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Evaluation of the interaction between phosphate-solubilizing bacteria and mycorrhizae, with phosphorus doses in corn crop

Avaliação da interação entre bactérias solubilizadoras de fosfato e micorrizas, com doses de fósforo na cultura do milho

Eliete de Fátima Ferreira da Rosa [1(ORCID 0000-0001-9131-8118)], Cristiano do Nascimento Andrade [1(ORCID 0009-0001-4269-689X)], Steffani da Luz [1(ORCID 0000-0001-5442-7403)], Jéssica Fernandes Kaseker *[2(ORCID 0000-0001-5487-7439)], Marcos André Nohatto [1(ORCID 0000-0002-0820-330X)], Lenoir Eder Teixeira Nagel [1 (ORCID 0000-0002-9671-9022)]

[1]Instituto Federal Catarinense, Santa Rosa do Sul, SC, Brasil.
[2]Autonomous Researcher, Três Cachoeiras, RS, Brazil. *Author's email: jessikaseker@gmail.com

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RESUMO

As doses de P a serem aplicadas na cultura do milho comumente são elevadas em decorrência do baixo aproveitamento do nutriente devido a sua adsorção ao solo. O objetivo deste trabalho foi avaliar o uso das bactérias solubilizadoras de fosfato *Bacillus megaterium* e *Bacillus subtilis*, e do fungo micorrízico *Rhizophagus intraradices*, bem como a interação entre eles, e a possibilidade de redução da adubação fosfatada na cultura do milho. O delineamento experimental foi em blocos casualizados, em um fatorial 4x3, sendo o fator A composto por diferentes microrganismos solubilizadores de P (micorrizas e BiomaPhos®) e o fator B composto por diferentes doses de P (100, 75 e 50% da dose recomendada). A redução das doses de P, sem o uso de inoculantes, resultou em menor área foliar e teor de clorofila, porém não foi observado efeito significativo na altura das plantas. Nos componentes de rendimento número de grãos por espiga, peso de mil grãos e produtividade, a inoculação compensou a redução das doses. A aplicação de 50% da dose de P juntamente com a inoculação das bactérias foi o tratamento com maior margem bruta, sendo viável para a redução da adubação mineral.

PALAVRAS-CHAVE: análise econômica; *Bacillus megaterium*; *Bacillus subtilis*; *Rhizophagus intraradices*; *Zea mays.*

ABSTRACT

The doses of P to be applied in the corn crop are commonly high due to the low use of the nutrient due to its adsorption to the soil. The objective of this study was to evaluate the use of the phosphate solubilizing bacteria *Bacillus megaterium* and *Bacillus subtilis*, and the mycorrhizal fungus *Rhizophagus intraradices*, as well as the interaction between them, and the possibility of reducing phosphate fertilization in corn. The experimental design was in randomized blocks, in a 4x3 factorial, with factor A composed of different P-solubilizing microorganisms (mycorrhizae and BiomaPhos®) and factor B composed of different doses of P (100, 75 and 50% of the recommended dose). The reduction of P doses, without the use of inoculants, resulted in lower leaf area and chlorophyll content, but no significant effect was observed on plant height. In the yield components number of grains per cob, thousand grain weight and yield, inoculation compensated for the dose reduction. The application of 50% of the P dose together with the inoculation of bacteria was the treatment with the highest gross margin, being viable for the reduction of mineral fertilization.

KEYWORDS: economic analysis; *Bacillus megaterium*; *Bacillus subtilis*; *Rhizophagus intraradices*; *Zea mays*.

INTRODUCTION

Corn cultivation is a major global agricultural commodity, with Brazil ranking as the third-largest producer, harvesting over 112 million tons in the 2021/2022 crop year. While not among the country's top producers, in Santa Catarina this crop plays a crucial role in agricultural development, serving as a foundation for poultry and pig farming. However, the state still relies on imports to meet its demand and supply animal feed factories (EPAGRI 2020, CONAB 2022). Therefore, tools that boost productivity and lower production costs are crucial for enhancing the corn production chain in the state.

