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Efficiency of phosphate fertilizers containing growth promoters in plant fertilization

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ABSTRACT

Optimizing water and nutrient absorption in plants can be achieved through a practical strategy: the combination of plant growth promoters (PGPs) with phosphate fertilizers. This synergistic approach holds promise for enhancing agricultural productivity and sustainability. The objectives of this work were to produce new phosphate fertilizers containing PGPs and to evaluate the efficiency in promoting the growth of the roots and shoots and increased phosphorus (P) uptake of plants. The fertilizers produced contained reactive natural phosphate rock from Morocco and PGPs indole-3-butyric acid, humic substances or *Trichoderma afroharzianum*, and a control fertilizer, without adding PGPs. The agronomic efficiency of this fertilizers were evaluated in two contrasting soils. The additional treatment with the monoammonium phosphate was included. The experimental unit consisted of rhyzobox containing 350 cm^3 of soil, where a soybean plant was grown. Thirty days after sowing, the root length, average root diameter, root tips, and fine roots were measured using WinRHIZO software. The dry matter and P content of the roots and shoots were determined. PGPs have a great potential to increase the efficiency of P absorption by plants and the photosynthetic rate, and have a great potential to increase phosphorus use efficiency. The effects of PGPs were observed in the architecture of the root system, leaf area, and increased P content by plants. In sandy soil, the application of phosphate rock containing humic substances, *Trichoderma afroharzianum*, and indole-3-butyric increases the total phosphorus uptake by 26.5%, 16.3%, and 12.2%, respectively.

1. Introduction

The limitation of P availability in very weathered soils, due to specific adsorption in Fe and Al oxyhydroxides (Novais et al., 2007) makes the nutrient less mobile in the soil, making it difficult for plant absorption (Silva and Delatorre, 2009). Research shows that the P absorption by plants does not exceed 15–20% of the total P applied to the soil through soluble phosphate fertilizers, such as monoammonium phosphate (MAP) (Zhan et al., 2004). The exploration of a larger volume of soil and the interception of roots are mechanisms used by plants to increase the efficiency of absorption of P (Novais and Mello, 2007). Therefore, it is very important to in-

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crease the efficiency of P absorption by plants from phosphate fertilization management strategies or new technologies, such as the association of plant growth promoters (PGPs) and phosphate fertilizers.

The PGPs are considered substances produced exogenously from plants, which influence the physiological process of growth (cell elongation, division, and differentiation), through an action analogous to auxin, cytokinin, and gibberellin hormones (Silva et al., 2007; Taiz et al., 2017). Several synthetic or natural substances, such as indole-3-butyric (IBA) and humic substances (HS), are analogous to auxins action and can be considered PGPs. IBA is considered a synthetic PGP widely used in the rhizogenic induction process (Damodaran and Strader, 2019; Ferriani et al., 2006; Miranda et al., 2004). HS are natural substances composed of humic acid, fulvic acid, and humine, the action on cell expansion and rooting being attributed to fulvic acid (Borcioni et al., 2016; Canellas et al., 2009; García et al., 2019). Humic and fulvic acids have other actions in the soil/plant system; these substances are also considered physical, chemical, and microbiological conditions of soils, reduce stresses in plants, stimulate the synthesis of enzymes related to the cell wall, and can stimulate seed germination (Caron et al., 2015; García et al., 2019). Similar to IBA and HS, some microorganisms can be considered PGPs because their metabolites can act as plant hormones.

The PGPs promote the growth and development of plants through direct mechanisms involving the synthesis of phytohormones (indole-3-acetic acid, cytokinins, ethylene, and gibberellins), siderophores, and ACC deaminase, nitrogen fixation, phosphorus and potassium solubilization. Additionally, they indirectly promote plant growth through induced systemic resistance, production of siderophores, antibiotics, exopolysaccharides, volatile oils, lytic and protective enzymes (Singh et al., 2019).

The PGPs are studied to promote the development of the root system of different cultures and increase the efficiency of nutrient absorption (Borcioni et al., 2016; Chagas et al., 2017; Lana et al., 2009). They can act by solubilizing phosphates precipitated in host plants through the synthesis of organic acids or protons (Paredes and Lebeis, 2016; Verma et al., 2007), chelation, and substitution interactions (Hameeda et al., 2008).

Plant growth-promoting fungi (PGPF) have the potential to enhance nutrient absorption from the soil by plants and facilitate the decomposition of organic matter (Khalil et al., 2015). These microorganisms can be employed to boost the defensive capabilities and physiological immunity of plants (Etesami and Maheshwari, 2018; Khan et al., 2019). PGPF can produce chemical compounds with various benefits for the plant, such as HCN, an antifungal compound that acts in biological control, based on its attributed toxicity against plant pathogens (Ali et al., 2020; Attia et al., 2020). Studies by Attia et al. (2022) demonstrate that PGPFs exhibited antifungal activity for the *in vitro* biocontrol of *Fusarium* wilt in tomato plants; and in vivo results confirmed that all PGPFs have the ability to stimulate the growth of healthy tomato plants and enhance the efficiency of tomato immunity against *Fusarium* wilt.

