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Rock dust and phosphorus-solubilizing biostimulants in maize development

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ABSTRACT

Phosphate rock is a finite resource, which underscores the need to improve phosphorus-use efficiency and sustainability in crop production. Therefore, this study aimed to evaluate the effect of rock dust combined with biostimulants on the development of maize (*Zea mays* L.). The experiment was conducted in a greenhouse at the Chapadão do Sul Campus using a randomized complete block design with seven treatments and seven replications: 1) Single superphosphate (control); 2) Rock dust (PR); 3) Rock dust + arbuscular mycorrhizal fungi (PR+AMF); 4) Rock dust + plant biostimulant (PR+EST); 5) Rock dust + *Azospirillum* (PR+AZO); 6) Rock dust + arbuscular mycorrhizal fungi + plant biostimulant (PR+AMF+EST); 7) Rock dust + *Azospirillum* + plant biostimulant (PR+AZO+EST). At the V9 stage of maize, the following variables were evaluated: plant height, stem diameter, leaf area, relative chlorophyll index, leaf dry mass, stem dry mass, root dry mass, leaf phosphorus, and percentage of mycorrhizal colonization. Compared with rock dust alone, the treatment combining rock dust with the plant biostimulant (PR+EST) resulted in higher stem diameter, leaf dry mass, stem dry mass, relative chlorophyll index, and leaf area. Furthermore, the treatments PR+AZO, PR+AMF+EST, and PR+AZO+EST showed the highest percentages of mycorrhizal colonization.

Keywords: Bioinputs, Mycorrhizal colonization, Soil microbiology.

Pó de rocha e bioestimulantes solubilizadores de fósforo no desenvolvimento do milho

RESUMO

O fosfato é um recurso finito e isso demanda mais eficiência de uso e sustentabilidade nos cultivos agrícolas. Diante disso, o presente estudo teve como objetivo avaliar o efeito do uso do pó de rocha associado a bioestimulantes no desenvolvimento do milho. O experimento foi conduzido em casa de vegetação no Câmpus Chapadão do Sul com delineamento em blocos casualizados, com sete tratamentos e sete repetições: 1) Superfosfato simples (testemunha- TEST); 2) Pó de Rocha (PR); 3) Pó de Rocha + Fungos Micorrízicos Arbusculares (PR+FMA); 4) Pó de Rocha + Produto fisiológico (PR+EST); 5) Pó de Rocha + *Azospirillum* (PR+AZO); 6) Pó de Rocha + Fungos Micorrízicos Arbusculares + Produto fisiológico (PR+FMA+EST); 7) Pó de Rocha + *Azospirillum* + Produto fisiológico (PR+AZO+EST). No estádio V9 do milho foram avaliadas as seguintes variáveis: altura da planta, diâmetro do colmo, área foliar, índice relativo de clorofila, massa seca de folha, a massa seca de colmo e a massa seca de raiz, fósforo foliar e a porcentagem de colonização micorrízica. Observou-se que para as variáveis diâmetro do colmo, massa seca de folha, massa seca de colmo, índice de clorofila e área foliar foram influenciadas pelo tratamento com o pó de rocha associado ao produto fisiológico (PR+EST), quando comparado à sua aplicação isolada. Além disso, os tratamentos PR+AZO, PR+FMA + EST e PR+AZO+EST apresentaram as maiores porcentagens de micorrização. A associação de bioestimulantes ao pó de rocha proporcionou resultados positivos no desenvolvimento do milho.

Palavras-chave: Bioinsumos, Micorrização, Microbiologia do solo.

1. Introduction

Maize (*Zea mays* L.) is a fundamental component of human and animal diets and plays a significant role in industry, particularly in biofuel production (Assaf et al., 2024; Sidahmed et al., 2025). However, with the growth of the global population and the livestock sector, agriculture faces the challenge of adopting more sustainable practices that preserve both soils and the environment (Shanmugam et al., 2024).

Due to its rapid growth cycle, maize cultivation requires substantial amounts of nutrients, a demand typically met through frequent applications of chemical fertilizers composed primarily of nitrogen (N), phosphorus (P), and potassium (K). In tropical soils, phosphorus availability is limited by adsorption onto iron and aluminum oxides and by precipitation reactions, making the element less accessible to plants (Paiva et al., 2022; Szerlag et al., 2022; Bedassa, 2023).