In this regard, nutrient availability is a key factor. Corn plants require substantial nutrients, particularly nitrogen and potassium, as well as phosphorus. The high rate of nutrient transfer to the grains and subsequent removal necessitates fertilizer application to replenish these essential elements (COELHO 2006). Corn plants grow rapidly in a short time and require higher levels of available phosphorus compared to perennial crops (BASTOS et al. 2010).

Another consideration is the potential scarcity of phosphorus in certain soil types. Free phosphate anions in the soil solution are quickly bound through interactions with other ions such as calcium, aluminum, and iron (TIMOFEEVA et al. 2022, Li et al. 2023). At higher pH levels, calcium phosphate precipitates may form, while iron and aluminum oxides and hydroxides adsorb the nutrient through covalent bond complexation with ligand exchange. This process is stronger and favored by low pH (PENN & CAMBERATO 2019). Plants absorb only 5-25% of phosphate from chemical fertilizers (LI et al. 2023).

These interactions are particularly important in highly weathered soils, which are common in Brazil. Due to the nutrient's affinity for minerals found in these conditions, the bonds are difficult to break, making potential residual fertilization minimally available for subsequent crops (MENEZES-BLACKBURN et al. 2018, ZHU et al. 2018).

The accumulated soil phosphorus could potentially nourish crops, reducing the need for mineral fertilizers, but plants' access to this source depends on various factors (DOYDORA et al. 2020). As an alternative to enhance plant P uptake and decrease reliance on soluble fertilizers, plant-microbe partnerships show promise. Mycorrhizal fungi, known for boosting phosphorus absorption in various crops, and phosphate-solubilizing bacteria are particularly noteworthy in this regard.

Many plant species form symbiotic relationships with arbuscular mycorrhizal fungi in their rhizosphere, and corn is considered highly dependent on this symbiotic association under low phosphorus conditions (SALGADO et al. 2017). In a study examining the effectiveness of arbuscular mycorrhizae in corn cultivation, researchers observed increased phosphorus uptake and grain yield in plants that were inoculated compared to those without inoculation (STOFFEL et al. 2020). Moreover, inoculated treatments showed higher economic yield than fertilized and non-inoculated ones, even when receiving only half the recommended P dose, suggesting better utilization of soil nutrients.

In addition to mycorrhizal fungi inoculation, phosphate-solubilizing bacteria also show promise for reducing soluble fertilizer use(OWEN et al. 2015). These organisms can directly solubilize phosphorus and release soluble phosphates through their chelating action (PAIVA et al. 2020). Among the diverse range of P-solubilizing microorganisms, *Bacillus* bacteria stand out, such *as B. megaterium* isolated from corn rhizosphere, which can produce phosphatase and solubilize calcium and rock phosphates, and *B. subtilis*, an endophyte that generates high production of gluconic acid and phytase enzyme, while also solubilizing calcium and iron phosphates (PAIVA et al. 2020). The use of these bacteria resulted in an average productivity gain of 8.9% and a 19% increase in P export (PAIVA et al. 2020).

Microbial consortia have gained prominence, leveraging complementary traits among organisms for synergistic interactions, beyond individual applications. This combination can occur within strains of the same group, or between different groups, such as fungi and bacteria (CARNEIRO et al. 2023). However, it's crucial to assess organism compatibility to prevent any adverse interactions that could hinder plant growth.

