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Impact of Phosphorus Fertilizer and Microbial Inoculants on Maize and Soil Microbiota

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Abstract

Maize (*Zea Mays*) is an important crop that strongly depends on soil phosphorus availability. Therefore, this study aimed to assess the compatibility of two commercial microbial inoculants that assist in soil phosphorus absorption and their effects on soil microbiota and the early development of maize plants. The experiment was conducted in a greenhouse at the Experimental Farm of the State University of Goiás, Ipameri, Goiás, Brazil. A completely randomized design with four replications and seven treatments was used, totaling 28 experimental units (pots). The treatments consisted of applying the recommended phosphorus (P) rate (100 kg P_2O_5 ha^{-1}) alone or combined with seed inoculation with an arbuscular mycorrhizal fungus (AMF) (*Rhizophagus intraradices*; Rootella BR®), phosphate-solubilizing bacteria (PSB) (*Bacillus subtilis and B. megaterium*; BiomaPhos®), or AMF + PSB; and half the recommended P rate (50 kg P_2O_5 ha^{-1}) combined with seed inoculation with AMF, PSB, or AMF + PSB. Microbial biomass carbon, soil basal respiration, microbial metabolic quotient, mycorrhizal colonization, plant height, stem diameter, and shoot and root dry weights were assessed 42 days after emergence (DAE). The best microbiological results were observed in the treatments applying the recommended P rate without inoculation and half this rate combined with PSB or AMF inoculation. The recommended P rate alone and half this rate combined with PSB, AMF, or AMF + PSB had a satisfactory effect on phytotechnical parameters up to 42 DAE. However, an apparent incompatibility between AMF and PSB was observed when combined with the recommended P rate.

Keywords: *Bacillus*, Cerrado, phosphorus, mycorrhiza, *Zea Mays*

1. Introduction

Brazil has stood out globally in maize-planted area, which was estimated in 20.96 million hectares for the 2024-2025 crop season, with a production of 119.55 million Mg, representing a 3.3% increase compared to the previous crop season (CONAB – Companhia Nacional de Abastecimento, 2025). Maize production in the country is concentrated in regions of the Cerrado biome, where most soils have low natural fertility with a high demand for phosphorus (P), as it is an immobile element in the soil and is often adsorbed as Fe and Al phosphates, making it unavailable to plants (Zhang et al., 2021).

P fertilizer requirements has typically supplied using soluble phosphate fertilizers, which are readily available sources for plants. However, most of these fertilizers are imported, leading to high production costs and a strong dependence on the external market, as the domestic market does not meet national demand (Sobral et al., 2018). Currently, 21% of monoammonium phosphate (MAP) imports come from Russia, and the onset of the conflict between Russia and Ukraine led to an 88% increase in MAP prices in Brazil (CEPEA – Centro de Estudos Avançados em Economia Aplicada, 2022). Furthermore, the frequent use of soluble sources can impact the soil microbial community (JOTE et al., 2023) due to the accumulation of toxic residues from acids used in fertilizer production, such as simple superphosphate, which is obtained by reacting rock phosphate with sulfuric acid (Sherkuziev, 2021). Thus, excessive fertilizer application rates and formulations that are inappropriate for crop needs have caused imbalances and, consequently, environmental degradation (Rahman et al., 2019).

Technological advances have consolidated the benefits from microorganisms by contributing to better plant performance under stress conditions, resulting in an increased crop yield (Silva et al., 2024). Some microorganisms can solubilize phosphate from P sources, releasing it into the soil solution to be absorbed by plant roots (Ribeiro et al., 2018). Several studies have demonstrated that the use of inoculants based on phosphate-solubilizing bacteria significantly increases P availability and absorption by plants (Irshad & Yergeau, 2018).

Additionally, certain fungi enhance plants' P uptake through mycorrhizal associations, which extend the root system and increase the root surface area (Etesami et al., 2020). These fungi develop a network of hyphae that grow in the soil and/or within the roots, differentiating into structures for absorption, growth, or infection. The absorption structure comprises extraradical hyphae that actively absorbs phosphates from the soil, which are then transported to intraradical hyphae within the root, where they are actively transferred to plant cells through phosphate transport proteins, primarily located in the arbuscular membrane (Jansa et al., 2019).