The fungi of the genus *Trichoderma* stand out because of their capacity to colonize roots of plants and produce metabolites that show an action similar to plant hormones of the auxins group (Cubillos-Hinojosa et al., 2009; Vinale et al., 2008). In addition, *Trichoderma* spp. they are widely studied and used as biocontrol agents for diseases caused by different pathogens, such as *Verticillium, Phytophthora, Pythium, Sclerotinia, Fusarium*, and *Botrytis cinerea* (Abdelaziz et al., 2022; Harman, 2006; Isaias et al., 2014; Khan et al., 2020; Oliveira et al., 2021; Rajani et al., 2021). The metabolites of these fungi can also act as phosphate solubilizers and increase the availability of P for plants (Bononi et al., 2020).

PGPs are commonly used in very small concentrations just like phytohormones (du Jardin, 2015). Therefore, the combination of these substances and phosphate fertilizers can also guarantee homogeneity in the granule and easier application in field conditions.

Biodegradable polymers, such as sodium alginate, have been used as a matrix to synthesize beads containing nutrients with different properties (Castro et al., 2020; Ni et al., 2010; Shan et al., 2016; Wang et al., 2012), due to its low cost, ease of manipulation and chemical stability (Ni et al., 2010; Shan et al., 2016; Wang et al., 2012). This polymer can aggregate several compounds through the extrusion process of the polymeric matrix, containing compounds of interest, in a solution of divalent cations (Baki and Abedi-Koupai, 2018; Gombotz and Wee, 1998; Kay, 2004; Paula et al., 2010; Salmieri and Lacroix, 2006). Thus, alginate polymer can be an alternative to synthesize new phosphate fertilizers containing PGPs with different physical, chemical, and biological properties compared to the single materials. The synthesis of alginate beads containing phosphate fertilizers and PGPs is still incipient and has not been reported in the literature.

The efficiency of phosphorus absorption from low-solubility sources may increase with the enhancement of plant root growth, both due to the greater absorption surface area and the capacity to modify the rhizosphere for phosphorus acquisition. Thus, the objectives of this work were to produce new phosphate fertilizers containing PGPs and to evaluate their efficiency in promoting the growth of the roots and shoots and increasing the P uptake by plants.

2. Material and methods

2.1. Synthesis of fertilizers

The synthesis of alginate beads containing phosphate fertilizers and PGPs was performed by the complex coacervation method, adapted from Zhang et al. (2014). First, 50 mL of the solution of alginate in water (20 g L^{-1}) were prepared and kept under constant stirring for 30 min at 25 °C. Immediately afterward, were added in this solution, 10 g of PR (ground and passed through a 48 mesh sieve), 1.0 g of activated charcoal, and the PGPs indole-3-butyric acid – IBA (PR_IBA), humic substance – HS (PR_HS), or *Trichoderma afroharzianum* – TA (PR_TA) in concentrations 2.91 mg kg^{-1} for IBA, 0.48 dag kg^{-1} for HS or 4.13×10^{10} conidia kg^{-1} for TA. The suspension was kept under constant agitation during the production of fertilizers. Then, the suspension was extruded, using a dropper, into the beaker containing 100 mL of $Ca(NO_3)_2 \cdot 4H_2O$ (5 % w/v), immediately forming the fertilizer spheres. The fertilizers were separated from the solution using a sieve, placed on a clean surface, and dried at room temperature (25 °C) for 48 h. The synthesis of PR_TA was carried out in sterile conditions to avoid contamination. For this purpose, a laminar flow chamber, and autoclaved solutions and materials were used. The fertilizer without PGPs (PR_CONTROL) was produced using the same methodology as described above with only PR and activated charcoal. The images of the synthesized fertilizers are presented in Fig. 1.

The IBA (purity = 99 %) was purchased from Sigma-Aldrich and the HS were used in the form of Leonardite. The fungus used in this research was isolated from a cadaver of plant parasitic nematode. To identify this isolate, was used 3 regions: ACT, ITS and TEF1-α. The *Trichoderma afroharzianum* was obtained from the Phytonematodes Control Laboratory of the Federal University of Viçosa in the form of mycelium grown in a Petri dish for seven days. The fungus was added in a bag with a biological filter containing 200 g of rice and 30 mL of water, both autoclaved. After ten days, 10 g of colonized rice were added to a beaker containing 15 mL of distilled and autoclaved water. Then, the suspension was stirred and filtered using sterile gauze. The aliquot of the filtrate corresponding to 1 mL was transferred to a beaker containing 9 mL of water and the concentration of conidia was determined through a Neubauer chamber.

All reagents used in this work have a degree of analytical purity. Sodium alginate ($(C_6H_7(NaO)_6)_n$, purity = 90%) was purchased from Dinâmica Quimica Contemporanea Ltda. $Ca(NO_3)_2.4H_2O$ (purity >99%) was purchased from Vetec. Activated charcoal was purchased from Vetec. The water used in the synthesis reactions was distilled and deionized (Milli-Q® system).