While numerous studies have examined phosphate-solubilizing microorganism inoculation in annual crops, few have investigated the interaction between these microorganisms and mycorrhizal fungi, particularly in the southernmost region of Santa Catarina. This study aimed to assess the potential of phosphate-solubilizing bacteria *Bacillus megaterium and Bacillus subtilis*, along with the mycorrhizal fungus *Rhizophagus intraradices*, and their interactions, in reducing phosphate fertilizer requirements for corn cultivation

MATERIAL AND METHODS

The experiment was carried out at the Federal Institute of Santa Catarina - Santa Rosa do Sul Campus. According to Köppen's classification, the region's climate is Cfa type, with an average temperature of 19,5 °C and evenly distributed rainfall, averaging 1789 mm. annually. Figure 1 shows the daily rainfall and temperature data throughout the experiment's duration. The soil in the experimental area was classified as MELANIC GLEYSOL (SANTOS et al. 2018)where chemical properties and clay content are shown in Table 1.

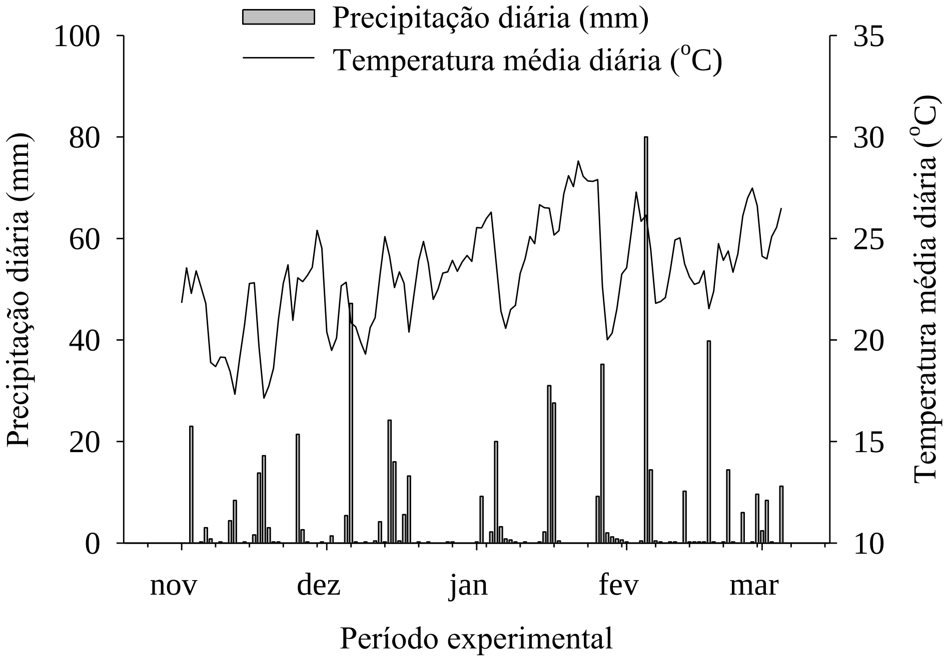

Figure 1. Daily rainfall and average temperature recorded during the experiment. Data obtained at the Araranguá - SC experimental station from 01/11/2021 to 05/03/2022. Source: Brazilian Agricultural Research Corporation of Santa Catarina (EPAGRI).

Table 1. Chemical analysis and percentage of soil clay in the 0-0.20 m layer in the experimental area.

Clay	pH	pH	MO*	P	K	Al	Ca	Mg	CTC	V	m
(%)	SMP	H_2O	(%)	(mg dm^{-3})			(cmolc dm-3)			(%)	
20	6,5	5,3	2,1	108,5	79	0,1	3,6	1,8	8,1	68,5	2,1

*SMP = SMP index; OM = organic matter; P = phosphorus extracted by Mehlich-1 solution; K = exchangeable potassium; Al = exchangeable aluminum; Ca = exchangeable calcium; Mg = exchangeable magnesium; CEC = cation exchange capacity; BS = base saturation; AS = aluminum saturation.

