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Biosolubilization of phosphate by strains of *Trichoderma in vitro* and in greenhouse in three varieties of *Coffea arabica*

Biosolubilización de fosfatos por cepas de *Trichoderma in vitro* y en invernadero sobre tres variedades de *Coffea arabica*

Biosolubilização de fosfatos por cepas de *Trichoderma in vitro* e em invernada sobre três variedades de *Coffea arabica*

Rosa María Arias Mota[1]*
Alberto Donaldo Torres Salas[1]
Yamel del Carmen Perea Rojas[2]
Yadeneyro de la Cruz Elizondo[3]

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Crop production

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University of Zulia, Faculty of Agronomy
Bolivarian Republic of Venezuela

[1]Instituto de Ecología A.C. km 2.5 antigua carretera a Coatepec 361 Congregación el Haya, CP 91073, Xalapa, Veracruz, México.
[2]Doctorado en Micología Aplicada, Centro de Investigación en Micología Aplicada, Universidad Veracruzana Médicos No. 5, U. H. del Bosque, CP 91010, Xalapa, Veracruz, México.
[3]Facultad de Biología. Universidad Veracruzana, circuito Gonzalo Aguirre Beltrán s/n. Zona Universitaria, CP 91000, Xalapa, Veracruz, México.

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Abstract

Coffee soils have a low availability of phosphorus, the use of phosphate-solubilizing *Trichoderma* strains is a promising sustainable strategy for the management of phosphorus deficiencies. In this study, we evaluated 10 strains of *Trichoderma* from the andosol soil of coffee plantations in Mexico and their capacity for phosphate solubilization *in vitro*, and their impact on the growth of coffee seedlings of three varieties (Anacafé, Costa Rica and Marsellesa) were evaluated. The tested microorganisms showed high phosphorus solubilization, the phosphorus solubilization ranged between 2.41 and 7.40 $mg.mL^{-1}$. The maximum phosphate solubilizing activity was observed using two strains of *Trichoderma harzianum* 75.73 (Th53) and 74.62 $mg.mL^{-1}$ (Th48) for calcium phosphate (Ca_2PO_4) and three strains of *T. asperellum* 22.99 (Th57), 22.90 (Th49) and 21.55 $mg.mL^{-1}$ (Th40) for aluminum phosphate ($AlPO_4$). In both calcium (Ca_2PO_4) and aluminum phosphate ($AlPO_4$), a decrease in the pH of the medium was detected, from 4.81 to 3.73 and from 3.38 to 2.75, respectively. In the Anacafé variety, the application of two strains of *T. harzianum* (Th48 and Th53) favored greater availability of phosphorus in the substrate, while in the Costa Rica and Marsellesa varieties the available phosphorus of the substrate was greater with *T. harzianum* (Th48). Inoculation with these *Trichoderma* strains is potentially important for the solubilization of insoluble phosphorus and the development of coffee plants.

*Corresponding author: rosa.arias@inecol.mx

Resumen

Los suelos cafetaleros presentan una baja disponibilidad de fósforo, el uso de cepas de *Trichoderma* fosfato solubilizadoras es una estrategia sostenible prometedora para el manejo de las deficiencias de fósforo. En este estudio, se evaluaron 10 cepas de *Trichoderma* provenientes de suelos andosoles de cafetales en México para determinar su capacidad de solubilización de fosfato *in vitro*, y su impacto en el crecimiento de plántulas de café de tres variedades (Anacafé, Costa Rica y Marsellesa). Los microorganismos probados mostraron alta solubilización de fósforo, la solubilización de fósforo osciló entre 2,41 y 7,40 $mg.mL^{-1}$. La máxima actividad solubilizadora de fosfato se observó utilizando dos cepas de *Trichoderma harzianum* 75,73 (Th53) y 74,62 $mg.mL^{-1}$ (Th48) para fosfato de calcio (Ca_2PO_4) y tres cepas de *T. asperellum* 22,99 (Th57), 22,90 (Th49) y 21,55 $mg.mL^{-1}$ (Th40) para fosfato de aluminio ($AlPO_4$). Tanto en fosfato de calcio (Ca_2PO_4) como de aluminio ($AlPO_4$) se detectó una disminución del pH del medio, de 4,81 a 3,73 y de 3,38 a 2,75, respectivamente. En la variedad Anacafé, la aplicación de dos cepas de *T. harzianum* (Th48 y Th53) favorecieron una mayor disponibilidad de fósforo en el sustrato, mientras que en las variedades Costa Rica y Marsellesa el fósforo disponible del sustrato fue mayor con *T. harzianum* (Th48). La inoculación con estas cepas de *Trichoderma* es potencialmente importante para la solubilización del fósforo insoluble y el desarrollo de las plantas de café.