2.2. Characterization of fertilizers

Moisture content, pH, and salt index were determined according to (Brasil, 2017). For total P concentration analysis, samples of 0.5 g of fertilizers were added to Falcon tubes (50 mL) containing 5 mL of HNO_3 concentrate and 25 mL of deionized water. Then, the tubes were sealed and stirred on a shaking table at 60 rpm for 1 h. The extracts were filtered with rapid quantitative filter paper (125-μm pore sizer) and aliquots were used to determine P by a colorimetric method (Braga and Defelipo, 1974).

Sodium alginate, activated charcoal, phosphate rock, PR_CONTROL, PR_IBA, PR_HS, and PR_TA, were characterized by scanning electronic microscopy (SEM). For SEM analyses, the precursor materials (powder form) and fertilizers synthesized were supported on conductive double-sided adhesive tape and a Zeiss EVO 50 scanning electron microscope was used.

2.3. Viability of Trichoderma afroharzianum

The survival test of the TA in the fertilizers was carried out by the "*in vitro*" method. The test was performed by adding one granule of PR_TA fertilizer in the central region of Petri dishes containing agar-water (2 %) culture medium with antibiotic Streptomycin sulfate salt from Sigma-Aldrich. The survival of the TA was evaluated after 1, 10, 20, 30, 40, and 50 days of fertilizer production with four replicates.

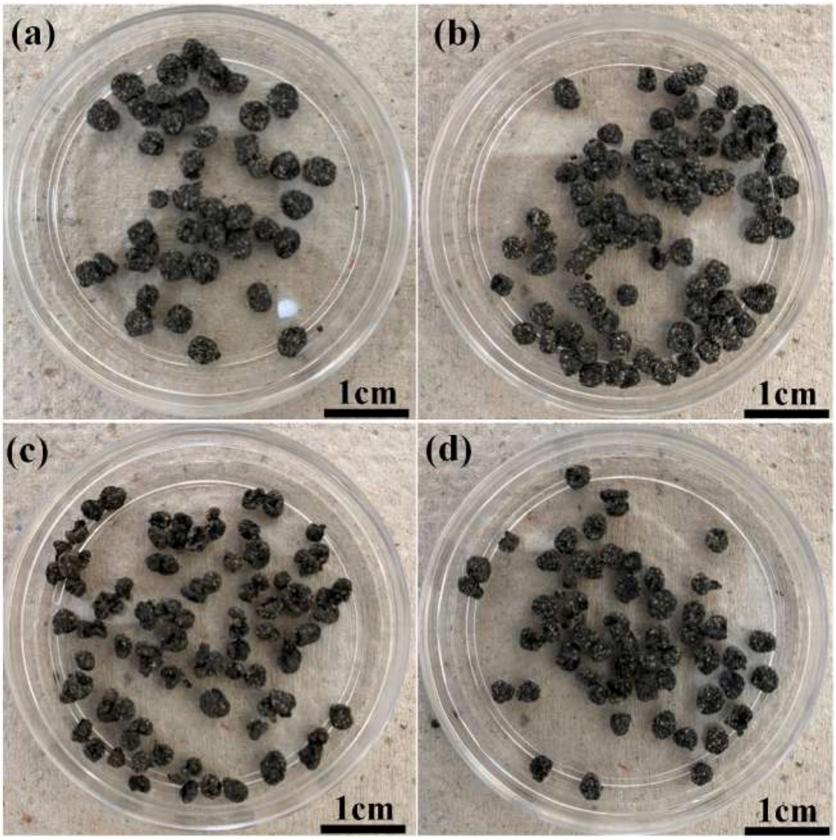

Fig. 1. Images of PR_CONTROL (a); PR_IBA (b); PR_HS (c); and PR_TA (d) fertilizers.

The Petri dishes were stored at 25 °C in an incubation chamber and the survival of the fungus was evaluated through the presence or absence of hyphal growth after five days of incubation. All process was performed under sterile conditions.

2.4. *Rhizobox test*

A rhizobox experiment was carried out to evaluate root growth with the application of phosphate fertilizers containing PGPs. The assay was performed using a [(3 + 1+1) × 2] factorial scheme: three fertilizers with PGPs (PR_IBA, PR_HS, and PR_TA), a control fertilizer, without PGPs (PR_CONTROL), an additional treatment, using monoammonium phosphate (MAP) fertilizer; and two contrasting soils (clayey and sandy soil). The treatments were distributed in a randomized block design, with five replications. The experimental unit consisted of a rhyzobox (Fig. 2) containing 350 cm^3 of soil, where a soybean plant ('BMX Ipro 8579') was cultivated.

Initially, 1.9 and 1 g dm^{-3} of a mixture of $CaCO_3$ and $MgCO_3$ (4:1) were applied in the clayey and sandy soil, respectively. The soil moisture was maintained at 70% of the field capacity for thirty days. Subsequently, the soils were air-dried, ground, and sieved (2 mm), and samples were collected for chemical and physical characterization (Table 1).

The rhyzobox was filled with soils and the fertilizers were applied 3 cm deep at three equidistant points. For PR_IBA, PR_HS, PR_TA, and PR_CONTROL fertilizers, a complementary P fertilization was carried out with a soluble source (MAP) to ensure an adequate initial supply of phosphorus to the plants. For example, 16.8 mg dm^{-3} of P was supplied by PR contained in the fertilizers produced, and, 11.5 mg dm^{-3} of P was supplied by MAP. Moreover, in the additional treatment, with the only MAP, a total dose of 28.3 mg dm^{-3} of P was established.

