

RAFAEL LUCAS COCA CUESTA

PHOSPHATE FERTILIZERS CONTAINING PLANT GROWTH PROMOTERS

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Solos e Nutrição de Plantas, para obtenção do título de Magister Scientiae.

Orientador: Edson Marcio Mattiello

Coorientador: Leandro Grassi de Freitas

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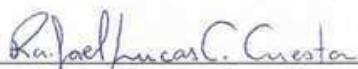
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RESUMO

CUESTA, Rafael Lucas Coca, M.Sc., Universidade Federal de Viçosa, fevereiro de 2021. **Phosphate fertilizers containing plant growth promoters**. Orientador: Edson Marcio Mattiello. Coorientador: Leandro Grassi de Freitas.

Promotores de crescimento vegetal (PCVs) são substâncias cuja ação nas plantas é análoga aos hormônios vegetais pertencentes aos grupos das auxinas, citocinicas e giberelinas. Estas substâncias apresentam grande potencial de uso na agricultura pois podem contribuir com o aumento da eficiência de absorção de água e nutrientes, por meio do estímulo ao desenvolvimento radicular, e aumento da taxa fotossintética, através do crescimento vegetativo das plantas. A associação de PCVs e fertilizantes fosfatados é uma forma eficiente e prática de utilização destas substâncias, tornando-se viável através do processo de granulação dos compostos utilizando o polímero alginato de sódio. Portanto, os objetivos deste trabalho foram produzir fertilizantes fosfatados contendo PCVs e avaliar a eficiência em promover o crescimento da raiz e parte aérea e aumentar a absorção de P pelas plantas. Os fertilizantes produzidos continham fosfato natural reativo e os PCVs ácido-3-idol butírico (PR_IBA), substância húmica (PR_HS) ou *Trichoderma afroharzianum* (PR_TA). Além disso, um fertilizante controle (PR_CONTROL) foi produzido sem adição de PCVs. Para avaliar a eficiência dos fertilizantes em promover crescimento vegetal, um ensaio foi realizado em esquema fatorial [(3 + 1 + 1) × 2], sendo três fertilizantes contendo PCVs (PR_IBA, PR_HS e PR_TA), um fertilizante controle, sem PCVs (PR_CONTROL), um tratamento adicional, com o fertilizante fosfato monoamônico (MAP) e dois solos contrastantes (solo argiloso e arenoso). Os tratamentos foram distribuídos em delineamento de blocos casualizados, com cinco repetições. A unidade experimental consistiu em um rizobox contendo 350 cm³ de solo, onde foi cultivada uma planta de soja (BMX Ipro 8579). Após trinta dias de condução, foram mensurados nas raízes o comprimento, diâmetro médio, número de extremidades e raízes finas através do software WinRHIZO. Além disso, foram determinados massa de matéria seca e teor de P das raízes e parte aérea. Os dados obtidos foram submetidos a análise de variância e teste de médias pelo teste Scott Knott ($p < 0,05$). Os PCVs têm um grande potencial para aumentar a eficiência da absorção de P pelas plantas e a taxa fotossintética. Os efeitos dos PVC's foram observados na arquitetura do sistema radicular, na área foliar e no aumento do teor de P pelas plantas. O processo de granulação de

matérias-primas com alginato de sódio torna-se uma forma eficaz de produzir fertilizantes cada vez mais eficientes. A eficiência dos promotores de crescimento de plantas em rocha fosfática aumenta na seguinte ordem: substâncias húmicas > *Trichoderma afroharzianum* > ácido indol-3-butírico.

Palavras-chave: Auxinas. Ácido indol-3-butírico. Substâncias húmicas. *Trichoderma* spp. Absorção de P.

ABSTRACT

CUESTA, Rafael Lucas Coca, M.Sc., Universidade Federal de Viçosa, February, 2021. **Phosphate fertilizers containing plant growth promoters.** Adviser: Edson Marcio Mattiello. Co-adviser: Leandro Grassi de Freitas

Plant growth promoters (PGPs) are substances with actions in plants that are analogous to plant hormones belonging to the auxin, cytokine, and gibberellin groups. These substances have great potential for use in agriculture because they can contribute to increasing the efficiency of water and nutrient absorption, by stimulating root development and increasing the photosynthetic rate, through the vegetative growth of plants. The association of PGPs and phosphate fertilizers is an efficient and practical way of using these substances, which is viable through the granulation process of the compounds using the sodium alginate polymer. Therefore, the objectives of this work were to produce phosphate fertilizers containing PGPs and to evaluate the efficiency in promoting the growth of the roots and aerial parts, and increase the absorption of P by the plants. The available fertilizers contained reactive natural phosphate and PGPs indole-3-butyric acid (PR_IBA), humic substance (PR_HS), or *Trichoderma afroharzianum* (PR_TA). In addition, a control fertilizer (PR_CONTROL) was produced without PGPs. To evaluate the efficiency of fertilizers in promoting plant growth, a trial was carried out in a factorial scheme $[(3 + 1 + 1) \times 2]$, with three fertilizers containing PGPs (PR_IBA, PR_HS, and PR_TA), a control fertilizer, without PGPs (PR_CONTROL), and an additional treatment, with the monoammonic phosphate fertilizer (MAP) 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 rhizobox containing 350 cm³ of soil, where a soybean plant ('BMX Ipro 8579') was grown. After thirty days of growth, the root length, average root diameter, root tips, and fine roots were measured using WinRHIZO software. In addition, the dry matter mass and P content of the roots and shoots were determined. The data obtained were subjected to analysis of variance and test of means by the Scott Knott test ($p < 0.05$). 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 area, and increased P content by plants. The efficiency of plant growth promoters in phosphate rock increases in the following order: humic substances > *Trichoderma afroharzianum* > indole-3-butyric acid.