The National Program for Bioinputs, stablished by Decree no. 10.375 of May 26, 2020, has significantly promoted the implementation of ecologically based technologies in large-scale agriculture in Brazil (BRASIL, 2020). In this context, researchers at the Brazilian Agricultural Research Corporation (Embrapa Maize and Sorghum) have been studying and selecting phosphate-solubilizing microorganisms for approximately 20 years (Sousa et al., 2020; Velloso et al., 2020), leading to the development of a commercial product (BiomaPhos®) containing bacterial strains of *Bacillus subtilis* (CNPMS B2084) and *B. megaterium* (CNPMS B119). The use of this commercial bioinoculant in maize production areas, under the application of recommended P fertilizer rates, resulted in an average grain yield increase of 8.9% (Oliveira-Paiva et al., 2020).

In 2018, the company NovaTero, in partnership with the Federal University of Santa Catarina (UFSC), developed the first inoculant based on the arbuscular mycorrhizal fungus *Rhizophagus intraradices* (Rootella BR®), approved in Brazil for agricultural crops, including maize and soybean. Satisfactory results have been observed with field applications of this bioinoculant, showing grain yield increases of 11% to 38% for maize (Stoffel et al., 2020a) and 11% to 58% for soybean (Stoffel et al., 2020).

The compatibility of chemical fertilizers has been well documented; however, the process of mixing components in a fertilizer formulation can lead to incompatibilities, such as caking and loss of solubility, resulting in reduced efficacy (Karagöz, 2021). However, information on the compatibility of biological products is limited.

In this context, this study aimed to assess the compatibility of bioinoculants based on phosphate-solubilizing bacteria (*Bacillus subtilis and B. megaterium*; BiomaPhos®) and an arbuscular mycorrhizal fungus (*Rhizophagus intraradices*; Rootella BR®) as a complement to the application of phosphorus fertilizer rates to soils for maize crop cultivation, based on microbiological and phytotechnical parameters.

2. Materials and Methods

The experiment was conducted in a greenhouse at the Experimental Farm of the State University of Goiás, South Campus, in Ipameri, Goiás, Brazil (17°41'S, 48°11'W, and altitude of 800 m). The region has an Aw, tropical savanna climate, according to the Köppen classification, with a mean temperature of 21.9 °C, relative air humidity ranging from 58% to 81%, and a mean annual rainfall depth of 1,447 mm.

A completely randomized experimental design with four replications and seven treatments was used, totaling 28 experimental units (pots). The treatments consisted of applying the recommended phosphorus (P) rate (100 kg P_2O_5 ha^{-1}) alone or combined with seed inoculation with an arbuscular mycorrhizal fungus (AMF), phosphate-solubilizing bacteria (PSB), or AMF + PSB; and half the recommended P rate (50 kg P_2O_5 ha^{-1}) combined with seed inoculation with AMF, PSB, or AMF + PSB.

Simple superphosphate (18% of P_2O_5) was used as P source at a rate of 560 kg ha^{-1}, based on the soil chemical and granulometric analysis (Table 1). The commercial microbial inoculants used were: an arbuscular mycorrhizal fungus-based product (Rootella BR®), containing the species *Rhizophagus intraradices*, at the manufacturer's recommended rate of 120 g ha^{-1}, providing approximately 2,496,000 propagules ha^{-1}; and a phosphate-solubilizing bacteria-based product (BiomaPhos®), containing strains of *Bacillus subtilis* (CNPMS B2084) and *B. megaterium* (CNPMS B119), at the manufacturer's recommended rate of 100 mL per 60,000 seeds, providing 4×10^9 viable cells mL^{-1}.