The concentrations of N in the treatments containing PR_IBA, PR_HS, PR_TA, and PR_CONTROL were balanced with urea (13.7 mg dm^{-3} N), according to the concentration of N in the treatment fertilized with MAP (6.3 mg dm^{-3} N). One soybean seed was sown at 0.5 cm depth in the rhyzobox. During the experiment, the sides of the rhyzobox were covered with aluminum foil and the soil moisture was maintained at 60 % of the field capacity using deionized distilled H_2O.

A basal nutrient solution was applied, giving K (66 mg dm^{-3}), S (30 mg dm^{-3}), B (0.8 mg dm^{-3}), Zn (3 mg dm^{-3}), Cu (1.3 mg dm^{-3}), Mn (3.6 mg dm^{-3}), and Mo (0.2 mg dm^{-3}). The inoculation with *Bradyrhizobium japonicum* was done through the application of the commercial product Masterfix L® (300 mL ha^{-1}).

Thirty days after emergence (DAE), the aluminum foil was removed from the sides of the rhyzobox and the root and shoot development were photographed. Subsequently, plant height and leaf area were measured using a ruler and *LI-COR* LI^{-3}100AC area meter, respectively. The roots were removed from the soil, washed, and conditioned in a solution containing 40 % of alcohol in water (v/v). Subsequently, the root system was scanned in Scanner Epson Expression 11,000 XL, and the total length, fine roots (diameter less than 0.5 mm), root tips, and average diameter were measured using WinRHIZO Pro software.

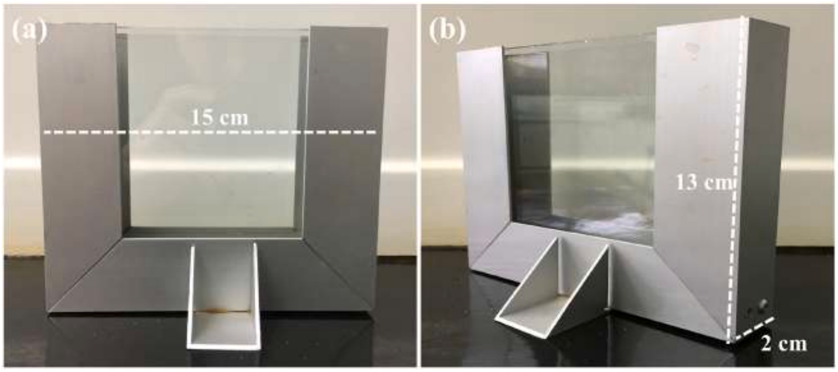

Fig. 2. Images of Rhyzobox in a front (a) and side (b) view.

Table 1
Physical and chemical soil characteristics after liming.

Soil characteristic	Sandy soil	Clayey soil
Sand (g kg^{-1})	821	363
Silt (g kg^{-1})	25	106
Clay (g kg^{-1})	154	531
pH (water)	6.5	5.9
P (mg dm^{-3})	0.3	0.2
Al^{3+} ($cmol_c$ dm^{-3})	0.0	0.0
m (%)	0.0	0.0
OM (dag kg^{-1})	1.3	1.5
P-rem (mg L^{-1})	19.1	6.2

pH: soil/water ratio of 1:2.5; P: Mehlich-1 extractant; Al^{3+}: 1.0 mol L^{-1} KCl extractant; m: saturation by aluminum; OM: Organic matter content (Walkley and Black, 1934); P-rem: remaining phosphorus.

Shoots and roots were dried in a forced-air oven at 60 °C for 72 h, weighed, and ground. Plant samples were subject to perchloric nitric digestion (v/v 4:1) to determine total P in shoots and roots (Braga and Defelipo, 1974).

The data were subjected to analysis of variance and Scott Knott test of means ($p < 0.5$). The R environment was used in the statistical analysis (R Core Team, 2020).

3. Results

3.1. Characterization of fertilizers

Physical (moisture content) and chemical (pH and salt index) characterizations (Table 2) showed that all synthesized fertilizers had characteristics like the PR. The total P content in the synthesized fertilizers was slightly lower than in PR due to the addition of other raw materials in these fertilizers.

The morphology of the synthesized fertilizers and the materials used in the synthesis were analyzed by SEM (Fig. 3). SEM images of PR_CONTROL, PR_IBA, PR_HS, and PR_TA showed microspheres with diameters varying between 2500 and 3000 mm (Fig. 3a, b, c and d). The surface images of the morphology of all fertilizers indicated the distribution of raw materials (active charcoal and PR) in the alginate polymeric network (Fig. 3e, f, g and h), which can be identified and compared with surface images of the morphology of the alginate, activated charcoal and PR (Fig. 3j and k).

The cationic exchange in the fertilizer synthesis process (Na^+ present in sodium alginate “in natura” by Ca^{2+} in the solution used in the synthesis of fertilizers), alters the structure of sodium alginate, turning it into a gel. Therefore, it is not possible to identify the “in natura” structure of the polymer (Fig. 3i) in the SEM image (Fig. 3e, f, g and h).