Palabras clave: hongos solubilizadores de fosfato (PSF), plantas de café, biofertilización.

Resumo

Os solos cafeeiros apresentam baixa disponibilidade de fósforo, o uso de cepas de *Trichoderma* solubilizadoras de fosfato é uma estratégia sustentável promissora para o manejo das deficiências de fósforo. Neste estudo, foram avaliados cepas 10 *Trichoderma* do solos andossolos de plantações de café no México e sua capacidade de solubilização de fosfato *in vitro*, e seu impacto no crescimento de mudas de café de três variedades (Anacafé, Costa Rica e Marsellesa). Os microrganismos testados apresentaram solubilização de fósforo variou entre 2,41 e 7,40 $mg.mL^{-1}$. A máxima atividade solubilizadora de fosfato foi observada utilizando duas cepas de *Trichoderma harzianum* 75,73 (Th53) e 74,62 $mg.mL^{-1}$ (Th48) para fosfato de cálcio (Ca_2PO_4) e três cepas de *T. asperellum* 22,99 (Th57), 22,90 (Th49) e 21,55 $mg.mL^{-1}$(Th40) para fosfato de alumínio ($AlPO_4$). Tanto no fosfato de cálcio (Ca_2PO_4) quanto no fosfato de alumínio ($AlPO_4$) foi detectada diminuição do pH do meio, de 4,81 para 3,73 e de 3,38 para 2,75, respectivamente. Na variedade Anacafé, a aplicação de duas cepas de *T. harzianum* (Th48 e Th53) favoreceu maior disponibilidade de fósforo no substrato, enquanto nas variedades Costa Rica e Marseillaise o fósforo disponível do substrato foi maior com *T. harzianum* (Th48). A inoculação com estas cepas de *Trichoderma* é potencialmente importante para a solubilização do fósforo insolúvel e o desenvolvimento dos cafeeiros.

Palavras-chave: fungos solubilizadores de fosfato (PSF), café, Biofertilização.

Introduction

Numerous studies have classified species of the genus *Trichoderma* as beneficial microorganisms for plants because they exhibit antagonistic activity against phytopathogenic fungi, promote plant growth, improve crop yield, promotes abiotic stress tolerance and increases nutrient utilization (Zin and Badaluddin, 2020). In recent years, phosphorus-solubilizing fungi (PSF) have been considered as an alternative phosphorus fertilizer that can promote plant growth (Yang *et al*., 2022). In Mexico, one of the main problems in coffee production is the low availability of phosphorus in the soil, as this crop is often grown on volcanic soils characterized by acidic pH and low availability of essential macronutrients, including phosphorus (Geissert and Ibanez, 2008). The average phosphorus content in the coffee soils of the region is 1.75 $mg.kg^{-1}$; according to Sadeghian *et al*. (2019) is below the optimal level for coffee cultivation (10-30 $mg.kg^{-1}$). Generally, soil phosphorus deficiency can be solved by adding phosphorus fertilizers; however, these lack efficiencies due to the rapid conversion of available phosphorus to insoluble forms. Therefore, it is very important to study the phosphorus solubilizing potential of *Trichoderma* species, because of their activity they can act as biofertilizers. The objectives of this research were to estimate the *in vitro* phosphorus solubilization capacity of ten *Trichoderma* strains from the rhizosphere of coffee trees and to evaluate their effect on phosphorus availability and coffee growth under greenhouse conditions.

Materials and methods

Fungal material

Trichoderma strains were isolated from rhizospheric soil samples from coffee plantations in the center of the state of Veracruz (Arias *et al.,* 2022) using the soil particle washing technique. The strains were reactivated in potato dextrose agar (PDA) culture medium and kept in incubation (Thermo Scientific® 150) for 10 days at 25 °C in the dark.