The experiment used a randomized block design with four replications, employing a 4x3 factorial arrangement. Factor A consisted of inoculant treatments, while factor B involved varying levels of phosphate fertilization. Factor A treatments included: BiomaPhos® phosphate solubilizer inoculation (*Bacillus megaterium* BRM 119 and *Bacillus subtilis* BRM 2084 strains) at 100 ml/ha^{-1}; mycorrhizal fungus *Rhizophagus intraradices* inoculation at 120 g/ha^{-1} (20,800 propagules/g); co-inoculation with BiomaPhos® and mycorrhizal fungus; and no inoculation. Both inoculants were applied as seed treatments at planting according to the manufacturers' technical recommendations. For factor B, phosphorus doses were set at 100%, 75%, and 50% of the recommended amount based on soil analysis, following the Fertilization and Liming Manual for Rio Grande do Sul and Santa Catarina (CQFS 2016). This corresponded to 74, 55, and 37 kg ha^{-1} of P_2O_5, applied as triple superphosphate, totaling 180, 135, and 90 kg ha^{-1} of fertilizer, respectively.

Corn was planted in October 2021 using no-till methods on white oat (*Avena sativa*) residue. The Syngenta Feroz cultivar, an early-maturing single-cross hybrid with caterpillar and glyphosate resistance technology, was used. The row spacing was 0.5 m, aiming for a final plant population of 60,000 plants ha^{-1}. Each plot consisted of six planting rows, covering a usable area of 10 m^2.

Nitrogen and potassium fertilization was applied based on soil analysis results (Table 1), following the Fertilization and Liming Manual for Rio Grande do Sul and Santa Catarina States (CQFS 2016), aiming for a yield expectation of 10 tons. 168 kg/ha^{-1} of N was applied as urea, with 40 kg/ha^{-1} at sowing and the remainder as top dressing, split between growth stages V4 and V6. 154 kg/ha^{-1} of K_2O as potassium chloride was applied, with 62 kg/ha^{-1} at sowing and the remainder as topdressing along with nitrogen fertilization.

The analyzed parameters included plant height, leaf chlorophyll content, leaf area, average number of kernels per ear, thousand-grain weight, and yield. Plant height was measured at the R1 phenological stage, according to the phenological scale developed by RITCHIE et al. (1993), using a measuring tape, from the base of the stem to the uppermost fully expanded leaf. At this same growth stage, leaf chlorophyll content was measured using a handheld ClorofiLOG CFL1030 chlorophyll meter on the index leaf located at the same node as the plant's main ear. Leaf area was measured from all leaves with over 50% green surface on four plants per plot, and calculated using the formula (SANGOI et al. 2007):

$AF = CxLx0{,}75$ (1)

In which: LA is the total leaf area (cm²), L is the leaf length (cm), and W is the leaf width (cm).

The harvest took place in March 2022, manually collecting from the four central rows of each plot. The three plants at each end of the row were discarded as they served as borders. The grains were harvested when they reached about 22% moisture content. After harvesting, the ears of corn were manually shelled. The average number of kernels per ear was determined by manually counting the grains. The thousand-grain weight was measured by counting and weighing grain samples from each plot using a precision analytical balance. Grain yield was estimated by weighing a thousand kernels and converting to kg/ha^{-1}, standardized at 130 g kg^{-1}.

The data were subjected to analysis of variance at the 5% probability level. When significant differences between treatments were observed, means were compared using Tukey's test ($p \leq 0.05$).

Statistical analysis was followed by a descriptive assessment of the total operational cost (TOC), based on the high-tech corn production cost spreadsheet developed by Epagri's Center for Socioeconomics and Agricultural Planning (EPAGRI/CEPA 2022). This assessment factored in input expenses, operational services, and overall production costs. The Brazilian real amounts were converted to US dollars using an average exchange rate of R$5.05 per dollar. This figure represents the average dollar exchange rate for the first half of March 2022.

RESULTS AND DISCUSSION

The treatments showed no statistically significant differences, and there was no interaction between factors regarding plant height (Table 2).

The results align with previous reports for this cultivar (ARAÚJO et al. 2016)and the results indicated that plant growth was not adversely affected in any of the treatments. This outcome may be linked to the high initial phosphorus levels found in the experimental area's soil (Table 1), which were adequate for this attribute.