3.2. Viability of *Trichoderma afroharzianum* in fertilizer

The viability test of *Trichoderma afroharzianum* in fertilizer showed, through the growth of hyphae in the Petri dish, that the microorganism survived all times evaluated (Fig. 4). Visually, the fungus germination was higher in the first incubation times (1, 10, 20, and 30 days after synthesis of fertilizers). From the fortieth day, the presence of hyphae began to decrease, although there was still the development of fungi in the Petri dish.

Table 2
Characterization of fertilizers containing phosphate rock (PR) and growth plant promoters: indole-3-butyric (PR_IBA), humic substances (PR_HS), or *Trichoderma afroharzianum* (PR_TA), and control fertilizer, without plant growth promoters (PR_CONTROL).

Fertilizer	Moisture content	Total P	pH	Salt index
	dag kg^{-1}	dag kg^{-1}	-	%
PR	3.1	11.2	6.7	4.5
PR_CONTROL	3.3	10.0	6.6	3.8
PR_IBA	4.3	9.3	6.7	3.9
PR_HS	3.2	9.7	6.5	4.2
PR_TA	4.4	9.7	6.5	4.1

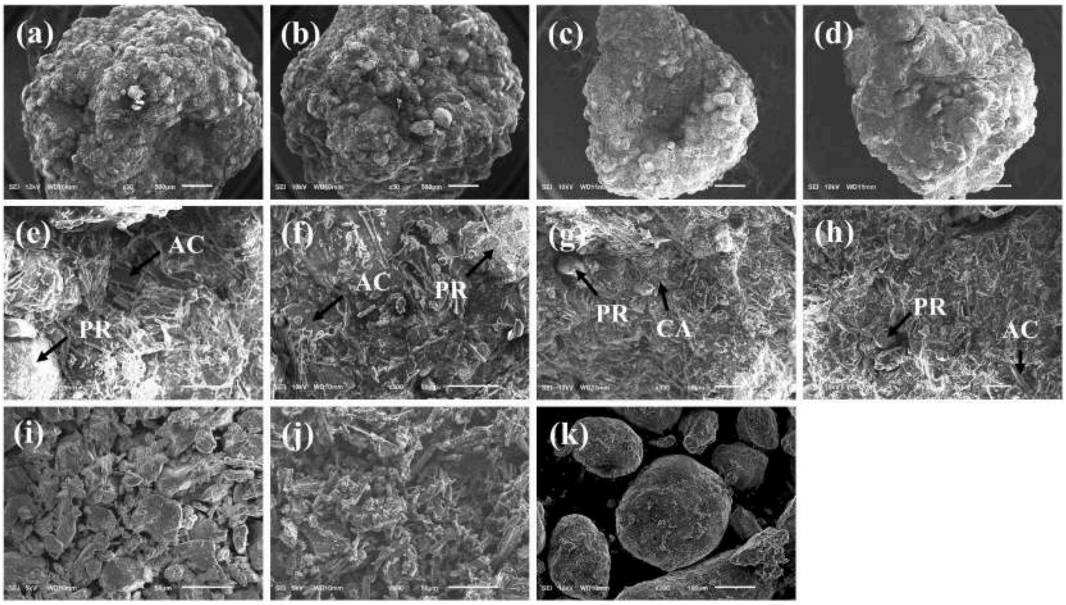

Fig. 3. SEM images of the intact microspheres of PR_CONTROL (a); PR_IBA (b); PR_HS (c) and PR_TA (d); and surface morphology of PR_CONTROL (e); PR_IBA (f); PR_HS (g) and PR_TA (h); sodium alginate “in natura” (i); charcoal activated (j); phosphate rock (k). PR: Phosphate rock; AC: activated charcoal.

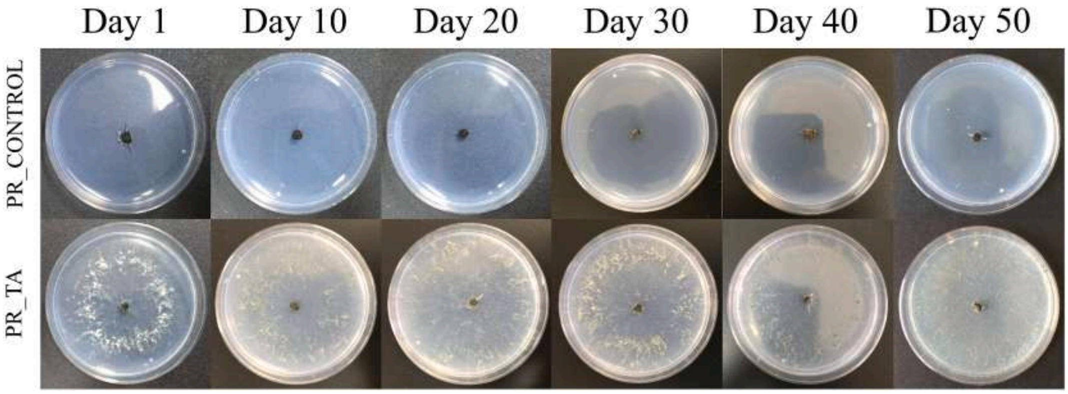

Fig. 4. *Trichoderma afroharzianum* survival test in a petri dish containing agar-water culture medium (2%) with antibiotic Streptomycin sulfate salt at 1, 10, 20, 30, 40 and 50 days after fertilizer synthesis. PR_CONTROL: Control fertilizer without adding plant growth promoters. PR_TA: Fertilizer containing phosphate rock and *Trichoderma afroharzianum.*