Identification of the *Trichoderma* isolates

For molecular identification, mycelial DNA was extracted from pure cultures and recover by scraping with a sterile needle. Genomic DNA extraction was performed using the Wizard® Genomic DNA Purification Kit from Promega (Madison, USA) according to the manufacturer's instructions. The nuclear rDNA ITS region (ITS1-5.8S-ITS2) was amplified using ITS5/ITS4 primers (White *et al*., 1990). Amplification was performed by PCR (polymerase chain reaction) using MyTaq DNA polymerase (Meridian Bioscience). For sequencing, PCR products were dispatched to Macrogen, Inc (Seoul, Korea). The genetic affinity of the obtained sequences was determined using the BLASTn algorithm of the National Center for Biotechnology Information GenBank (NCBI, 2024). Furthermore, *Trichoderma* strains are identified according to the method of Gams and Bisset (2002) by the morphological characteristics (mycelial structure, concentric ring formation and conidial pigmentation of the colonies) and the microscopic (shape and arrangement of conidia, phialides and conidiophores).

Study quantitative of the ability of *Trichoderma* strains to solubilize calcium phosphate and aluminum phosphate

For this measurement, Pikovskaya (1948) liquid culture medium was used with tricalcium phosphate ($Ca_3(PO_4)_2$) (0.5 $g.L^{-1}$) or aluminum phosphate ($AlPO_4$) (0.5 $g.L^{-1}$) as a source of insoluble phosphorus. Each *Trichoderma* strain was inoculated with four discs

(5 mm diameter) of active mycelium (three replicates for each strain) (Prasad *et al*., 2023). The cultures were kept in incubation (Thermo Scientific®150) at 26 °C for 10 days. A culture with Pikovskaya medium without fungus was used as a control. After 10 days, the content of the flasks of each strain was filtered (Whatman® 42). The extract obtained from each of the strains was measured for soluble phosphorus content and pH.

Measurement of soluble phosphorus in the extracts of *Trichoderma* strains

Was evaluated by the ascorbic acid method (Clesceri *et al.,* 1992). The absorbances were measured in a spectrophotometer (JENWAY® 6305) at 880 nm phosphorus solubilization was calculated against a standard curve prepared with KH_2PO_4 (concentration 1-40 $mg.L^{-1}$). The data obtained were expressed in $mg.mL^{-1}$.

Study of the ability of *Trichoderma* strains to solubilize phosphate in the greenhouse

Inoculum production

Trichoderma strains were transferred to PDA and then incubated at 26 °C for 7 days to promote the production of conidia. The culture was immersed in solution of Inex A 0.1% (Cosmocel®) to prepare concentration to 1.10^{-8} $CFU.mL^{-1}$ by counting in a Neubauer chamber.

Inoculation of *Trichoderma* isolates on coffee plants of three varieties

Six months old coffee plants of three varieties of *C. arabica* (Anacafe, Costa Rica and Marsellesa) were used. The plants were transplanted into 5 kg pots with fresh soil (table 1) from the coffee plantations, and sterile sand was added in a ratio of 2:1 v/v.

Table 1. Chemical characteristics of the soil used.

Soil	pH	Phosphorus $mg.kg^{-1}$	Nitrogen (%)	Carbon (%)
Anacafe	4.82	0.73	0.45	3.80
Costa Rica	4.92	0.97	0.42	3.70
Marsellesa	4.98	0.69	0.47	3.80

The design was a randomized block design with 11 treatments [10 *Trichoderma* strains and 1 control (no fungus)] with 5 replications. The inoculum of the strains of *Trichoderma* are added directly to the substrate and plant roots at a concentration of $1x10^{-8}$ $CFU.mL^{-1}$ according to Souchie *et al*. (2010). The plants were kept in the greenhouse for six months, at the end of this time the soluble phosphorus content of the substrate and the height of the plants were measured.