Phosphorus plays a crucial role in various cellular processes, including membrane maintenance, biomolecule synthesis, and energy production. It's essential for forming ATP, the primary energy source for plant metabolic functions such as photosynthesis and cell division. Thus, when P is deficient, plants exhibit reduced growth due to decreased photosynthesis or increased energy expenditure (MALHOTRA et al. 2018). This effect is demonstrated in the findings reported by PATIL et al. (2012), where plants receiving higher nutrient doses exhibited greater growth compared to others under initially low availability conditions.

Table 2. Plant height in treatments involving inoculations of various phosphate-solubilizing microorganisms combined with different percentages of the recommended phosphorus (P) dose.

	100% of the P dose	75% of the P dose m	50% of the P dose
Without inoculant	2.36^{ns}	2.39^{ns}	2.37^{ns}
Mycorrhizae	2,39	2,39	2,36
BiomaPhos®	2,39	2,41	2,43
Mycorrhizae + BiomaPhos®	2,37	2,42	2,44
CV (%)		2,26	

^{ns} Differences not significant by Tukey test (p<0.05).

Analyzing the data on leaf chlorophyll content (Table 3), there was a significant effect only in the treatment that received 75% of the recommended dose of P and without inoculation, which was lower than the treatments with the same dose with inoculation and also the treatment without inoculant with 100% of the P dose.

Table 3. Leaf chlorophyll content in treatments with inoculations of different phosphate-solubilizing microorganisms combined with different percentages of the recommended dose of phosphorus (P).

	100% of the P dose	75% of the P dose	50% of the P dose
Without inoculant	64.42 Aa[1]	58.15 Bb	61.97 Aab
Mycorrhizae	64.52 Aa	62.25 ABa	66.07 Aa
BiomaPhos®	65.85 Aa	64.22 Aa	66.35 Aa
Micorrizas+ BiomaPhos®	67.07 Aa	66.75 ABa	66.37 Aa
CV(%)		5,51	

[1]Means followed by the same letter are not statistically different, uppercase in the column and lowercase in the row, according to Tukey's test (p<0.05).

While phosphorus levels in plants influence chlorophyll activity and photosynthesis, variations in chlorophyll content are more closely linked to nitrogen availability (PAVLOVIĆ et al. 2014, FRYDENVANG et al. 2015).

Regarding leaf area (Table 4), when half the recommended P dose was applied, the non-inoculated treatment underperformed compared to inoculated ones, and was also inferior to the non-inoculated treatment with 100% of the dose.

Table 4. plants in treatments with inoculations of different phosphate solubilizing microorganisms combined with different percentages of the recommended dose of phosphorus (P).

	100% of the P dose	75% of the P dose	50% of the P dose
		cm^2	
Without inoculant	586.25 Aa[1]	548.25 Aab	509.86 Bb
Mycorrhizae	562.41 Aa	563.01 Aa	579.97 Aa
BiomaPhos®	539.02 Aa	572.70 Aa	568.74 ABa
Mycorrhizae + BiomaPhos®	568.24 Aa	572.61 Aa	568.74 ABa
CV(%)		5,77	

[1]Means followed by the same letter are not statistically different, uppercase in the column and lowercase in the row, according to Tukey's test (p<0.05).

Insufficient phosphorus supply hinders cell division, reducing leaf emergence, expansion, and lifespan, as well as leaf area index and solar radiation interception (SICHOCKI et al.). 2014, MALHOTRA et al. 2018). The microorganisms effectively increased phosphorus availability for plants under limited supply conditions.

Significant differences were noted in yield components, ultimately affecting the area's final productivity. Regarding average grain count per ear (Table 5), mycorrhizal + BiomaPhos® inoculation underperformed compared to BiomaPhos® alone at 100% P dosage. However, it outperformed others at 75% P and surpassed the uninoculated control at 50% P.