3.3. Rhyzobox test

Soybean root growth was visualized using the rhyzobox observation technique (Fig. 5). In sandy soil, root growth was significantly lower when plants were fertilized with PR_CONTROL compared to PR_IBA, PR_HS, PR_TA, and MAP. For the clayey soil, there were no visual differences between fertilizer treatments.

The parameters used to assess shoot and root development and P uptake are shown in Table 3. In sandy soil, the plants showed higher shoot length when fertilized with PR_IBA and PR_TA in comparison with PR_CONTROL, PR_HS, or MAP. The leaf area was higher when the PGPs were used. However, there were no significant differences in shoot dry matter between treatments in sandy soil.

Fertilizers also differently influenced the development and morphology of the root systems of plants in sandy soil. Plants fertilized with PR_IBA had a longer root length, while the PR_HS promoted the same increase as MAP, and PR_TA promoted root length greater than the PR_CONTROL. The fine roots were higher in fertilizations with PR_TA and MAP, consequently, the average diameter was smaller for the treatment containing PR_TA. In this soil, plants developed more root tips when they were fertilized with PR_HS and PR_TA compared to other P sources. The fertilization with PR_IBA promoted a greater development of root tips than PR_CONTROL. However, the production of root dry matter was higher when we used the MAP, followed by PR_HS.

Shoot P uptake was higher when the plants were fertilized with MAP followed by PR_HS, and both sources showed higher shoot P uptake than PR_CONTROL, PR_TA, and PR_IBA for the cultivation on sandy soil. In the root system, all treatments with fertilizers containing PGPs increased the content of P relative to other sources of P. The sum of P shoot and root uptakes resulted in a total P uptake that was higher in treatments fertilized with PR_HS and MAP. In this soil, PR_TA and PR_IBA had higher P uptake than the PR_CONTROL.

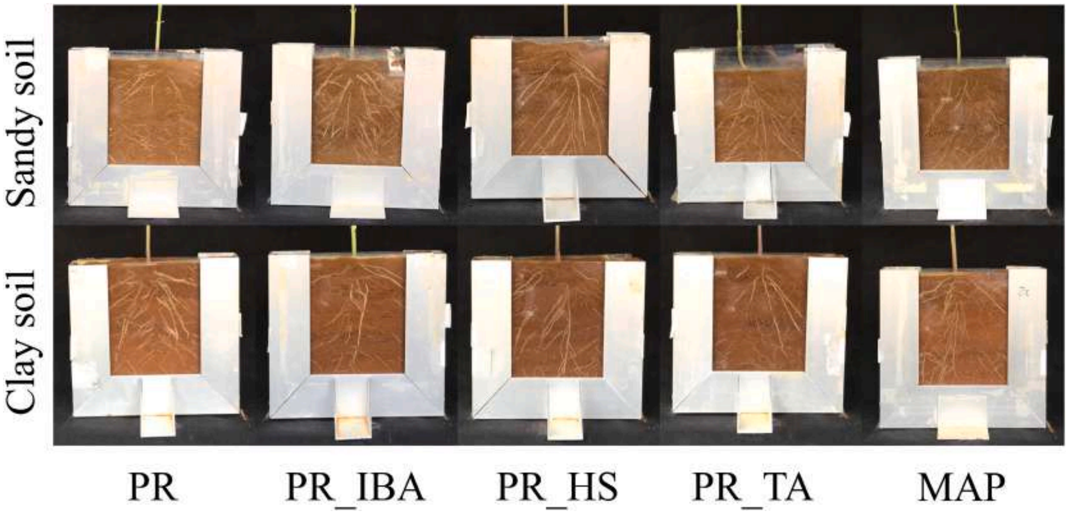

Fig. 5. Images of soybean root development in sandy and clayey soil. PR_CONTROL: Control fertilizer without adding plant growth promoters. PR_IBA: Fertilizer containing phosphate rock and indole-3-butyric acid. PR_HS: Fertilizer containing phosphate rock and humic substances. PR_TA: Fertilizer containing phosphate rock and *Trichoderma afroharzianum.* MAP: monoammonium phosphate.

Table 3

Shoot length, leaf area, shoot dry matter, root length, fine root, average diameter, root tips, root dry matter and shoot, root and total P uptake for phosphate rock fertilizer without PGP's (PR_CONTROL), fertilizers containing phosphate rock and with PGP's indole-3-butyric acid (PR_IBA), humic substances (PR_HS), or *Trichoderma afroharzianum* (PR_TA) and MAP, and mean values, for the sandy and clayey soil.