To compensate for this low availability, large quantities of phosphate fertilizers are applied, which increases production costs and contributes to soil and water contamination (Gatiboni et al., 2020). Consequently, phosphate scarcity has become a global concern, as the deposits from which these fertilizers are derived are finite resources (Illakwahhi et al., 2024). Therefore, it is necessary to adopt management strategies that maximize nutrient-use efficiency while simultaneously reducing the environmental impacts associated with intensive fertilizer application (Guelfi et al., 2022). Rock dust application has gained prominence as an alternative phosphate source; it involves the use of ground rocks that release nutrients in a slower, more controlled manner, offering an attractive solution for nutrient-demanding crops such as maize (Teles et al., 2020). However, its efficiency can be enhanced through biological interactions between plants and microorganisms. Microbial inoculation can play an important role in mobilizing residual soil phosphorus and phosphorus from applied materials (Timofeeva et al., 2022). Such microorganisms can contribute to phosphorus mobilization through mechanisms including the production of organic acids and enzymes that act on mineral-bound nutrients (Ahmed et al., 2025).

Arbuscular mycorrhizal fungi (AMF) enhance mineral nutrient acquisition by extending soil exploration through extraradical hyphae and transferring nutrients to host roots. They can also act synergistically with mineral-solubilizing bacteria, which produce organic acids and enzymes, thereby indirectly contributing to the solubilization process (Fall et al., 2022). This mechanism is particularly effective for acquiring elements with low mobility and high soil retention, such as phosphorus (P) (Han et al., 2025).

Another relevant group of microorganisms for maize cultivation consists of associative nitrogen-fixing bacteria of the genus *Azospirillum*. These bacteria promote plant growth by synthesizing growth-related hormones and enhancing resistance to stress and disease. They also solubilize phosphorus sources (Condori et al., 2024; Oliveira et al., 2025). Plant biostimulants derived from various organic materials have gained widespread use because they contribute to more sustainable and environmentally friendly agricultural practices (Batelja Lodeta et al., 2025). These products are considered a viable option because they alleviate biotic and abiotic stresses; they contain substances such as seaweed extracts, hormones, nutrients, plant growth regulators, and vitamins that aid in nutrient solubilization and promote healthy plant growth and development (Araújo et al., 2022). Consequently, they represent a promising alternative for mitigating negative effects that disrupt crop cycles, particularly those caused by abiotic factors (Almeida et al., 2020).

Given this context, the present study aimed to evaluate the effect of rock dust combined with biostimulants on maize development.

2. Material and Methods

The experiment was conducted in a greenhouse at the Federal University of Mato Grosso do Sul, Chapadão do Sul Campus (UFMS/CPCS), located in the municipality of Chapadão do Sul (18°46′17.7″ S, 52°37′27.7″ W; 813 m above sea level). According to the Köppen classification, the climate is tropical savanna (Aw), with a rainy summer and a dry winter.

The soil used in the pots was a Latossolo Vermelho distrófico from the CPCS experimental area, collected from the surface layer (0–20 cm); 5.2 kg of soil was used per pot. Chemical analysis yielded the following results: 31 mg dm^{-3} P (resin); 27 g dm^{-3} organic matter; pH 5.2 ($CaCL_2$); K, Ca, Mg, and H+Al = 2.2, 28, 7, and 30 mmolc dm^{-3}, respectively; Cu, Fe, Mn, and Zn (DTPA) = 1.5, 35, 1.6, and 2.5 mg dm^{-3}, respectively; 0.31 mg dm^{-3} B (hot-water extraction); and 55% base saturation.

The experimental design was a randomized complete block design with seven treatments and five replicates. The treatments consisted of combinations of rock dust with microbial inoculants or a plant biostimulant associated with phosphorus acquisition, plus a control receiving a soluble mineral phosphorus source. The treatments were: 1) single superphosphate (control); 2) rock dust (PR); 3) rock dust + arbuscular mycorrhizal fungi (PR + AMF); 4) rock dust + plant biostimulant (PR + EST); 5) rock dust + *Azospirillum* (PR + AZO); 6) rock dust + arbuscular mycorrhizal fungi + plant biostimulant (PR + AMF + EST); and 7)

rock dust + *Azospirillum* + plant biostimulant (PR + AZO + EST).

The seeds were immersed for one hour in a solution prepared by diluting 10 mL of the product in 1 L of distilled water. For the *Azospirillum* treatments, the product Azotrop was used, containing *Azospirillum brasilense* strains Ab-V5 and Ab-V6 at a concentration of 2×10^8 CFU mL^{-1}; the inoculant was applied at a rate of 100 mL per 60,000 seeds before sowing and thoroughly mixed with the seeds in plastic bags. For the arbuscular mycorrhizal fungi treatments, a soil-based inoculum was used, containing an average of 102 spores per 50 mL of soil (produced specifically for the experiment using native regional spores cultured on the host plant *Urochloa decumbens* for three months). At sowing, 50 mL of this inoculum was placed directly beneath the maize seeds.