Keywords: Auxins. Indole-3-butyric acid. Humic substances. Trichoderma spp. P uptake.

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ARTIGO

PHOSPHATE FERTILIZERS CONTAINING PLANT GROWTH PROMOTERS

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ABSTRACT

The association of plant growth promoters (PGPs) and phosphate fertilizers is an efficient and practical way of using these substances to increase the efficiency of water and nutrients absorption and increase the photosynthetic rate of plants. This association can become viable through the granulation of PGPs and fertilizers using the sodium alginate polymer. Therefore, 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 P uptake of plants. The fertilizers produced contained reactive natural phosphate rock from Morocco (PR) and PGPs indole-3-butyric acid (PR_IBA), humic substances (PR_HS), or *Trichoderma afroharzianum* (PR_TA), and a control fertilizer, without adding PGPs (PR_CONTROL). To evaluate the efficiency of fertilizers in promoting plant growth, an experiment was carried out in a factorial scheme [(3 + 1 + 1) × 2], with three fertilizers containing PGPs, a control fertilizer, additional treatment with the monoammonium phosphate fertilizer (MAP) 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 rhizobox containing 350 cm³ of soil, where a soybean plant ('BMX Ipro 8579') was grown. Thirty days after sowing, the root length, average root diameter, root tips, and fine roots were measured using WinRHIZO software. In addition, dry matter and P content of the roots and shoots were determined. The data obtained were subjected to analysis of variance and test of means by the Scott Knott test ($p < 0.05$). PGPs have a great potential to increase the efficiency of P absorption by plants and the photosynthetic rate. 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 area, and increased P content by plants. The efficiency of plant growth promoters in phosphate rock increases in the following order: humic substances > *Trichoderma afroharzianum* > indole-3-butyric acid.

Keywords: Auxins. Indole-3-butyric acid. Humic substances. *Trichoderma* spp. P uptake.

INTRODUCTION

The limitation of P availability in very weathered soils, due to specific adsorption in Fe and Al oxyhydroxides (NOVAIS; SMYTH; NUNES, 2007), makes the nutrient less mobile in the soil, making it difficult for plant absorption (SILVA; DELATORRE, 2009). Research shows that the P absorption by plants does not exceed 15 to 20% of the total P applied to the soil through soluble phosphate fertilizers, such as MAP (ZHAN et al., 2004). Therefore, it is very important to increase 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 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; MELLO, 2007). For this reason, PGPs are studied to promote the development of the root system of different cultures and increase the efficiency of nutrient absorption. (LANA et al., 2009; BORCIONI; MÓGOR; PINTO, 2016; CHAGAS et al., 2017).

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; ZAIGER, 2013).

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 (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 (BARCIONI; MÓGOR; PINTO, 2016; CANELLAS et al., 2009). Humic and fulvic acids have other actions in the soil/plant system; these substances are also considered physical, chemical, and mycobiological conditions of soils, reduce stresses in plants, stimulate the synthesis of enzymes linked to the production of porcupines in plants, and can stimulate seed germination (CARON; GRAÇAS; CASTRO, 2015)

Similar to IBA and HS, some microorganisms can be considered PGPs because their metabolites can act as plant hormones, for example, soil-borne fungi. 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; VALERO; MEJÍA, 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* (HERMAN, 2005; 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 (BANOBİ 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., 2011), due to its low cost, ease of manipulation and chemical stability (NI et al., 2010; SHAN et al., 2016; WANG et al., 2011). 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; ABEDI-KOUPAI, 2018; DE PAULA et al., 2010; GOMBOTZ; WEE, 1998; KAY, 2006; SALMIERI; 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, as an organic-inorganic hybrid material, is still incipient and has not been reported in the literature. 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 of plants.

MATERIAL AND METHODS

A preliminary study was performed to obtain the best PGP concentrations for indole⁻³-butyric acid (IBA), humic substances (HS), and *Trichoderma afroharzianum*, (TA) to be added in a phosphate fertilizer for promoting shoot (shoot length and leaf area) and root (length, fine roots, tips, and average diameter) development of soybean plants. The results of this assay are presented in the supplementary material.

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 minutes at $25 \text{ }^{\circ}\text{C}$. 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 IBA (PR_IBA), HS (PR_HS), or T10 (PR_TA) in concentrations 2.91 mg kg^{-1} for IBA, 0.48 dag kg^{-1} for SH or $4.13 \times 10^{10} \text{ conidia kg}^{-1}$ for T10. 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 $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (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 ($25 \text{ }^{\circ}\text{C}$) for 48 hours. 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 Figure 1.

IBA (purity = 99 %) was purchased from Sigma-Aldrich and the HS were used in the form of Leonardite. Conidia of TA were obtained from rice colonized with the fungus in the Biological Control Laboratory for Phytomatodes at the Federal University of Viçosa. For this, *Trichoderma afroharzianum* was obtained from the Phytomatodes 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 ($\text{C}_6\text{H}_7(\text{NaO})_6$)_n, purity = 90%) was purchased from Dinâmica Química Contemporânea Ltda. $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (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).