Measurement of soluble phosphorus in the substrate of coffee plants

To determine the amount of soluble phosphorus using the technique of Bray and Kurtz (1945). Phosphorus concentration was measured with a spectrophotometer (JENWAY ®6305) at 882 nm and was calculated against a standard curve prepared with KH_2PO_4 (concentration 0-20 ppm). The obtained data were expressed in $mg.kg^{-1}$. To calculate the amount of soluble phosphorous use the following formula: P (mg.Kg): ppm in CC x DM x DV. Where: ppm in DC: parts per million in the calibration curve, DM: mass dilution (volume of extractant solution)/(grams of sample) and DV: volume dilution (capacity)/(sample aliquot added).

Statistical analysis

The trial used a completely randomized design, and data were analyzed using ANOVA (one-way). Fisher's test was treated to separate means. The level of significance for analysis was $p \leq 0.05$. To know the relationship between the content of solubilized phosphorus and the pH in the extracts of the *Trichoderma* strains as well as the soluble phosphorus content of the substrate with the height of the plants of the three coffee varieties a Pearson evaluation analysis was performed. All analysis were done using Statistica 8.0 statistical software.

Results and discussion

Identification of the *Trichoderma* isolates

Species were assigned by comparing the sequences obtained with Gen Bank databases. The species identified with 100 % identity corresponded to *T. asperellum* (Th40, Th49) and *T. harzianum* (Th48, Th53 and Th55); the rest were categorized as related because they did not have 100 % identity (table 2). Table 2 presents the data obtained in the analysis del BLASTn algorithm of the National Center for Biotechnology Information GenBank (NCBI, 2024).

Table 2. Species assignment of *Trichoderma* strains using BLASTn algorithm of the National Center for Biotechnology Information GenBank.

Key	Species	Gene bank accession numbers	Max Score	Query cover (%)	Identity Score (%)
Th12	*T.* aff *crassum*	PQ516492	436	85	90.62
Th24	*T.* aff *silva-virgineae*	PQ514865	726	79	98.78
Th26	*T.* aff *virens*	PQ514858	1074	99	99.34
Th40	*T. asperellum*	PQ514866	1038	100	100
Th48	*T. harzianum*	PQ514859	814	100	100
Th49	*T. asperellum*	PQ514860	1062	98	100
Th53	*T. harzianum*	PQ514861	1109	99	100
Th55	*T. harzianum*	PQ514862	1110	98	100
Th57	*T.* aff *asperellum*	PQ514863	1090	98	99.83
Th78	*T.* aff *piramidales*	PQ514864	1098	99	98.86

The ten evaluated strains had morphological and microscopic features characteristic of *Trichoderma* spp. The mycelial color of all strains starts off white with a cottony appearance and turns light to dark green after sporulation. Most of them form colonies with concentric rings. After 5 days of cultivation in PDA medium, the colonies rapidly grew and matured. Microscopic observation revealed slightly ovoid conidia arranged in rosettes, apical phialides arranged in three to four transverse arcs.

Study quantitative of the ability of *Trichoderma* strains to solubilize calcium phosphate and aluminum phosphate.

The soluble phosphorus concentrations of ten *Trichoderma* strains evaluated in the culture medium with calcium phosphate (Ca_2PO_4) after 10 days of incubation varied significantly ($p>0.0005$), from 54.42 to 75.73 $mg.mL^{-1}$. In the control treatment, the soluble phosphorus value was 7.42 $mg.mL^{-1}$. The highest value was detected in the culture of the strains Th53 (75.73 $mg.mL^{-1}$) and Th48 (74.62 $mg.mL^{-1}$), and the lowest in the strain Th55 (54.42 $mg.mL^{-1}$). The solubilized phosphorus content was negatively correlated with the pH ($R= -0.78$; $p< 0.05$) (figure 1a). The pH values were 6.35 in the control and 4.81-3.73 with the different *Trichoderma* treatments (figure 1a).