The plant biostimulant used was FisiOn (from JC Selecta), composed of soy molasses, magnesium nitrate, and the following additives: 0.4% dispersing/emulsifying/surfactant agent; 1.5% stabilizing/preservative agent; 0.15% absorption enhancer and foliar protectant; complexing agents (hydroxycarboxylic acids and natural amino acids); seaweed extract; and water.

For the control treatment, single superphosphate fertilizer was used as the phosphorus source, applied at a rate of 100 kg ha^{-1} of P_2O_5. For the other treatments, rock dust was applied at a rate of 400 kg ha^{-1}; this source contained 6.07% P_2O_5.

Sowing involved placing four seeds per pot in 1-cm-deep holes, which were then covered with soil. The maize cultivar used was FS 575 PWU, characterized by an early maturity cycle and high yield potential. Basal fertilization was applied to all treatments at the following rates: 80 kg ha^{-1} urea, 150 kg ha^{-1} K_2O, 10 mg kg^{-1} soil zinc sulfate, 0.5 mg kg^{-1} soil boric acid, 5 mg kg^{-1} manganese sulfate, and 10 mg kg^{-1} soil magnesium sulfate.

The pots were arranged according to the randomized complete block design, with treatments randomized within each block. Throughout the experiment, the pots were irrigated twice daily.

At 19 days after emergence (DAE), a supplemental nitrogen dose was applied: 4.4 g diluted in 1 L of water, with 20 mL applied per pot, at the V4 growth stage. At 23 DAE, *Diabrotica speciosa* (green-yellow beetle) and *Dalbulus maidis* (corn leafhopper) were controlled by applying the insecticide Perito 970 SG (acephate), which acts by contact and ingestion; the solution was prepared at 2 g per 1 L of water, and 20 mL was applied per pot. At 41 DAE, when the plants were at the V9 stage, evaluations were performed for plant height (H), measured with a flexible tape from the plant base to the highest part of the plant, expressed in centimeters (cm) and stem diameter (SD), measured with a caliper, expressed in millimeters (mm). Leaf area (LA), expressed in square centimeters (cm^2), was calculated from leaf width and length measured with a tape. The relative chlorophyll index (CL) was assessed with a chlorophyll meter (ClorofiLOG CFL1030) on leaves from the upper and middle thirds of the plant during the morning.

After removing the plants from the pots, a 1-g sample of fine roots was collected to determine mycorrhizal colonization (MIC), following the method of Giovannetti and Mosse (1980). The remaining plant material was washed with water, separated into leaves, stems, and roots, placed in paper bags, and oven-dried at 65 °C for 72 h or until constant weight. Leaf dry mass (LDM), stem dry mass (SDM), and root dry mass (RDM) were determined using a precision balance and expressed as g pot^{-1}. After drying, a subsample of leaf tissue was ground for the determination of leaf phosphorus concentration according to Malavolta et al. (1989).

Data were subjected to analysis of variance (ANOVA), and, when the F-test detected significant treatment effects, means were grouped using the Scott-Knott test at the 5% probability level. Analyses were performed using SISVAR software (Ferreira, 2019).

3. Results and Discussion

Significant treatment effects were detected for stem diameter, leaf dry mass, stem dry mass, root dry mass, leaf area, relative chlorophyll index, and mycorrhizal colonization (Table 1). No significant differences among treatments were observed for plant height (H) or leaf phosphorus (LP).

The PR+EST treatment was notable for stem diameter, leaf dry mass, and stem dry mass, whereas the PR+AZO+EST treatment was notable for root dry mass (Table 2).

Although the highest stem diameter and leaf dry mass values were obtained in the control treatment (soluble P source), the PR+EST combination produced values 11% and 19% higher than those of the other treatments (Table 2). For stem dry mass, this same treatment showed a mean increase of 23% compared with the others, except for the control, suggesting a beneficial combined effect on maize development. In addition, greater biomass accumulation in the stem improves plant resistance to lodging and breakage (Boutahiri et al., 2024).

Table 1. Analysis of variance for the variables plant height (H), stem diameter (SD), leaf dry mass (LDM), stem dry mass (SDM), root dry mass (RDM), leaf area (LA), relative chlorophyll index (CL), leaf phosphorus (LP), and mycorrhizal colonization (MIC) in maize grown under different combinations of rock dust and phosphorus-cycling biostimulants.