Parameter	PR_CONTROL	PR_IBA	PR_HS	PR_TA	MAP	Average
Sandy soil						
Shoot length (cm)	12.0 b	12.8 a	12.1 b	12.7 a	12.0 b	12.3 A
Leaf area (cm^2)	99.1 b	104.7 a	108.9 a	111.6 a	84.8 c	101.8 B
Shoot dry matter (g)	0.52 a	0.51 a	0.52 a	0.53 a	0.51 a	0.50 B
Root length (cm)	3546 d	4479 a	4185 b	3808 c	4294 b	4063 B
Fine root (%)	82.9 c	86.7 b	82.7 c	88.7 a	89.7 a	86.1 A
Avg root diameter (mm)	1.9 b	1.8 b	2.2 a	1.6 d	1.7 c	1.9 B
Root tips	4215 d	4793 c	6224 a	5935 a	5223 b	5278 B
Root dry matter (g)	0.39 c	0.41 c	0.48 b	0.43 c	0.51 a	0.35 B
Shoot P uptake (mg $plant^{-1}$)	0.40 c	0.41 c	0.48 b	0.41 c	0.51 a	0.45 B
Root P uptake (mg $plant^{-1}$)	0.10 b	0.14 a	0.13 a	0.14 a	0.09 b	0.12 B
Total P uptake (mg $plant^{-1}$)	0.49 d	0.55 c	0.62 a	0.57 b	0.61 a	0.57 B
Clayey soil						
Shoot length (cm)	12.2 b	11.2 c	11.2 c	12.1 b	15.5 a	12.2 A
Leaf area (cm^2)	122.5 b	73.6 d	123.1 b	109.1 c	166.41 a	119.0 A
Shoot dry matter (g)	0.56 b	0.48 c	0.59 b	0.57 b	0.90 a	0.62 A
Root length (cm)	4190 b	3613 c	4364 b	4355 b	5959 a	4496.7 A
Fine root (%)	80.7 d	89.3 a	87.93 b	86.2 c	90.0 a	86.7 A
Avg root diameter (mm)	2.6 a	2.4 b	2.4 b	2.2 b	2.5 b	2.4 A
Root tips	6846 c	4545d	10,173 b	7281 c	13,064 a	8382 A
Root dry matter (g)	0.40 d	0.48 b	0.43 c	0.43 c	0.79 a	0.39 A
Shoot P uptake (mg $plant^{-1}$)	0.40 d	0.48 b	0.43 c	0.43 c	0.79 a	0.51 A
Root P uptake (mg $plant^{-1}$)	0.13 c	0.06 d	0.16 b	0.13 c	0.27 a	0.15 A
Total P uptake (mg $plant^{-1}$)	0.53 c	0.55 c	0.60 b	0.56 c	1.0 a	0.65 A

Avg root diameter: Average root diameter. Values followed by the same lowercase letter within lines indicate that the mean is not significantly different at $p < 0.05$ according to Scott Knott test between P fertilizer sources. Lowercase letters, comparison of a parameter between fertilizers and capital letters, of a parameter between soils.

In clayey soil, all parameters evaluated for shoot and root development and P uptake were better fertilizing plants with MAP, except for the fine root and average diameter. Fertilization with PR_IBA promoted effect significantly equal to the MAP for fine roots, followed by PR_HS and PR_TA, which resulted in a higher proportion of fine roots than PR_CONTROL. All fertilizers reduced the average diameter when compared to PR_CONTROL and there were no significant differences among them.

Still, in the clayey soil, the root tips of plants fertilized with PR_IBA were higher than PR_CONTROL and the other fertilizers containing PGPs. In this same reasoning, all fertilizers containing PGPs increased shoot P uptake relative to the PR_CONTROL. The root P and total P uptake were greater than the control when the plants were fertilized with PR_HS.

Generally, the shoot and root development of plants, accessed from the means of the values of the parameters presented in Table 3, were greater in the clayey soil compared to sandy soil. Only shoot length and fine roots were statistically equal for the two soils studied.

4. Discussion

The architecture of the root system plays a pivotal role in influencing nutrient uptake from the soil, with P absorption being particularly impacted by factors such as root growth, root average diameter, and the presence of adventitious roots (fine roots). These characteristics are responsible for increasing the contact between plants and soil, contributing significantly to nutrient acquisition (Crusciol et al., 2005; Silva and Delatorre, 2009; Vejchasarn et al., 2016). In sandy soil, characterized by lower phosphorus adsorption capacity, the influence of root architecture on P uptake becomes even more pronounced. In our study, the application of PGPs led to discernible alterations in root architecture in sandy soil, promoting increased contact between the roots and the soil matrix. This was accompanied by a notable enhancement in the root interception mechanism, as outlined in Table 3.

Our findings provide strong evidence for the efficacy of PGPs in enhancing the efficiency of phosphorus absorption compared to fertilization without PGPs, as indicated by the data presented in Table 3. This underscores the potential of PGPs not only in influencing root characteristics but also in positively impacting phosphorus uptake. Such effects contribute significantly to the improvement of phosphorus use efficiency, especially in environments with limited phosphorus availability, such as sandy soils. In addition, the fertilizer PR_HS demonstrated comparable efficiency to the soluble P source (MAP) used in this study, highlighting its effectiveness. Furthermore, fertilizers containing PGPs (PR_IBA and PR_TA) exhibited increased efficiency compared to fertilizers without PGPs. These results position these PGP-containing fertilizers as promising alternatives to enhance phosphorus absorption by plants.