The soil used in the pots was a Latossolo Vermelho Amarelo distrófico (Typic Hapludox) of medium texture (EMBRAPA – Empresa Brasileira de Pesquisa Agropecuária, 2018), collected from the 0–0.20 m layer in an area cultivated under a no-tillage system for ten years. The soil was air-dried, sieved, homogenized, and placed in 14.3-liter pots. Soil samples were subjected to chemical analysis (Table 1), and the results were used for soil acidity correction and basal dressing 60 days before sowing and at sowing (Sousa & Lobato, 2004).

Seeds of the MG607PWU maize hybrid were treated with a fungicide based on fludioxonil, metalaxyl-M, and thiabendazole at 150 ml 100 kg^{-1}, along with pirimiphos-methyl and deltamethrin insecticides at 1.6 and 8 ml 100 kg^{-1}, respectively, before applying the fungal and bacterial inoculants. The seeds were sown using five seeds per pot. Thinning was performed seven days after germination, leaving one plant per pot. Thiamethoxam insecticide was applied at 250 mL ha^{-1} 30 days after emergence (DAE) for controlling corn leafhopper (*Dalbulus maidis*).

Table 1. Chemical and granulometric characteristics of the soil (0–0.20 m layer) used in a greenhouse experiment at the Experimental Farm of the State University of Goiás, Ipameri, GO, Brazil

P-meh	OM	pH	K	Ca	Mg	H+AL	Al	CEC	V	Clay	Silt	Sand
mg dm^{-3}	g dm^{-3}	$CaCl_2$	--------------cmol$_c$ dm^{-3}--------------						%		g kg^{-1}	
9.3	20.0	5.1	0.5	2.1	0.8	0.0	2.5	5.9	57.9	310.0	60.0	630.0

P-meh = phosphorus content determined using the Mehlich extraction method; OM = organic matter; CEC = cation exchange capacity; V = base saturation.

During the experimental period, the maize plants were monitored, weeds were removed manually, and irrigation was performed based on field capacity. The plants were evaluated at 42 DAE for assessing microbiological parameters (microbial biomass carbon, soil basal respiration, microbial metabolic quotient, and mycorrhizal colonization) and phytotechnical parameters (plant height, stem diameter, and shoot and root dry weights).

Soil microbial biomass carbon (Cmic) was assessed using the irradiation-extraction method (Islam & Weil, 1998) with 0.5 mol L^{-1} potassium sulfate, oxidation with 0.066 mol L^{-1} potassium dichromate, and titration with 0.033 mol L^{-1} ammonium ferrous sulfate (Vance et al., 1987). The microbial biomass carbon contents in the soil were expressed as mg kg^{-1}.

Soil basal respiration was estimated by the amount of C-CO_2 released from the soil, following the methodology of (Anderson & Domsch, 1993). A soil sample of 100 g were stored in glass jars with screw caps, and a vial containing 10 mL of 0.1 mol L^{-1} NaOH was placed in the center. The incubation period was determined using a calibration curve for opening the jars, followed by titration with 0.1 mol L^{-1} HCl. The values were expressed as mg C-CO_2 kg^{-1} of soil day^{-1}.

The microbial metabolic quotient was calculated by the microbial respiration to microbial biomass carbon ratio (Anderson and Domsch, 1993), and the values expressed in mg C-CO_2 mg^{-1} Cmic day^{-1}.

Mycorrhizal colonization was evaluating by separating the finer roots from the plant, washing them in running water, and preserving them in a 50% alcohol solution. These roots were clarified and stained using the method proposed by (Phillips & Hayman,1970), which consists of collecting 0.5 g of roots and subjecting them to heat in a 10% KOH solution, acidification with diluted HCl, and staining with 0.05% trypan blue. Quantification was performed using the grid-plate method under a stereomicroscope, as described by (Giovannetti & Mosse, 1980), where the roots are evenly distributed on a plate with quadrants measuring 1.1 × 1.1 cm, counting all segments, including those that did not contain fungal structures (arbuscules and/or vesicles) and those that did, which intercepted the lines of the plate. The percentage of mycorrhizal colonization (MC; %) was determined using the following equation (1):

$$MC = \frac{cs}{ns + cs} \times 100 \tag{1}$$

where *ns* and *cs* are the number of non-colonized and colonized segments, respectively.