The soluble phosphorus concentrations of ten *Trichoderma* strains in the culture medium with aluminum phosphate ($AlPO_4$) after 10 days of incubation varied significantly (p>0.005), from 7.04 to 22.99 mg.mL^{-1}. In the control treatment, the soluble phosphorus value was 7.04 mg.mL^{-1}. The highest value was observed in the strains Th57 (22.99 mg.mL^{-1}), Th49 (22.90 mg.mL^{-1}), and Th40(21.55 mg.mL^{-1}). The strains Th55 and Th48 presented intermediate values, with no significant difference between them. The lowest solubilization values were found in Th12 (9.1584 mg.mL^{-1}), Th26 (8.52 mg.mL^{-1}), and Th78 (7.04 mg.mL^{-1}) (figure 1b). The solubilized phosphorus content was negatively correlated with the pH (R= -0.47; p< 0.05). The pH values were 5.21 in the control and 3.38-2.75 with the different *Trichoderma* treatments. Based on the calcium phosphate (Ca_2PO_4) results, Th53, Th40 y Th26 were selected for the greenhouse study since they showed the highest solubilization of phosphorus.

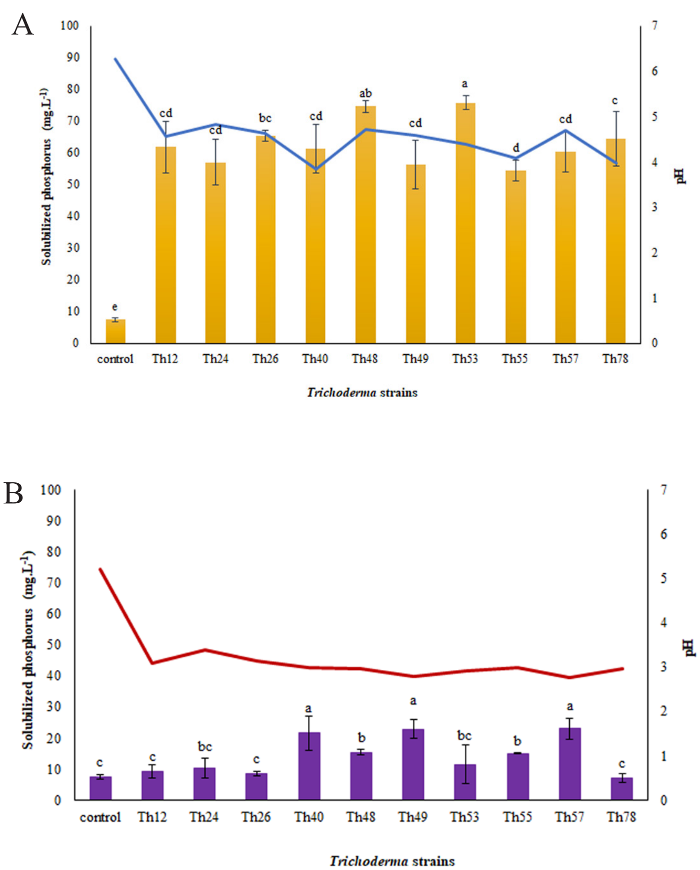

Figure 1. Solubilized phosphorus concentration (bars) and pH (line) of the extracts of *Trichoderma* strains (Th12: *T.* aff *crassum*, Th24: *T.* aff *silva-virgineae,* Th26: *T.* aff *virens*, Th40: *T. asperellum,* Th48: *T. harzianum*, Th49: *T. asperellum*, Th53: *T. harzianum*, Th55: *T. harzianum*, Th57: *T.* aff *asperellum*, Th78: *T.* aff *piramidales*) and control in liquid culture medium with calcium phosphate (a) and aluminum phosphate (b) after 10 days of incubation. Bars values represent the mean of three repetitions ± standard deviation, shared letters in a column do not indicate significant differences when compared with Fisher's means tests (p<0.05). The line values represent the mean of three repetitions.