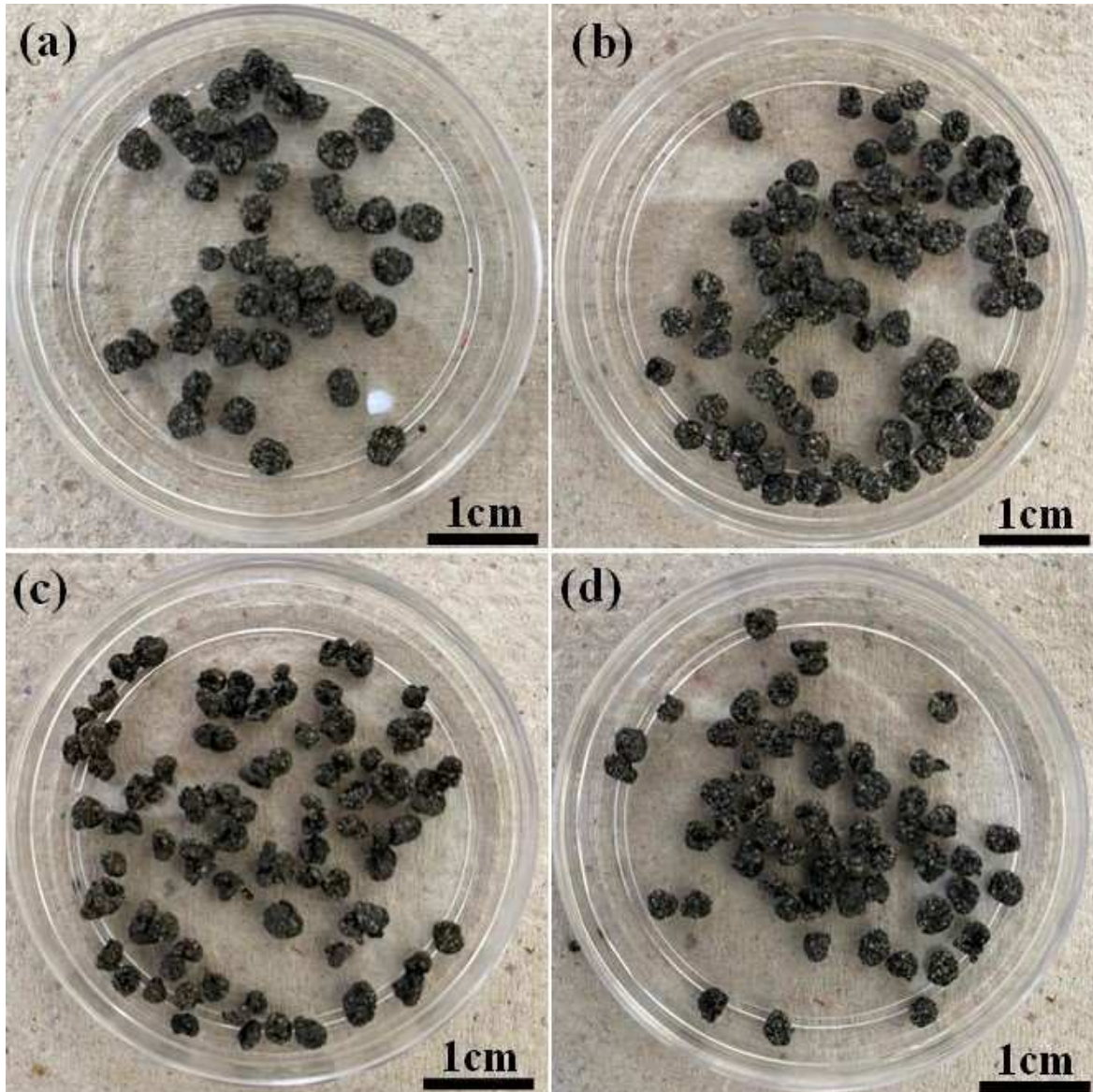


Figure 1. Images of PR_CONTROL (a); PR_IBA (b); PR_HS (c); and PR_TA (d) fertilizers.

Characterization of fertilizers

Moisture content, pH, and salt index were determined according to Brasil (2014). For total P concentration analysis, samples of 0.5 g of fertilizers were added to Falcon tubes (50 mL) containing 5 mL of HNO₃ concentrate and 25 mL of deionized water. Then, the tubes were sealed and stirred on a shaking table at 60 rpm for 1 hour. 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; 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.

Viability of *Trichoderma* sp. in fertilizers

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.

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.

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) \times 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 rhizobox (Figure 2) containing 350 cm³ of soil, where a soybean plant (‘BMX Ipro 8579’) was cultivated.