Plant height (cm) was measured using a tape measure, whereas stem diameter (mm) was measured 10 cm above ground level using a caliper.

Shoot and root dry weights (SDW and RDW, respectively) were determined by separating the shoot and root system, placing them in labeled paper bags, and drying in a forced-air oven at 65 °C for 48 hours. The resulting materials were then weighed on an analytical balance (precision of 0.001 g), the weight of each sample was divided by the number of plants (Fenner & Barnes, 1965), and the results of SDW and RDW were expressed as g plant^{-1}.

The data were subjected to analysis of variance, and the means were compared using Tukey's at a 5% significance level. All statistical analyses were conducted using the statistical software SISVAR (Ferreira, 2011).

3. Results and Discussion

Analysis of variance revealed a significant effect of the treatments on soil microbial biomass carbon and mycorrhizal colonization at 1% significance level, and on soil basal respiration and microbial metabolic quotient at 5% significance level, 42 days after emergence (DAE) of maize plants (Table 2).

The treatment with the application of the recommended P rate (100 kg P_2O_5 ha^{-1}) without seed inoculation with microorganisms differed significantly from the other treatments at 42 DAE, with the highest mean soil microbial biomass carbon (408.2 mg C kg^{-1}). This treatment also resulted in higher mean basal respiration (30.00 mg C-CO_2 kg^{-1} day^{-1}) compared to the other treatments (Table 3). Fiuza et al. (2022) reported contrasting results in a study evaluating the effect of soil and seed inoculation with rhizobacteria on soybean plants grown in soils different cultivation systems. A higher soil microbial biomass carbon (MBC) was observed throughout the crop cycle in plants treated with phosphate-solubilizing bacteria (PSB) (BiomaPhos®) and other bacterial species compared to non-inoculated plants.

Table 2. Analysis of variance of microbiological parameters (soil microbial biomass carbon – MBC, soil basal respiration – SBR, microbial metabolic quotient – qCO_2, and mycorrhizal colonization – MC) for different treatments (application of P rates and microbial inoculants) during the early development of maize plants (42 days after emergence)

Source of variation	MBC	SBR	qCO_2	MC
Treatments	23.72^{**}	3.13^{*}	5.73^{*}	7.16^{**}
Error	-	-	-	-
CV (%)	13.65	15.54	29.27	9.34

*, **, and ^{ns} = significant at 5%, significant at 1%, and not significant by the F-test, respectively. CV = coefficient of variation.

Table 3. Mean values of soil microbial biomass carbon (MBC), soil basal respiration (RBS), microbial metabolic quotient (qCO_2), and mycorrhizal colonization (MC) for different treatments (application of P rates and microbial inoculants) during the early development of maize plants (42 days after emergence)

Treatments	MBC (mg C kg^{-1})	SBR (mg C-CO_2 kg^{-1} day^{-1})	qCO_2 (mg C-CO_2 mg^{-1} Cmic day^{-1})	MC (%)
P	408.2a	30.00a	0.074b	57.25cd
$P_{50\%}$ + AMF	265.2b	26.26ab	0.100b	73.25ab
$P_{50\%}$ + PSB	223.5b	27.81ab	0.084b	75.50a
$P_{50\%}$ + AMF + PSB	203.8bc	27.54ab	0.145ab	68.00bcd
P + AMF	259.8b	20.17b	0.087b	54.00d
P + PSB	281.3b	22.53ab	0.093b	62.75bcd
P + AMF + PSB	133.7c	23.14ab	0.177a	60.75bcd

P = recommended P rate (100 kg P_2O_5 ha^{-1}); $P_{50\%}$ = half of the recommended P rate (50 kg P_2O_5 ha^{-1}); AMF = arbuscular mycorrhizal fungus; PSB = phosphate-solubilizing bacteria. Means followed by same letter are not significantly different from each other by the Tukey's test at a 5% significance level.

