilis* inoculation, even without mineral fertilization, increased the phosphorus concentrations in plant roots and shoots compared with fertilized plants. In the present study, even a 50% reduction in mineral fertilizer did not have detrimental effects on the maize plant growth.

It is noteworthy that, despite receiving 100% mineral fertilizers, treatment T1 exhibited the lowest concentration of available phosphorus in the soil in comparison to treatment T3, which received 50% mineral fertilizers + *B. subtilis,* T7, which received 100% mineral fertilizers + *T. harzianum*, and T10, which received 100% mineral fertilizers + *B. subtilis* + *T. harzianum.* These results suggest that the application of mineral fertilizers along with microbial inoculation may improve the phosphorus availability in the soil. Phosphorous fertilizer can be adsorbed by the soil, making it unavailable to plants. The lowest value of phosphorus being observed in T1 could be explained by this. The adsorption capacity of soil varies significantly, depending on its composition and properties. Aluminum and organic matter contents are primarily responsible for phosphorus adsorption, with iron playing a secondary role [35].

The phosphorus content in treatment T3 was higher than that in T6. Both treatments received 50% mineral fertilizer; however, treatment T3 received inoculation with *B. subtilis*, whereas treatment T6 received inoculation with *T. harzianum*. This result suggests that *T. harzianum* likely has a limited ability to solubilize phosphorus compared to *B. subtilis.* However, some studies have reported benefits of using both *B. subtilis* and *T. harzianum*. When applied together or individually, *T. harzianum* and *B. subtilis* have been shown to suppress plant pathogens, enhance nutrient uptake, and promote plant growth [36]. For instance, their application has significantly improved the plant growth parameters and nutrient uptake efficiency in maize [37].

Interestingly, inoculation with *B. subtilis* and *T. harzianum*, coupled with the application of rock powder, increased the soil fertility for nutrients such as phosphorus, iron, sulfur, calcium, and potassium. This combination also enhanced plant growth parameters, even with a 50% reduction in the chemical fertilization dose compared to the control treatments [36]. Similarly, other studies have shown that certain plant growth-promoting bacteria, including *B. subtilis*, can be used in maize crops with reduced chemical fertilization doses while maintaining good plant performance [38].

Regarding the phosphorus content in the shoots and roots of maize, the treatment T10, that received 100% mineral fertilizer along with both *B. subtilis* and *T. harzianum*, showed the highest values compared to the other treatments. Silva et al. [36] also reported similar results. In their study, the authors evaluated the inoculation of *T. harzianum*, *B. subtilis*, and rock powder as fertilizers, and the treatments with *T. harzianum* and rock powder also promoted a higher leaf area and shoot dry weight compared to the control. The highest values were found in treatments with 100% conventional fertilization plus rock powder and *T. harzianum*, and 50% conventional fertilization plus rock powder and *T. harzianum*. Interestingly, the treatment T7, which received 100% mineral fertilizer + *T. harzianum*, showed a high amount of phosphorous in the soil; however, it showed low amount of the phosphorous in the shoots and roots. These results reinforce the suggestion that *T. harzianum* has a limited ability to increase the phosphorus content of the studied plant. In another study, maize seedlings inoculated with *B. subtilis* AZS2 exhibited the highest root dry weights, with an improvement of 30.17% compared with uninoculated control plants [39]. Similarly, another study found that *B. subtilis* FZB24 enhanced the root biomass production by 38–65% compared to the uninoculated control [40]. Furthermore, *T. harzianum* has been shown to significantly increase the root dry weight in maize plants. In a greenhouse experiment, maize plants inoculated with *T. harzianum* exhibited an 80% increase in root dry weight compared with uninoculated controls [41].

Interestingly, the lowest values of chlorophyll a and b and carotenoid content in the plants were found in treatments that did not receive mineral fertilizers, showing a positive correlation between mineral fertilizer use and chlorophyll content. Interestingly, treatment T9 showed a low concentration of chlorophyll content and received 50% mineral fertilizer along with inoculation with *B. subtilis* and *T. harzianum*. Treatments T3 and T6 also received 50% mineral fertilizer and had higher chlorophyll contents. The difference was that treatment T9 was inoculated with both microorganisms, and this effect was harmful for this particular parameter. However, several studies have reported that the ability of *T. harzianum* to promote plant growth extends beyond its capacity to solubilize phosphorus. Plant growth promotion may be associated with the production of indole acetic acids (IAAs). As mentioned, this study found that the chlorophyll content decreased in the treatment that received 50% mineral fertilizer combined with *B. subtilis* and *T. harzianum.* However, a study showed that *T. harzianum* strains produce indole-3-acetic acid (IAA), which boosts root and shoot growth, thus enhancing photosynthesis [42]. In the present study, there was no difference between the evaluated parameters and the results obtained when the microorganisms were applied together or in combination. The same result was found in the mentioned study in that, in general, the application of rock powder and the mixture of *B. subtilis* and *T. harzianum* did not present higher values than the treatments that received the same microorganisms separately.

5. Conclusions

The findings of this study showed that there was no difference between the treatments that received 100% of the mineral fertilization compared to the treatments that received 50% of the mineral fertilization along with the inoculation of *B. subtilis* and *T. harzianum*, showing that a reduction in the mineral dose, including phosphorous fertilization, could be possible without harming maize plant development. Reductions in the mineral fertilization dose reduce the environmental impact and production cost. These microorganisms are promising alternatives for sustainable maize crop production.

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