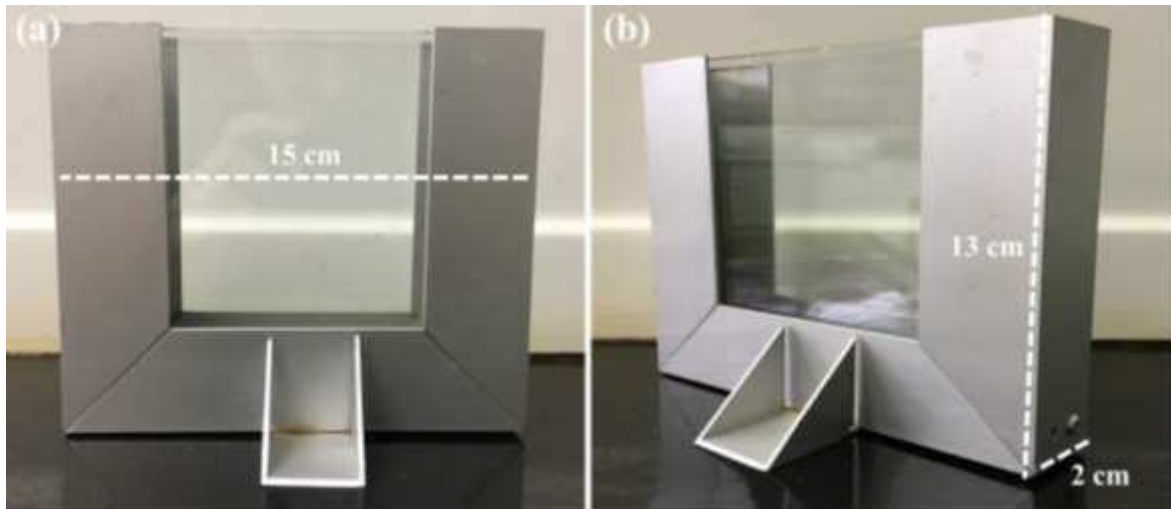


Figure 2. Images of Rhyzobox in a front (a) and side (b) view.

Initially, 1.9 and 1 g dm⁻³ of a mixture of CaCO₃ and MgCO₃ (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).

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; Al³⁺: 1.0 mol L⁻¹ KCl extractant; m: saturation by aluminum; OM: Organic matter content (Walkley & Black, 1934); P-rem: remaining phosphorus.

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 ($13.7 \text{ mg dm}^{-3} \text{ N}$), according to the concentration of N in the treatment fertilized with MAP ($6.3 \text{ mg dm}^{-3} \text{ N}$). One soybean seed was sown at 0.5 cm depth in the rhizobox. During the experiment, the sides of the rhizobox 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 rhizobox and the root and shoot development were photographed. Subsequently, plant height and leaf area were measured using a ruler and LI-COR LI⁻³100AC 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 11000 XL, and the total length, fine roots (diameter less than 0.5 mm), root tips, and average diameter were measured using WinRHIZO Pro software.

Shoots and roots were dried in a forced-air oven at 60 °C for 72 hours, weighed, and ground. Plant samples were subject to perchloric nitric digestion (v/v 4:1) to determine total P in shoots and roots (BRAGA; 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, 2016).

RESULTS

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.

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	3.12	11.24	6.70	4.53
PR_CONTROL	3.26	10.05	6.64	3.84
PR_IBA	4.31	9.34	6.67	3.91
PR_HS	3.25	9.68	6.52	4.23
PR_TA	4.45	9.73	6.53	4.10

The morphology of the synthesized fertilizers and the materials used in the synthesis were analyzed by SEM (Figure 3). SEM images of PR_CONTROL, PR_IBA, PR_HS, and PR_TA showed microspheres with diameters varying between 2500 and 3000 nm (Figure 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 (Figure 3 e, f, g, and h), which can be identified and compared with surface images of the morphology of the alginate, activated charcoal and PR (Figure 3 j and k, respectively).

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 (Figure 3 i) in the SEM image (Figure 3 e, f, g, and h).