Ferreira et al. (2024) evaluated the effects of an inoculant containing the arbuscular mycorrhizal fungus (AMF) *Rhizophagus intraradices* on maize plants and reported a significant increase in MBC compared to non-inoculated plants. These results differ from those observed at 42 DAE in the present study. However, lower MBC values after inoculation with commercial mycorrhizal fungi-based products do not necessarily indicate inefficacy. According to Zhang et al. (2022), mycorrhizal fungi inoculation may primarily alter the structure of the soil microbial community, increasing its diversity without significantly affecting the carbon content immobilized by soil microbiota. Similarly, Jin et al. (2013) reported that inoculation can alter the composition of arbuscular mycorrhizal fungal communities, partially or completely replacing autochthonous mycorrhizal fungi.

Overall, the treatments with microbial inoculations did not result in a significant increase in soil basal respiration

(SBR) (Table 3). However, Sarathambal et al. (2022) reported a significant increase in SBR after co-inoculating AMF (genus *Rhizophagus*) and *Bacillus megaterium* in turmeric cultivation. Similarly, Zhang et al. (2018) observed increased SBR following microbial inoculation with *Azospirillum brasilense* and *Pseudomonas fluorescens* in rice crops.

The evaluated co-inoculation treatments (recommended P rate and half this rate combined with AMF + PSB) resulted in the highest microbial metabolic quotient ($q\mathrm{CO_2}$) values (0.177 and 0.145 mg C-CO_2 mg^{-1} Cmic day^{-1}, respectively) (Table 3). High $q\mathrm{CO_2}$ values suggest environmental stress due to microbial competition in co-inoculation treatments, regardless of the applied P rate. Sharma et al. (2013) reported lower $q\mathrm{CO_2}$ when maize plants were grown under co-inoculation with *Azospirillum* and AMF species compared to bacterial or fungal inoculation. According to Al-Maliki and Ali (2022), adding mycorrhizal fungal inoculum to the soil for eggplant cultivation did not alter $q\mathrm{CO_2}$; however, when combined with poultry litter application, it resulted in an increase in $q\mathrm{CO_2}$.

Souza et al. (2022) studied the co-inoculation with AMF and rhizobacteria for lemongrass cultivation and reported lower $\mathrm{qCO_2}$ values compared to the control treatment, indicating that inoculation improved soil quality, differing from the results observed in the present work.

The highest mycorrhizal colonization values (75.50% and 73.25 %) were observed in inoculation treatments with PSB or AMF when applying half the recommended P rate (50 kg P_2O_5 ha^{-1}), respectively, i.e., without co-inoculation (Table 3). Similarly, Bourles et al. (2020) reported that the co-inoculation with AMF species (*R. neocaledonicus* and *Claroideoglomus etunicatum*) and the bacterium *Curtobacterium citreum* did not significantly increase mycorrhizal colonization in *Tetraria comosa* plants cultivated in ultramafic soil.

However, mycorrhizal colonization in plants can be stimulated by bacteria after 12 months (Long, 2017; Xie, 2018), primarily due to the promotion of spore germination and hyphal growth (Selvakumar et al., 2019). In this context, the time factor may have limited the induction of stimulation mechanisms, as mycorrhizal colonization was assessed in the maize plants at 42 DAE.

The analysis of variance of maize phytotechnical parameters showed a significant effect of the treatments on shoot and root dry weights at the 5% significance, and on plant height and stem diameter at the 1% significance, at 42 DAE (Table 4).

Table 4. Analysis of variance of phytotechnical parameters (plant height – PH, stem diameter – SD, shoot dry weight – SDW, and root dry weight – RDW) assessed at 42 days after emergence of maize plants subjected to different treatments (application of P rates and microbial inoculants)

Source of variation	PH	SD	SDW	RDW
Treatments	2.94^{*}	5.37^{*}	7.84^{**}	10.49^{**}
Error	-	-	-	-
CV (%)	8.04	7.34	11.39	10.16

*, **, and ^{ns} = significant at 5%, significant at 1%, and not significant by the F-test, respectively. CV = coefficient of variation.