SV	DF	H	SD	LDM	SDM	RDM
				MS		
Treatment	6	0.012^{ns}	12.45**	14.27**	14.51**	69.001**
Residual	34	0.006	0.639	0.896	1.152	8.780
Overall average	-	0.920	19.22	9.360	9.800	17.16
CV (%)		8.470	4.160	10.11	10.95	17.26
SV	DF	LA	CL	LP	MIC	
				MS		
Treatment	6	16070.7**	27.392**	0.066^{ns}	0.099**	
Residual	24	1519.0	7.2216	0.039	0.008	
Overall average	-	590.17	32.110	1.990	0.630	
CV (%)		6.600	8.3700	9.990	14.33	

**: significant at the 1% probability level by the F-test; *: significant at the 5% probability level by the F-test; ns: not significant; SV: source of variation; CV: coefficient of variation; DF: degrees of freedom; MS: mean square.

Table 2. Stem diameter (SD), leaf dry mass (LDM), stem dry mass (SDM), and root dry mass (RDM) under different combinations of rock dust and phosphorus-cycling biostimulants in maize cultivation.

Treatment	SD (mm)	LDM (g)	SDM (g)	RDM (g)
Control	22.08 a	12.67 a	12.70 a	21.72 a
Rock dust	18.95 c	8.65 c	9.59 b	13.85 b
PR+AMF	17.71 c	8.37 c	8.16 b	13.72 b
PR+EST	20.65 b	10.55 b	11.53 a	15.58 b
PR+AZO	18.83 c	8.99 c	9.10 b	16.48 b
PR+AMF+EST	18.17 c	7.80 c	8.19 b	15.78 b
PR+AZO+EST	18.15 c	8.51 c	9.36 b	23.02 a

Means followed by different letters in the column differ from each other according to the Scott-Knott test at the 5% significance level.

It is well established that rock dust acts as a slow-release nutrient source for plants (Ramos et al., 2022). Thus, combining it with the plant biostimulant used in this study, which contained, among other compounds, readily available carbon (molasses) and seaweed extract, may have facilitated nutrient solubilization (particularly phosphorus) through the action of soil microorganisms that produce organic acids and specific enzymes for this purpose (Massucato et al., 2016). Ali et al. (2021) reported that seaweed extracts act as biostimulants, influencing soil nutrient availability and enzymatic activity, which can enhance phosphorus nutrition. Additionally, the plant biostimulant contained magnesium nitrate, a source of magnesium and nitrogen that may promote plant growth by supporting photosynthetic activity and protein synthesis (Segatto et al., 2023).

Regarding root dry mass (RDM), the PR+AZO+EST treatment produced the highest value among the rock-dust-based treatments and was statistically similar to the control (Table 2). This performance can be attributed not only to the microbial activity intensified by the plant biostimulant but also to the action of *A. brasilense*, which can solubilize phosphorus (P) and produce compounds that promote root growth (Cassán et al., 2020). The presence of *Azospirillum* favors the development of finer roots and increases the absorptive surface area, contributing to greater root biomass accumulation (Mourouzidou et al., 2023). Root dry mass under PR+AZO+EST was 66.2% greater than under rock dust alone and 47.8% and 39.7% greater than under PR+EST and PR+AZO, respectively.

Although biostimulants do not replace the need for essential nutrients, they enhance mineral uptake efficiency, promoting leaf growth, stem diameter, and stem dry mass (Hamid et al., 2021). In treatments involving rock dust, biostimulants are particularly effective, promoting more efficient utilization of available nutrients (Corrêa et al., 2025). Thus, plant biostimulants represent a complementary and potentially sustainable strategy (Sun et al., 2024).

For the relative chlorophyll index (CL), all biostimulant combinations except PR+AMF produced higher values than rock dust alone and were statistically similar to single superphosphate (Table 3).

The PR+EST, PR+AZO, PR+AMF+EST, and PR+AZO+EST treatments increased the relative chlorophyll index by an average of 16.4% compared with the rock dust treatment. This increase, observed in treatments including biostimulants, can be attributed to the amino acids in the plant biostimulant, which provide metabolic support for pigment formation (Deveikytė et al., 2025), while seaweed extract enhances chlorophyll levels through betaines, nutrients, hormones, and

phenolic compounds that promote chlorophyll biosynthesis and delay its degradation (Spagnuolo et al., 2025).

Table 3. Relative chlorophyll index (CL), leaf area (LA), and percentage of mycorrhizal colonization (MIC) under different combinations of rock dust and phosphorus-solubilizing agents in maize cultivation.