The utilization of enhanced efficiency fertilizers, as investigated in this study, presents a promising avenue for reducing doses of soluble P sources. Our findings demonstrate a significant reduction in the required doses of soluble P sources, in was possible to replace 60% of the P dose derived from a readily soluble source, such as monoammonium phosphate (MAP), with a lower solubility alternative (PR). Notably, the supplementary P dose (11.5 mg dm^{-3}) provided in the form of MAP proved sufficient to ensure optimal P levels in the plants. This result holds particular significance for Brazil, a country heavily dependent on imported phosphate fertilizers (Dias et al., 2020; Ogino et al., 2021). With approximately 63% of P sources used in agriculture relying on imports (IFA, 2018), the ability to reduce the dosage of soluble P sources without compromising plant development and production emerges as a strategic approach. This not only addresses the challenge of external dependence on P sources but also contributes to cost reduction in fertilization practices. In essence, our study suggests that optimizing P application through the use of improved efficiency fertilizers not only enhances nutrient uptake by plants but also holds promise for mitigating the economic and strategic challenges associated with phosphorus dependency. By embracing such strategies, agriculture can move towards greater sustainability by reducing external dependence, lowering costs, and ensuring the efficient utilization of phosphorus resources.

The limited response of root development to PGPs in clayey soil may be associated with the adsorption process of molecules in soil colloids, similar to the interaction observed with auxinic herbicide molecules such as 2,4-D (Vieira et al., 1999). The authors suggest that the availability of these molecules can be influenced by the content of soil clay, minerals, organic matter, as well as Fe and Al oxyhydroxides. In the clayey soil utilized in our study, which had a clay content of 531 g kg^{-1}, it is plausible that the availability of PGPs for plants was lower than the optimal concentrations defined, consequently reducing their effects on plant development. It's important to note that the impact of PGPs extends beyond the root system alone. The stimulation of aerial part development can result in an increase in leaf area, which is crucial for photosynthesis. This, in turn, enhances various parameters such as transpiration intensity, absolute and relative growth rates, liquid assimilation rate, and leaf area index. These factors directly influence the overall productive performance of plants (Magalhães, 1979). Therefore, the complex interplay between soil characteristics and PGP availability the need for a comprehensive understanding of the soil environment to optimize the benefits of PGPs in plant development.

This study introduces an innovative and efficient method for utilizing substances that influence plant physiological processes more effectively. The granulation process of PGPs with the P source, as presented in this work, represents a groundbreaking approach to producing improved efficiency fertilizers. This innovative method not only enhances the efficiency of phosphate fertilization but also improves the absorption of phosphorus by plants, ultimately leading to enhanced plant development. The diverse functions of PGPs broaden the potential applications of the fertilizers developed in this study. Humic substances, known for their diverse functional groups, offer a wide spectrum of reactivity in plants (Caron et al., 2015). *Trichoderma afroharzianum*, included in the formulation, serves as a biological control agent against fungal and nematode diseases (Loureiro et al., 2020). These secondary actions further enhance the attractiveness of this technology.

The chemical and physical characteristics of the fertilizers (Table 2 and Fig. 3) indicate a homogeneous distribution of raw materials in the granules. This finding aligns with previous research by (Castro et al., 2020) on beads of sodium alginate containing layered double hydroxide intercalated with borate. Therefore, the synthesis of fertilizers with sodium alginate using the complex coacervation method proves to be an efficient approach for producing improved efficiency fertilizers with a uniform distribution of raw materials, even at low concentrations such as PGPs. Additionally, the described fertilizer synthesis process in this work ensures a significant shelf life for the fertilizer containing the fungus, further enhancing its practicality and applicability.

5. Conclusions

Plant growth promoters have a great potential to increase phosphorus use efficiency. The effects of PGPs were observed in the architecture of the root system, leaf areas, and increased P contents of plants. In sandy soil, the application of phosphate rock containing humic substances, *Trichoderma afroharzianum*, and indole-3-butyric increases the total phosphorus uptake by 26.5%, 16.3%, and 12.2%, respectively. The efficiency of plant growth promoter with phosphate rock follows the order: humic substances > *Trichoderma afroharzianum* > indole-3-butyric acid. These findings signify a positive impact of these promoters on phosphorus utilization by plants, with humic substances demonstrating the highest efficacy in this context.

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CRediT authorship contribution statement

Rafael Lucas Coca Cuesta: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Edson Marcio Mattiello:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Gustavo Franco de Castro:** Writing – review & editing, Visualization, Supervision, Methodology, Conceptualization. **Patrícia Cardoso Matias:** Writing – review & editing, Visualization, Software, Methodology, Investigation. **Thalita Suelen Avelar Monteiro:** Visualization, Conceptualization. **Leandro Grassi de Freitas:** Visualization, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2024.103019.

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