The highest mean plant height (PH; 52.75 cm) was observed in the non-inoculation treatment with 100 kg P_2O_5 ha^{-1} (Table 5), which did not differ statistically from all inoculation treatments, except for that combining 50 kg P_2O_5 ha^{-1} with PSB inoculation, which had the lowest mean (43.50 cm). These results were unexpected, as *B. subtilis*, present in the commercial inoculant BiomaPhos®, solubilizes phosphate through siderophores (Rawat et al., 2021) and exhibits plant growth-promoting traits, including the production of the auxin indole-3-acetic acid (IAA) (Milani, 2017). However, Hoelscher (2020) reported that PH is strongly influenced by the plant's genetics, which may be affected by external factors, potentially explaining the results of the present study.

In contrast, Brito et al. (2022) reported that PH of maize grown under greenhouse conditions and inoculation treatments combining different P sources (natural phosphate and triple superphosphate) at 100 kg ha^{-1} and phosphate-solubilizing bacteria (BiomaPhos®) did not differ significantly from that observed in the control treatment (without bacterial inoculation) at 42 DAE. Similarly, Rosa et al. (2024) found that co-inoculation with phosphate-solubilizing bacteria and mycorrhizae, combined with different P rates, in maize cultivation resulted in no significant differences in PH compared to non-inoculation treatments.

However, Lima and Buso (2022) found significant differences in PH of two maize hybrids (MG 408PWU and MG 607PWU) when testing five BiomaPhos® rates (0, 2, 4, 6, and 8 mL kg^{-1}) for seed inoculation.

The largest mean stem diameter (SD; 17.20 mm) was observed in the treatment combining 50 kg P_2O_5 ha^{-1} with PSB inoculation (Table 5), followed by the non-inoculation treatment with 100 kg P_2O_5 ha^{-1} (15.10 mm). The other treatments did not differ significantly from each other in SD. Conversely, Pereira et al. (2020) reported a positive linear response of SD to P_2O_5 rates, with the lowest mean in treatments inoculated with *B. subtilis* compared to the control. Similarly, Carvalho (2022) reported that co-inoculation with growth-promoting bacteria, combined with 75% of the recommended P rate, did not affect SD compared with P application without inoculation.

SD is important for achieving high grain yields, as a larger stem enhances the plant's capacity to store photoassimilates that contribute to grain filling (Lana et al., 2009). Jamidi et al. (2017) reported a positive correlation between maize SD and high grain yield.

Table 5. Mean values of plant height (PH), stem diameter (SD), shoot dry weight (SDW), and root dry weight (RDW) assessed 42 days after emergence of maize plants subjected to different treatments (application of P rates and microbial inoculants)

Treatment	PH (cm)	SD (mm)	SDW (g)	RDW (g)
P	52.75a	15.10ab	9.60a	5.04ab
$P_{50\%}$ + AMF	48.81ab	13.90b	8.00ab	4.09bc
$P_{50\%}$ + PSB	43.50b	17.20a	8.230ab	4.56abc
$P_{50\%}$ + AMF + PSB	49.43ab	14.33b	10.11a	5.25a
P + AMF	45.75ab	14.35b	6.41b	5.37a
P + PSB	46.50ab	13.33b	7.33b	3.55c
P + AMF + PSB	48.87ab	14.34b	9.55a	3.72c

P = recommended P rate (100 kg P_2O_5 ha^{-1}); $P_{50\%}$ = half of the recommended P rate (50 kg P_2O_5 ha^{-1}); AMF = arbuscular mycorrhizal fungus; PSB = phosphate-solubilizing bacteria. Means followed by same letter are not significantly different from each other by the Tukey's test at a 5% significance level.

Overall, the mycorrhizal inoculation did not result in a significant increase in SD, regardless of the P rate or co-inoculation with phosphate-solubilizing bacteria (Table 5). In contrast, Barros et al. (2024) evaluated the effect of AMF on plant development and cooper content in maize crops and reported that inoculation with *Rhizoglomus clarum* increased SD compared to inoculation with *Acaulospora scrobiculata* and the control treatment.