Treatment	CL	LA (cm^2)	MIC (%)
Control	33.34 a	666.80 a	38.2 d
Rock dust	27.96 b	569.20 b	62.4 b
PR+AMF	29.64 b	530.60 b	65.6 b
PR+EST	33.58 a	631.40 a	54.6 c
PR+AZO	33.94 a	646.20 a	80.4 a
PR+AMF+EST	33.56 a	532.80 b	71.4 a
PR+AZO+EST	32.76 a	554.20 b	74.6 a

Means followed by different letters in the column differ from each other according to the Scott-Knott test at the 5% significance level.

For leaf area (LA), the PR+EST and PR+AZO treatments produced higher values than the other rock-dust-based treatments and were statistically similar to the control (Table 3). These responses may be associated with *A. brasilense* inoculation and/or with the seaweed extract contained in the plant biostimulant. *A. brasilense* enhances nutrient supply by fixing nitrogen and improving nutrient uptake through a more extensive root system (Santos et al., 2024). Seaweed extract stimulates maize root elongation, increasing the plant's capacity to support leaf growth (Ertani et al., 2018). It also directly stimulates growth-regulating pathways that drive cell division and expansion (Mendez et al., 2023).

All rock dust treatments increased the percentage of mycorrhizal colonization (MIC) compared with the single superphosphate treatment (Table 3). The highest values occurred in the PR+AZO, PR+AZO+EST, and PR+AMF+EST treatments, which had an average root colonization of 75.4%—more than 37 percentage points higher than the single superphosphate treatment. These results reinforce the beneficial interaction between microorganisms and biostimulants, which enhances the symbiotic association with mycorrhizal fungi.

According to Cozzolino et al. (2021), high levels of mycorrhizal colonization are crucial for plants grown in low-fertility soils because they increase nutrient uptake—particularly phosphorus—thereby promoting root development and overall plant growth. Conversely, in environments with high nutrient availability, such as the control treatment receiving single superphosphate, plants can regulate the establishment and extent of colonization by arbuscular mycorrhizal fungi (AMF) (Melloni et al., 2020). Shi et al. (2023) state that the bidirectional nutrient exchange during the symbiosis between plants and AMF follows a model in which both partners can exert control over their respective supplies. This bidirectional exchange of nutrients and carbohydrates, regulated by both partners, ensures a stable symbiotic relationship.

As expected, the use of single superphosphate, a soluble nutrient source, yielded satisfactory values for all variables evaluated in this study. However, when the natural rock dust source was combined with various biostimulants (including microbial inoculants and plant biostimulants), the results were comparable to those of the soluble source and superior to those of the rock dust treatment alone. These findings indicate that, within a sustainable cropping system, the use of plant biostimulants and microorganisms, such as *A. brasilense* and arbuscular mycorrhizal fungi, can compensate for the slow nutrient release associated with rock dust, thereby enhancing phosphorus uptake and promoting greater plant development (Etesami et al., 2021). These interactions, particularly those involving microorganisms, biostimulants, and alternative nutrient sources, require further investigation under both greenhouse and field conditions.

4. Conclusions

Selected combinations of rock dust with biostimulants improved stem diameter, leaf dry mass, stem dry mass, root dry mass, relative chlorophyll index, leaf area, and mycorrhizal colonization compared with rock dust alone. In particular, the PR+EST treatment resulted in higher stem diameter, leaf dry mass, stem dry mass, relative chlorophyll index, and leaf area. The PR+AZO, PR+AMF+EST, and PR+AZO+EST treatments resulted in the highest percentages of root mycorrhizal colonization.

Authors' Contribution

Conceptualization and methodology: Grazielle Nogueira da Silva, Evelyn Rodrigues de Lacerda; data collection and curation: Grazielle Nogueira da Silva, Evelyn Rodrigues de Lacerda; formal analysis: Grazielle Nogueira da Silva, Evelyn Rodrigues de Lacerda, Meire Aparecida Silvestrini

Cordeiro data interpretation: Grazielle Nogueira da Silva, Meire Aparecida Silvestrini Cordeiro, Sebastião Ferreira Lima; project administration: Grazielle Nogueira da Silva, Evelyn Rodrigues de Lacerda, Meire Aparecida Silvestrini Cordeiro; supervision: Meire Aparecida Silvestrini Cordeiro, Sebastião Ferreira Lima; preparation of the original draft: Grazielle Nogueira da Silva, Evelyn Rodrigues de Lacerda; writing -revision and editing: Grazielle Nogueira da Silva, Evelyn Rodrigues de Lacerda, Meire Aparecida Silvestrini Cordeiro, Sebastião Ferreira Lima. All authors read and approved the final version of the manuscript.

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