Table 5. Average number of grains per cob in treatments with inoculations of different phosphate solubilizing microorganisms combined with different percentages of the recommended dose of phosphorus (P).

	100% of the P dose	75% of the P dose	50% of the P dose
Without inoculant	490.73 ABa[1]	473.14 Ba	483.95 Ba
Mycorrhizae	497.01 ABab	473.12 Bb	503.23 ABa
BiomaPhos®	518.29 Aa	487.77 Ba	494.57 ABa
Mycorrhizae + BiomaPhos®	476.99 Bb	531.84 Aa	527.68 Aa
CV(%)		4,10	

[1]Means followed by the same letter are not statistically different, uppercase in the column and lowercase in the row, according to Tukey's test (p<0.05).

When comparing results across dosages, the treatment with mycorrhizal inoculation + BiomaPhos® at 100% dose yielded lower values compared to the same treatment at lower doses. Nevertheless, mycorrhizal inoculation alone yielded higher values at 50% compared to 75% of the recommended P dose.

The average thousand-grain weight was lower in the treatment with mycorrhizal inoculation + BiomaPhos® compared to BiomaPhos® alone when combined with 50% of the recommended P dose (Table 6). Comparing dosages, differences were only observed in the BiomaPhos® inoculation treatment, with the highest values obtained from the 50% dose application compared to the others.

Table 6. Average weight of thousand grains in treatments with inoculations of different phosphate solubilizing microorganisms combined with different percentages of the recommended dose of phosphorus (P).

	100% of the P dose	75% of the P dose	50% of the P dose
		g	
Without inoculant	202.65 Aa[1]	200.82Aa	197.72 ABa
Mycorrhizae	194.53 Aa	195.95Aa	201.89 ABa
BiomaPhos®	196.64 Ab	196.16Ab	208.52 Aa
Mycorrhizae + BiomaPhos®	198.64 Aa	201.15Aa	194.07 Ba
CV(%)		3,05	

[1]Means followed by the same letter are not statistically different, uppercase in the column and lowercase in the row, according to Tukey's test ($p<0.05$).

Productivity analysis reveals significant results across treatments and dosage levels (Table 7). When the full recommended phosphorus dose was applied, mycorrhizal inoculation treatment underperformed compared to others, and the non-inoculated treatment yielded better results than the treatment combining mycorrhizal inoculation and BiomaPhos®.

Mycorrhizal treatment also underperformed when combined with 75% P dosage, a condition where the non-inoculated treatment outperformed BiomaPhos®. When applied at half the recommended dose, BiomaPhos® treatment outperformed others, and mycorrhizal inoculation resulted in higher productivity compared to non-inoculated and co-inoculated treatments. Comparing dose results, separate inoculation with mycorrhizae and BiomaPhos® performed better when combined with the 50% dose compared to others. Conversely, treatments without inoculation and with co-inoculation of microorganisms resulted in lower productivity at 50% of the applied dose compared to the others.

Table 7. Grain productivity in treatments with inoculations of different phosphate-solubilizing microorganisms combined with different percentages of the recommended dose of phosphorus (P).

	100% of the P dose	75% of the P dose	50% of the P dose
		(kg ha^{-1})	
Without inoculant	12273.11 Aa[1]	12258.38 Aa	11242,06 Cb
Mycorrhizae	11503,45 Cb	11439.14 Cb	11920.11 Ba
BiomaPhos®	11918.22 ABb	11878.13 Bb	12726.89 Aa
Mycorrhizae + BiomaPhos®	11918.50 Ba	11971.39 ABa	11242,06 Cb
CV(%)		1,42	

[1]Means followed by the same letter are not statistically different, uppercase in the column and lowercase in the row, according to Tukey's test ($p<0.05$).