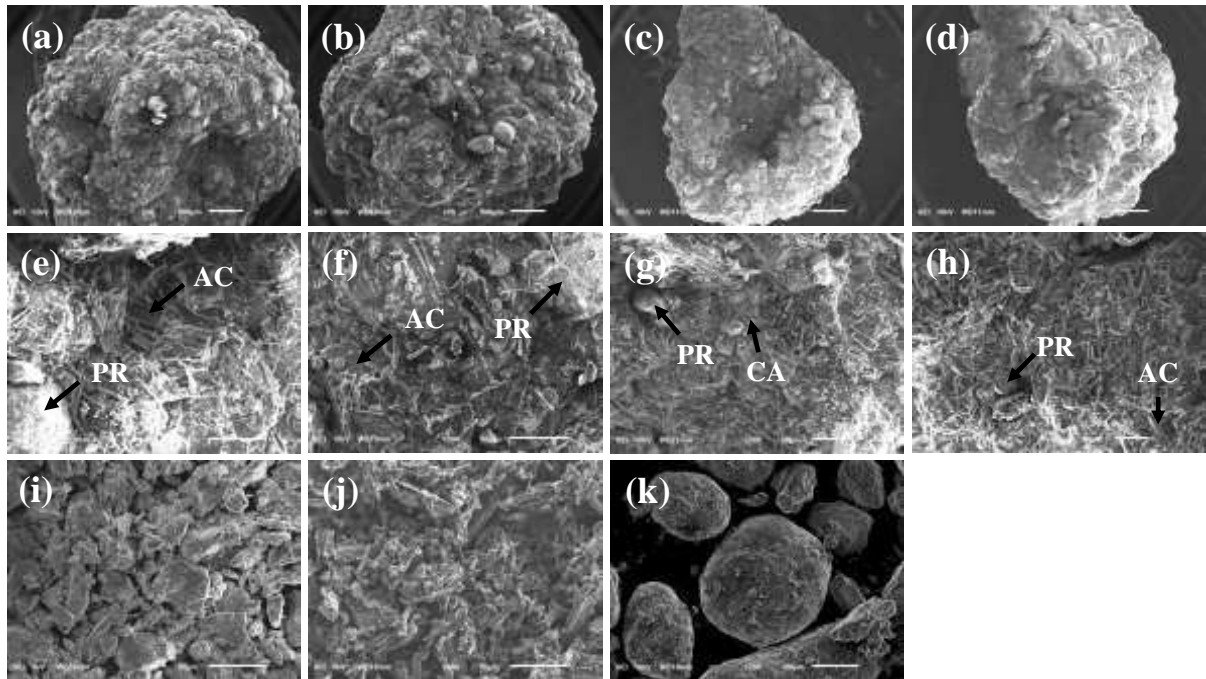


Figure 3. SEM images of the intact microspheres of PR_CONTROL (a); PR_IBA (b); PR_HS (c) and PR_TA (d); and surface morphology of PR_CONTROL (e); PR_IBA (f); PR_HS (g) and PR_TA (h); sodium alginate “in natura” (i); charcoal activated (j); phosphate rock (k). PR: Phosphate rock; AC: activated charcoal.

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 (Figure 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.

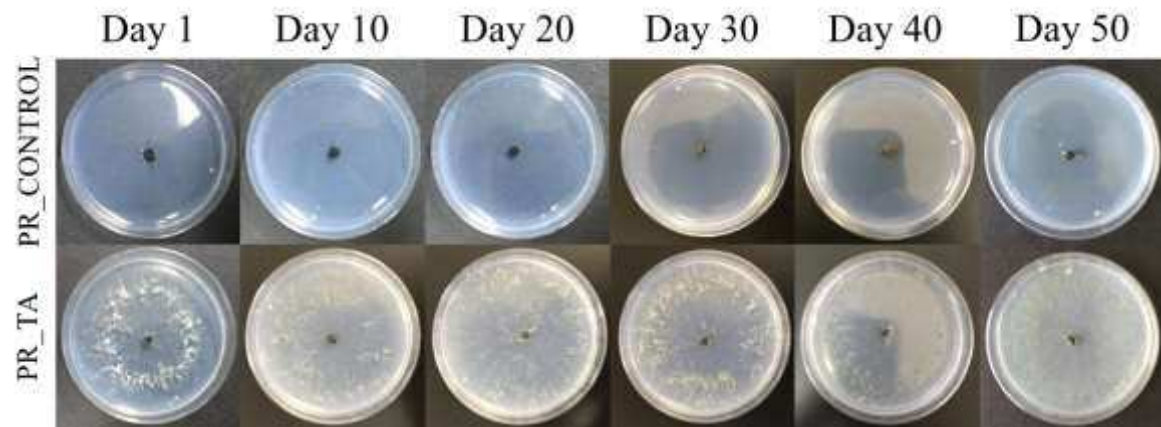


Figure 4. *Trichoderma afroharzianum* survival test in a petri dish containing agar-water culture medium (2%) with antibiotic Streptomycin sulfate salt at 1, 10, 20, 30, 40 and 50 days after fertilizer synthesis. PR_CONTROL: Control fertilizer without adding plant growth promoters. PR_TA: Fertilizer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

Rhizobox test

Soybean root growth was visualized using the rhizobox observation technic (Figure 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.

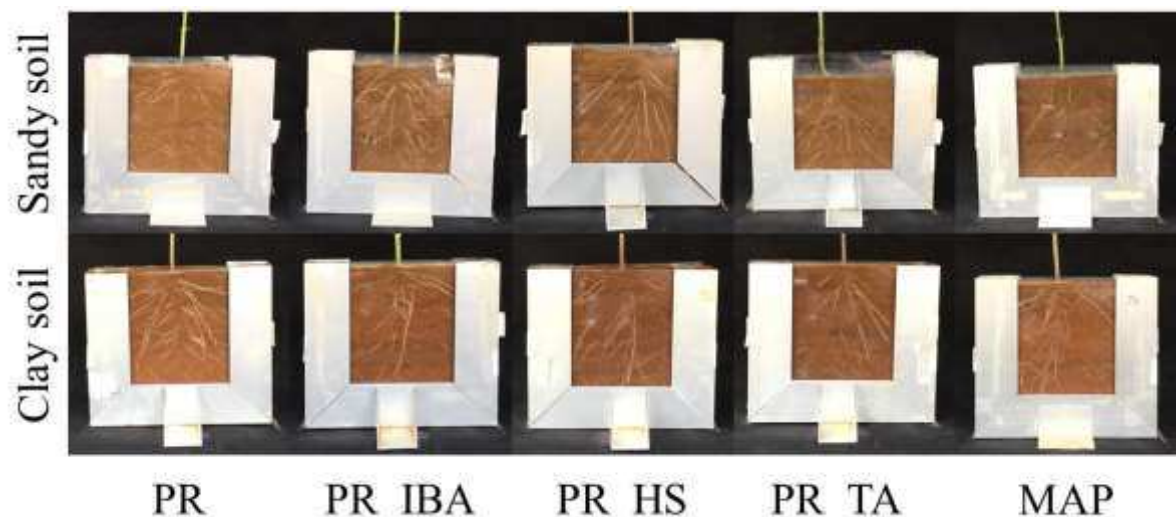


Figure 5. Images of soybean root development in sandy and clayey soil. PR_CONTROL: Control fertilizer without adding plant growth promoters. PR_IBA: Fertilizer containing phosphate rock and indole-3-butyric acid. PR_HS: Fertilizer containing phosphate rock and humic substances. PR_TA: Fertilizer containing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

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_SH, 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_SH 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_SH 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_SH.