For the *Trichoderma* strains analyzed in this study, the values of phosphorus resulting from the solubilization of aluminum phosphate were lower than those for calcium phosphate. Zhang *et al.* (2018) found the same with strains of *Talaromyces aurantiacus* and *Aspergillus neoniger*. Hernández-Leal *et al.* (2011) indicated that this can be explained through the Kps values (solubility product constant or equilibrium constant of ions in solution) for the different phosphates, the lower the value, the less soluble the compound. Calcium phosphate has a constant of 2.21x10^{-4}, while aluminum phosphate has a constant of 9.84x10^{-21}, making it more difficult to solubilize. The concentration of solubilized calcium phosphate (Ca_2PO_4) in this study occurred higher than those described for other *Trichoderma* strains by Kribel *et al*. (2019) (up to 12.42 µm.mL^{-1}), Tandon *et al*. (2020) (up to 70.8 µm.mL^{-1}), and Prasad *et al*. (2023) (48.0-56.0 µm.mL^{-1}); therefore, the strains evaluated in this study have great potential for use as biofertilizers. Strains *T. harzianum (*Th48 and Th53) provided the highest solubilization for calcium phosphate (Ca_2PO_4) and for aluminum phosphate ($AlPO_4$); the highest solubilization was achieved by *T. asperellum (*Th57), (Th49) and (Th40). With both sources of insoluble phosphate, a negative relationship with pH was detected, i.e., the greater the phosphate solubilization, the lower the pH. Several authors have associated phosphate solubilization in *Trichoderma* with the decrease in pH mediated by the production of organic acids (Bononi *et al.,* 2020); however, the production of phosphatase enzymes has also been shown. Li *et al*. (2015) reported redox, chelation (siderophore formation), and hydrolysis (phytase)-mediated phosphate solubilization by *Trichoderma*. These acids are products of microbial metabolism (lactic acid, diffuse acid, ascorbic acid, isocitric acid, malic acid, citric acid), mainly through oxidative respiration or by fermentation of carbonaceous substrates (e.g., glucose) (Menezes-Blackburn *et al.,* 2016). Although the secretion capacity and quality of organic acid is basically determined by genetic regulation, it can also be affected by the excretion of protons in the assimilation of NH_4^+ (Osorno and Osorio, 2014).

Study of the ability of *Trichoderma* strains to solubilize phosphate in the greenhouse

Phosphorus soluble

In general, compared to the control, a significant increase was observed in the available phosphorus content in the substrate of the plants of all varieties inoculated with the *Trichoderma* strains. However, the availability of phosphorus and the response of the plants inoculated with the *Trichoderma* strains differed among the three coffee varieties. In the plants of the Anacafe variety, the highest available phosphorus contents were 2.15 mg.kg^{-1} and 2.07 mg.kg^{-1}, with Th53 and Th48 strains, respectively. These values were significantly higher than those in plants inoculated with Th26. The response pattern of the available phosphorus content of the Costa Rica and Marsellesa variety plants to the inoculation of *Trichoderma* strains was similar; however, the values of soluble phosphorus were different. In both varieties, the highest available phosphorus content was detected in the plants inoculated with Th48 strain, followed by the plants inoculated with Th26 and Th53. The available phosphorus values in the Costa Rica variety were higher (1.80-3.49 mg.kg^{-1}) than those in the Marsellesa variety (1.42-2.97 mg.kg^{-1}) and Anacafe variety (1.13-2.15 mg.kg^{-1}) (figure 2a).

Height of coffee plants

The heights of the plants inoculated with *Trichoderma* strains were different in the three coffee varieties. In Anacafe plants, the height of the plants inoculated with *Trichoderma* strains was significantly greater than the height of the plants without *Trichoderma* (control). The height was significantly higher in those plants inoculated with Th53 than those inoculated with Th48 and Th26. The heights of the Costa Rica plants were similar under all treatments ($p>0.05$). In the plants of the Marsellesa variety, the heights were significantly different among all treatments. The greatest height was found in those inoculated with Th53, followed by Th48 and Th26 (figure 2b).

In the Pearson correlation analyzes a significant relationship of phosphorus content with height was only detected in the plants of the Anacafé variety ($R= 0.75$; $p< 0.05$).