Despite varied yield component results, BiomaPhos®-inoculated treatments generally showed superiority, with increased beneficial effects as applied P doses decreased. The findings align with existing research, which demonstrates the beneficial effects of plant growth-promoting and phosphate-solubilizing bacteria on plant development (HUSSAIN et al. 2013, BAIG et al. 2014, Li et al. 2017).

Bacillus bacteria in BiomaPhos® offer multiple plant benefits, including nutrient acquisition, phytohormone production, pathogen protection, and abiotic stress resistance. These bacteria can release previously unavailable phosphorus for plant uptake by producing organic acids that solubilize bound phosphorus or by mineralizing organic phosphorus (SAXENA et al. 2019). Thus, bacterial activity can offset reduced fertilization, ensuring adequate nutrient supply for plants and influencing the crop's final yield, as observed in this study.

AMANULLAH & KHAN (2015) found that using phosphate-solubilizing bacteria in corn cultivation increased the number of kernels per ear, thousand-grain weight, and overall yield. Additionally, it allowed for a 25% reduction in phosphorus application without compromising productivity.

In the experiments carried out by GUIMARÃES et al. (2021) the beneficial effects of inoculating corn crops with *B. subtilis* and *B. megaterium* were demonstrated in 2021, improving morphometric, nutritional, and production variables. Inoculation combined with half the recommended fertilizer dose yielded results comparable to those achieved with 100% of the dose.

Regarding the enhanced effectiveness of inoculation at lower P doses, this effect is also documented in existing research. Mycorrhizal inoculation symbiosis can enhance plant growth even when combined with higher fertilizer doses, though the improvements are less pronounced (ORTAS 2012). However, in conditions with initially low P availability, mycorrhizal inoculation enhances nutrient uptake in plants (BOWLES et al. 2016) research shows that when combined with moderate phosphorus application, yields match those achieved with higher doses. 2016).

In an experiment aimed at evaluating the use of arbuscular mycorrhizal fungi in corn cultivation with varying phosphorus levels, KAZADI et al. (2022) found that using these microorganisms enhanced plant growth and allowed for a 50% reduction in mineral phosphate fertilizer application.

In STOFFEL et al.'s research (2020), the effect of initial soil P content was evident, with greater effects observed at lower P levels. In soil with high P content, inoculation without phosphate fertilization did not produce statistically significant increases, but was sufficient to match the productivity obtained with 100% P dose without inoculation. This finding suggests that fertilizer application could be eliminated without compromising crop yields.

Given the initial P content in this study, it's reasonable to speculate that eliminating fertilization could also lead to greater inoculation effects and comparable yields. This assumption is particularly valid for BiomaPhos® inoculation, which has already shown higher productivity with reduced phosphate fertilization compared to higher doses.

The presence of soluble phosphate fertilization also reduces the efficiency of bioinoculation with bacteria (VIRUEL et al. 2014, ADNAN et al. 2020). However, when combined with alternative phosphate fertilizer sources, or with reduced doses of soluble fertilizers, it enhances the efficiency of these sources (HUSSAIN et al. 2013, BAIG et al. 2014, ADNAN et al. 2020) indicating that this combination could effectively replace soluble phosphate fertilizers.

The effectiveness of microbial inoculation relies on various environmental factors, including soil pH, moisture, temperature, and plant health, as well as the characteristics of the microorganisms themselves. Higher soil pH levels promote bacterial growth, while fungi thrive in more acidic environments (LOPES et al. 2021). Therefore, the soil conditions in this study (Table 1) are more conducive to bacterial growth, supporting the trend of superior results in treatments using *Bacillus* compared to mycorrhizae.

Co-inoculation of mycorrhizae and bacteria proved less effective than individual inoculation, particularly regarding productivity when phosphorus levels were halved. The findings diverge from those reported by SURI et al. (2011), where inoculation with isolated mycorrhizae or in combination with P-solubilizing bacteria, with or without additional fertilization, increased corn yield. However, it's worth noting that the soil was nutrient-deficient, unlike in the current study where initial levels were very high (Table 1).