Shoot P uptake was higher when the plants were fertilized with MAP followed by PR_SH, 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_SH and MAP. In this soil, PR_TA and PR_IBA had higher P uptake than the PR_CONTROL.

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_SH 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 P shoot 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_SH.

Generally, the shoot and root development of plants, accessed from the means of the values of the parameters presented in table 4, were greater in the clayey soil compared to sandy soil. Only shoot length and fine roots were statistically equal for the two soils studied.

Table 3. Shoot length, leaf area, shoot dry matter, root length, fine root, average diameter, root tips, root dry matter and P shoot, root and total 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)	12.01 b	12.82 a	12.12 b	12.73 a	12.04 b	12.31 A
Leaf area (cm ²)	99.12 b	104.74 a	108.91 a	111.63 a	84.77 c	101.8 B
Shoot dry matter (g)	0.52 a	0.51 a	0.52 a	0.53 a	0.51 a	0.5 B
Root length (cm)	3546 d	4479 a	4185 b	3808 c	4294 b	4063 B
Fine root (%)	82.92 c	86.66 b	82.71 c	88.75 a	89.72 a	86.1 A
Avg root diameter (mm)	1.94 b	1.82 b	2.21 a	1.56 d	1.66 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
P shoot uptake (mg plant ⁻¹)	0.40 c	0.41 c	0.48 b	0.41 c	0.51 a	0.45 B
P root 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)	12.22 b	11.21 c	11.24 c	12.11 b	15.53 a	12.2 A
Leaf area (cm ²)	122.52 b	73.64 d	123.11 b	109.12 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 (%)	80.72 d	89.31 a	87.93 b	86.25 c	89.97 a	86.7 A
Avg root diameter (mm)	2.62 a	2.44 b	2.37 b	2.22 b	2.46 b	2.4 A
Root tips	6846 c	4545d	10173 b	7281 c	13064 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
P shoot uptake (mg plant ⁻¹)	0.40 d	0.48 b	0.43 c	0.43 c	0.79 a	0.51 A
P root 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.

DISCUSSION

The architecture of the root system directly influences the uptake of nutrients in the soil. Phosphorus uptake is influenced by root growth, root average diameter, and adventitious root (fine roots), responsible for increasing the contact between plant and soil (CRUSCIOL et al., 2005; SILVA; DELATORRE, 2009). The PGPs used in this study were responsible for changes in the architecture of the roots in sandy soil, increasing the contact between soil and roots, and increasing the root interception mechanism (Table 3). It was also possible to verify the increase in the efficiency of P absorption relative to fertilization without PGPs (Table 3).

The efficiency of P uptake by plants can be easily observed under conditions of lesser P adsorption capacity in the soil (sandy soil) when the plants were fertilized with fertilizers containing PGPs. The fertilizer PR_SH stands out for having the same efficiency compared to the source of soluble P (MAP) used in this work, and other fertilizers containing PGPs (PR_IBA and PR_TA) showed increased efficiency relative to fertilizers without PGPs. Therefore, these fertilizers can be considered improved efficiency fertilizers and are alternatives to increase the absorption of P by plants.

The use of improved efficiency fertilizers, such as those studied in this work, can reduce doses of soluble P sources. In this study, it was possible to replace 60% of the dose of P (28.3 mg dm^{-3}), added from a soluble source (MAP) of P, with an alternative source of low solubility (PR). That is, the complementary dose of P (11.5 mg dm^{-3}) added in the form of MAP was sufficient to guarantee adequate levels of P in the plant.

Brazil is highly dependent on phosphate fertilizers from other countries (DIAS et al., 2020; OGINO et al., 2021). About 63% of the sources of P used in agriculture come from imports (ANDA, 2019). Therefore, reducing the dose of soluble P sources, without harming the development and production of the plant, is a strategy that can reduce external dependence on P sources, in addition to reducing fertilization costs.

The lack of responses of root development to PGPs in clayey soil may be related to the process of adsorption of molecules in soil colloids, as well as with auxinic herbicide molecules, such as 2,4 D (VIEIRA et al., 1999). According to the authors, the availability of these molecules can be influenced by the content of soil clay, minerals, organic matter, and Fe and Al oxyhydroxides. PGP availability for plants may have been lower than the optimal concentrations defined, reducing the effects of the substances on plant development, since that the clayey soil used in this study had 531 g kg^{-1} of clay.

The effects of PGP are not limited to the root system only. Stimulation of aerial part development can increase the leaf area useful for photosynthesis and, consequently, the intensity of transpiration, absolute and relative growth rate, liquid assimilation rate, and leaf area index, parameters that directly influence the productive performance of the plants (MAGALHÃES, 1985).

This work shows an innovative and efficient method of using substances that influence plant physiological processes more efficiently. The granulation process of PGPs with P sources presented in this work is an innovative way to produce improved efficiency fertilizers because it increases the efficiency of phosphate fertilization, absorption of P by plants, and plant development.

The multifunctionality of PGPs gives the fertilizers thus produced other applications. Humic substances have many functional groups that provide a broad spectrum of reactivity in plants (CARON; GRAÇAS; CASTRO, 2015). *Trichoderma afroharzianum* acts as a biological control agent for fungal and nematode diseases (LOUREIRO et al., 2020). These attributes as secondary actions of the new fertilizers presented in this work, make the use of this technology more attractive.