Co-inoculation treatments resulted in higher mean shoot dry weights (SDW) compared to treatments with single inoculation (AMF or PSB), showing 10.11 g (application of 50 kg P_2O_5 ha^{-1}) and 9.55 g (application of 100 kg P_2O_5 ha^{-1}). These values did not differ significantly from the mean (9.60 g) of the non-inoculation treatment with 100 kg P_2O_5 ha^{-1} (Table 5). This result was expected, as several studies have reported improved nutrient use efficiency due to microbial inoculation (Bueno et al., 2022). Huasasquiche et al. (2024) combined microbial inoculation (*B. subtilis* and *R. intraradices*) with different fertilizer rates in strawberry cultivation and observed the highest SDW (at 90 DAE) when the highest fertilizer rate was combined with the *B. subtilis* inoculum.

Additionally, Fuiza et al. (2022) reported significant increases in SDW when inoculating phosphate-solubilizing bacteria (BiomaPhos®) in soybean crops. Ferreira et al. (2024) evaluated the effect of a mycorrhizal fungal inoculant (*R. intraradices*) on maize plants in a two-year experiment and found distinct results. In the first year, SDW was significantly higher in plants under fungal inoculation than in non-inoculated plants, regardless of soil P content. However, in the second year, SDW was 27% higher in plants grow in soil with higher P content.

The highest mean root dry weight (RDW) was achieved when combining 100 kg P_2O_5 ha^{-1} with AMF inoculation (5.37 g), which did not differ significantly from the treatment combining 50 kg P_2O_5 ha^{-1} with co-inoculation (5.25 g) (Table 5). Nacoon et al. (2022) evaluated co-inoculation with phosphate-solubilizing bacteria and AMF (*Burkholderia vietnamiensis* and *R. aggregatus*) species in *Helianthus tuberosus* under water stress conditions and observed low synergism for root system-related parameters, with the best results obtained from fungal inoculation, which is partly consistent with the findings of the present study. According to Park et al. (2016), co-inoculation with phosphate-solubilizing bacterial strains *Burkholderia anthina* (PSB-15) and *Enterobacter aerogenes* (PSB-16) resulted in a higher RDW in green grasses compared to the control.

Fiuza et al. (2022) observed the highest SDW in soybean plants when inoculating seeds with phosphate-solubilizing bacterial species, including *B. subtilis* and *B. megaterium* (BiomaPhos®) and *Brevibacillus* sp. and *B. venezensis*.

These results suggest that while seed co-inoculating with arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria does not necessarily improve P uptake, the presence of these bacteria may stimulate the production of plant growth hormones.

4. Conclusions

The best microbiological results at 42 days after emergence of maize plants were observed in the non-inoculation treatment subjected to the application of the recommended P rate (100 kg P_2O_5 ha^{-1}), as well as in treatments with half this rate combined with inoculation with phosphate-solubilizing bacteria (PSB) (*Bacillus subtilis* and *B. megaterium*) or the arbuscular mycorrhizal fungus (AMF) *Rhizophagus intraradices*.

Applying 100 kg P_2O_5 ha^{-1} without inoculation and 50 kg P_2O_5 ha^{-1} combined with PSB, AMF, or AMF + PSB had a satisfactory effect on maize phytotechnical parameters at 42 DAE. However, an apparent incompatibility between AMF and PSB was observed when combined with the recommended P rate.

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Authors contributions

Prof. Dr. Talles Eduardo Borges dos Santos was responsible for the design and review of the study. Cleber Tavares da Rocha Filho, Icaro Alvarenga da Trindade, Maria Eduarda Borges Rodrigues Silva, Laiane Barbosa de Medeiros and Gabriel Duarte da Costa were responsible for data collection. Prof. Dr. Talles Eduardo Borges dos Santos, Cleber Tavares da Rocha Filho and Icaro Alvarenga da Trindade wrote the manuscript and Prof. Dr. Talles Eduardo Borges dos Santos and Maria Eduarda Borges Rodrigues Silva reviewed it. All authors read and approved the final manuscript.

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