In the study conducted by ROMERO-MUNAR et al. (2023) the co-inoculation of *Rhizophagus irregularis* and *Bacillus megaterium* enhanced corn plants' tolerance to water stress and high temperatures through physiological changes, suggesting potential benefits for plant development under adverse conditions. Since water availability and temperature were not limiting factors in this study (Figure 1), this effect was not observed.

The total operational expenses were calculated by assessing the economic value of inputs and variable costs. Table 8 summarizes these findings.

Table 8. Economic evaluation of treatments with inoculations of different phosphate-solubilizing microorganisms combined with different percentages of the recommended dose of phosphorus (P) in corn in the 2021/2022 harvest.

Treatments	Total Costs[1]	Gross Revenue[2]	Gross Margin[3]
	Dollars per hectare		
Without Inoculant + 100% of P dose	2148,46	3787,21	1638,76
Without Inoculant + 75% of P dose	2084,86	3782,58	1697,73
Without inoculant + 50% of the P dose	2018,48	3564,11	1545,63
Mycorrhizae + 100% of the P dose	2202,83	3628,17	1425,34
Mycorrhizae + 75% of the P dose	2138,01	3529,86	1391,85
Mycorrhizae + 50% of the P dose	2076,40	3678,16	1601,76
BiomaPhos® + 100% of the P dose	2167,40	3697,79	1530,39
BiomaPhos® + 75% of the P dose	2103,44	3665,39	1561,95
BiomaPhos® + 50% of the P dose	2043,31	3927,37	1884,06
Mycorrhizae + BiomaPhos® + 100% of the P dose	2223,58	3677,79	1454,21
Mycorrhizae + BiomaPhos® + 75% of the P dose	2160,25	3694,08	1533,83
Mycorrhizae + BiomaPhos® + 50% of the P dose	2093,79	3469,13	1375,34

[1]Sum of the costs of inputs and services in production. [2]Value obtained from production, considering the price of $18.51 per 60 kg bag. [3] Difference between total costs and gross revenue.

Given that production steps were uniform across the experimental area, cost differences stem from applied products. The lowest cost was observed in the treatment without inoculation and with 50% phosphorus dose, while the highest cost occurred in the treatment with 100% phosphate fertilization and co-inoculation. Costs increase proportionally with higher amounts of phosphate fertilizer used.

Revenues correlate with average productivity across treatments, highest in the 50% phosphate fertilization with BiomaPhos® inoculation treatment, which showed peak productivity in absolute terms, and lowest in the 100% fertilization with co-inoculation treatment. Gross profit margin, calculated as the difference between costs and revenues, follows the same pattern as revenues.

The standard treatment without inoculant using the full recommended phosphorus dose costs $106.93 more in operational expenses than using half the dose with BiomaPhos®, due to high fertilizer costs. When comparing profit margins, this difference exceeds $237.62.

The findings align with KAUR & REDDY's (2015) study, which also demonstrated greater economic viability in corn production using phosphate-solubilizing bacteria combined with rock phosphate application. This approach reduced costs and increased revenues, as the treatment enhanced growth and yield compared to other methods, including chemical phosphate fertilization.

Thus, reducing fertilizer dosage combined with bio-inoculation proves to be a viable alternative, making production more cost-effective and environmentally sustainable. It decreases mineral fertilizer use, which in excess increases eutrophication risk and depletes natural phosphate rock sources, also causing economic impact through rising fertilizer prices (ZHU et al. 2018).

CONCLUSION

The use of *Bacillus megaterium* and *Bacillus subtilis*-based inoculants allowed for a 50% reduction in mineral phosphate fertilizer while maintaining productivity levels, lowering costs, and increasing profit margins, proving to be a viable alternative for corn cultivation in the study area.

Co-inoculation of mycorrhizae and phosphate-solubilizing bacteria was less effective than individual inoculation, leading to lower productivity and profit margins.

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