The chemical and physical characteristics of fertilizers (Table 2 and figure 3) show that there was a homogeneous distribution of raw materials in fertilizers granules. This finding was also made by Castro et al. (2020) in beads of sodium alginate containing layered double hydroxide intercalated with borate. Therefore, the synthesis of fertilizers with sodium alginate, performed by the complex coacervation method, is an efficient way to produce improved efficient fertilizers with a homogeneous distribution of raw materials, including with low concentrations such as PGPs. In addition, the fertilizer synthesis process described in this work guarantees significant shelf life for the fertilizer containing the fungus.

CONCLUSION

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. The efficiency of plant growth promoters in phosphate rock increases in the following order: humic substances > *Trichoderma afroharzianum* > indole-3-butyric acid.

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SUPPLEMENTARY MATERIAL

A preliminary test was developed to determine the best concentrations of plant growth promoters (PGPs) to granulate with phosphate source by the complex coacervation method with sodium alginate, adapted from Zhang et al. (2014).

The fertilizers were composed of 2.5 g of monoammonium phosphate (MAP), 7.5 g of phosphate rock from Morocco (PR), 1.0 g of activated charcoal, 1.0 g of sodium alginate, and four different PGP concentrations of indole-3-butyric acid—IBA (0.4, 2.1, 3.2, or 4.2 mg kg⁻¹), *Trichoderma afroharzianum*—TA (1.7×10^{10} , 2.8×10^{10} , 4.1×10^{10} or 6.9×10^{10} conidia kg⁻¹), or humic substances—HS (0.5, 1.0, 2.0, or 3.0 dag kg⁻¹). In addition, a control fertilizer was produced without the addition of PGPs (CONTROL).

The characterizations of the fertilizers were carried out using the same methodology described in the “2.2 Characterization of fertilizers” section of this manuscript, and the N contents were determined according to Brasil (2014). The characterization of fertilizers is shown in Table 1.

A greenhouse experiment was performed with the treatments distributed in the factorial scheme [(3 × 4) + 1], with three PGPs (IBA, HS, and T10), four concentrations for each PGPs (above mentioned), and an additional treatment without PGPs (CONTROL). The experiment was carried out using randomized block designs with four replicates. The experimental units consisted of pots contained 150 mg dm⁻³ of washed and autoclaved sand.

Fertilizers (32, 3 mg dm⁻³ of P) were added to pots at a depth of 3 cm. Then one soybean seed (BMX Ipro 8579) was sown at a depth of 1 cm in the pots. A basal nutrient with K (80 mg dm⁻³), S (30 mg dm⁻³), Ca (90 mg dm⁻³), Mg (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) was added, divided into five applications.

Ten days after emergence (DAE), the roots were removed from the sand, washed, and conditioned in a solution containing 40 % of alcohol in water (v/v). Then, all roots were scanned in Scanner Epson Expression 11000 XL, and the total length, average diameter, tips, and fine roots (diameter less than 0.5 mm) were calculated using WinRHIZO software.

The results were submitted to analysis of variance and regression equations were adjusted between root development parameters (total length, average diameter, tips, and fine roots) and the concentration for each PGPS. From equations, were calculated the maximum or minimum points to find the concentration that ensured the best response of root development.

Table 1. Characterization of phosphate fertilizers containing MAP, phosphate rock (PR) and growth plant promoters indole-3-butyric acid (IBA), humic substances (HS) and *Trichoderma afroharzianum* (TA) in differences levels of plant growth promoters

Fertilizer	Humidity	Total P	Total N	pH	Salt index
	%	dag kg ⁻¹	dag kg ⁻¹	-	%
CONTROL	7.1	10.1	1.2	5.1	9.1
IBA_L1	5.3	10.2	1.3	4.9	10.0
IBA_L2	4.9	9.7	1.1	4.9	11.5
IBA_L3	5.3	9.5	1.3	4.9	10.0
IBA_L4	5.4	9.7	1.4	4.6	13.6
HS_L1	5.7	9.6	1.7	5.0	13.4
HS_L2	6.1	9.6	1.5	5.0	14.3
HS_L3	5.8	10.3	1.2	5.1	13.8
HS_L4	5.2	9.6	1.8	4.5	15.6
TA_L1	4.5	9.6	1.3	4.9	10.4
TA_L2	4.7	9.7	1.3	5.0	11.9
TA_L3	3.9	9.9	1.2	5.0	12.1
TA_L4	5.2	9.2	1.6	5.0	12.6
PR	3.2	11.3	0.0	6.7	4.5
MAP	4.8	20.9	8.4	4.8	65.3

IBA, HS and TA: Alginate beads containing monoammonium phosphate and phosphate rock of Morocco with indole-3-butyric acid, humic substances and *Trichoderma afroharzianum*, respectively. PR: phosphate rock of Morocco. MAP: monoammonium phosphate. L1, L2, L3 and L4: levels of PGPs concentration.

Root development increased until a certain concentration of plant growth promoters and, later, decreased due to the hormonal imbalance deleterious effects caused by excessive concentration of these substances (Figure 1). The best PGP concentrations were considered to be those that resulted in a greater increase in the parameter evaluated relative to the control (without PGPs) (Table 2). These concentrations were obtained from the maximum or minimum (for average diameter) of the regression equations.

The HS and TA concentrations (0.48 dag kg⁻¹ and 4.13 conidia kg⁻¹, respectively) used in the Material and Methods section of this manuscript were selected based on the best concentrations obtained in fine roots (Table 2). The average concentration for IBA (2.91 mg kg⁻¹) was calculated from root length, tips, and average diameter parameters (Table 2).

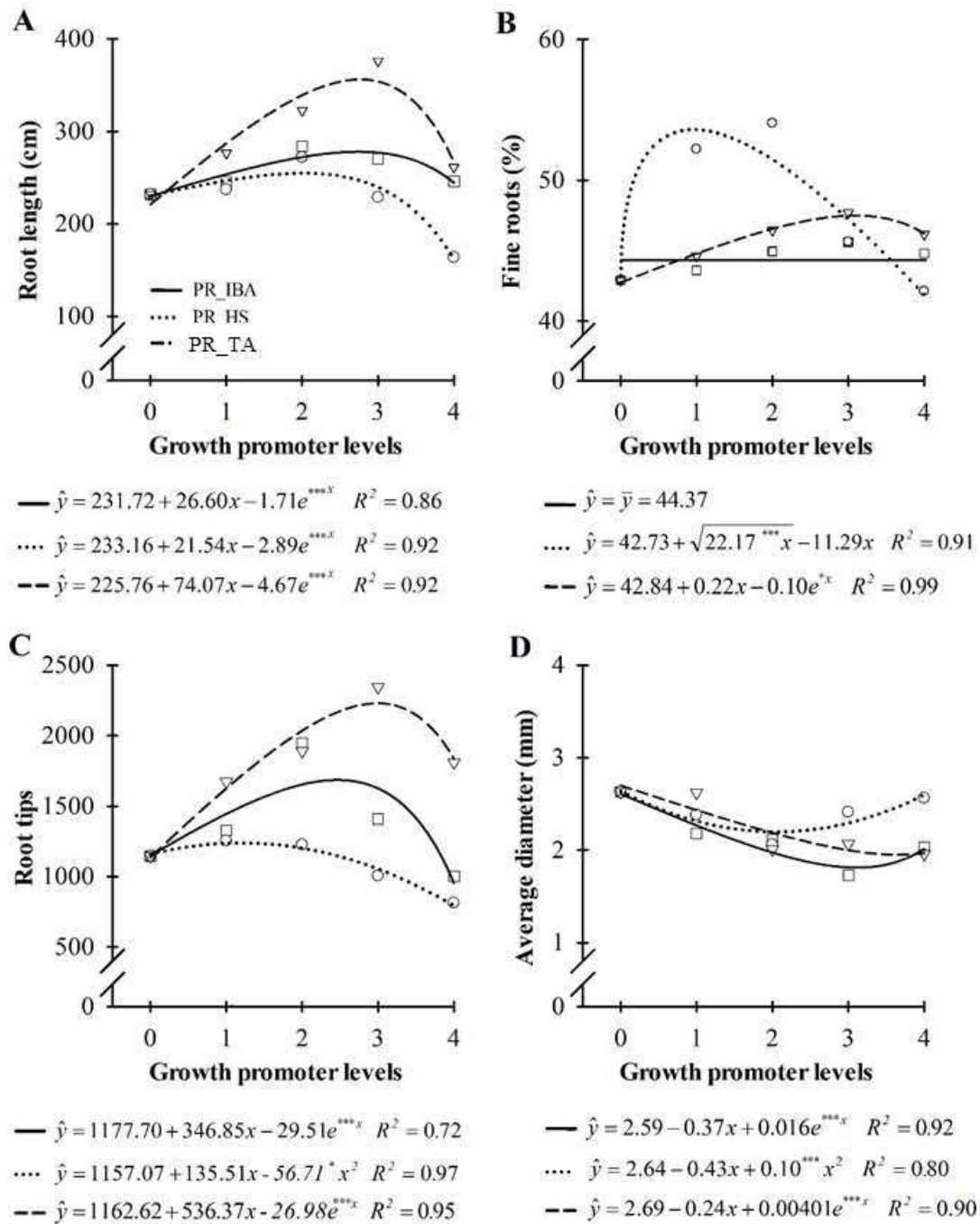


Figure 1. Regression between root length (A), fine root (B), root tips (C) and average diameter (D) and phosphate fertilizers containing phosphate rock, MAP and indole-3-butyric acid (IBA), humic substances (HS) or *Trichoderma afroharzianum* (TA) in different concentrations. °, * and *** indicated significant effect at 10 %, 5 % and 0.1 %, respectively.

Table 2. Best concentration of indole-3-butyric acid (IBA), humic substances (SH) and Trichoderma sp. (TA) and percentage of increase in the parameter evaluated in relation to control (without plant growth promoters)

Parameter	Growth promoter concentration		
	IBA	HS	TA
	mg kg ⁻¹	dag kg ⁻¹	conidia kg ⁻¹
Root length (cm)	2.88 (17 %)	1.00 (9 %)	3.18 × 10 ¹⁰ (35 %)
Fine root (%)	-	0.48 (20 %)	4.13 × 10 ¹⁰ (10 %)
Root tip	2.58 (30 %)	0.60 (7 %)	4.10 × 10 ¹⁰ (48 %)
Average diameter (mm)	3.27 (-31 %)	1.04 (-17 %)	6.94 × 10 ¹⁰ (-17 %)

Values in parentheses represent percentage increase of parameters in relation to control (without plant growth promoters). IBA: indole-3-butyric acid. HS: Humic substances. TA: Trichoderma afroharzianum.

REFERENCES OF SUPPLEMENTARY MATERIAL

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