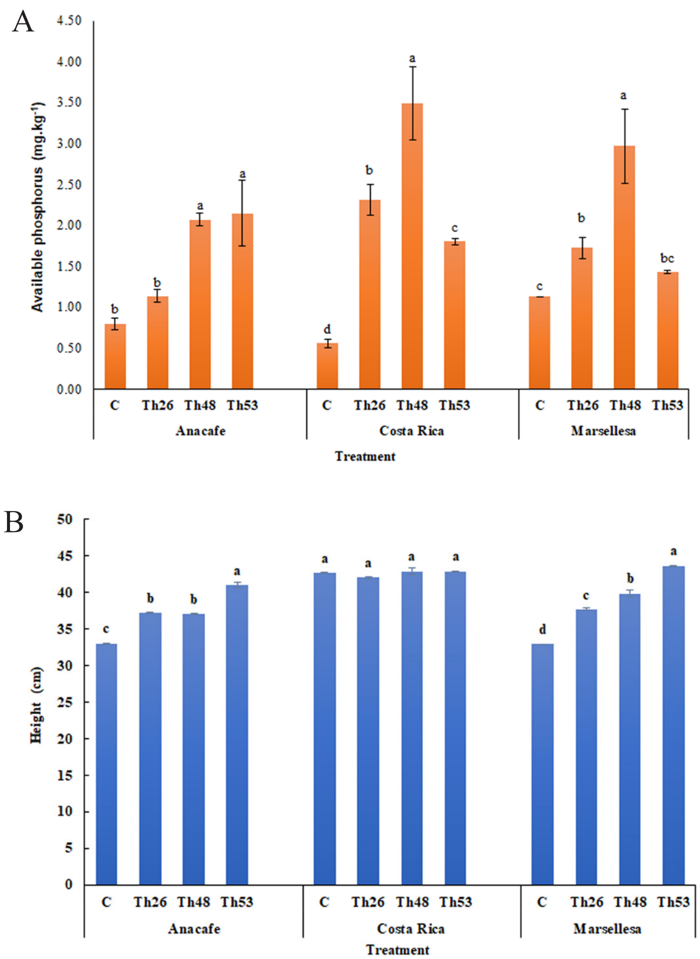

Figure 2. a) Soluble phosphorus in the substrate and b) height of coffee plants of coffee of the varieties Anacafe, Costa Rica and Marsellesa after 6 months of inoculation with three strains of *Trichoderma* (Th26: *T.* aff *virens*, Th48: *T. harzianum*, and Th53: *T. harzianum*) and control (C). The values are the mean of five repetitions ± standard deviation, shared letters in a column indicate no significant differences when compared with the Fisher mean tests ($p<0.05$).

Various studies demonstrate that species belonging to the genus *Trichoderma* improve the growth response of various crops (Galeano *et al.,* 2024; Kaissoumi *et al.,* 2024) because *Trichoderma* strains can produce phytohormones such as auxins and auxin-like secondary metabolites, as well as gibberellic acid and ethylene. The high diversity of *Trichoderma* species in the rhizosphere of coffee plantations has been demonstrated (Arias and Heredia, 2014; Mulatu *et al*., 2022), few studies have addressed the use of strains of this genus as phosphorus solubilizes. Most of the studies on phosphate solubilization by *Trichoderma* species have been performed *in vitro* (Tandon *et al*., 2020; Prasad *et al*., 2023). *In vivo* studies with *Trichoderma* have been reported with some cultures such as cowpea (*Vigna unguiculata*) (Chagas *et al.*, 2016); tomato (Bader *et al.*, 2020); soybean plant (Galeano *et al.,* 2024). Little research has addressed the stimulating effect of using phosphate-solubilizing *Trichoderma* species on coffee plants. In this work, three varieties of coffee plants (Costa Rica, Anacafe, and Marsellesa) were used; though the *Trichoderma* strains showed positive responses in the absorption of phosphorus, the responses of the three varieties of coffee plants to the inoculation of the *Trichoderma* strains were different. The delayed response is probably due to the coffee varieties having a different composition of the exudates that are released, which determines the interactions that may occur there (Huerta and Holguín, 2019). In general, the chemical compounds released by plant roots can interact directly with microorganisms and be directly responsible for the type of interaction and effect caused. However, the interactions are extremely complex and a matrix of variables that regulate the interaction can be generated, to such an extent that there is an overlap in the chemical language used (Oliveros-Bastidas *et al*., 2009). The rhizosphere is an environment rich in nutrients (sugars, organic acids, amino acids, etc.), probably different carbon sources associated with root exudates could have a significant effect on the solubilization of phosphorus that occurs in the rhizosphere (Liebersbach *et al*., 2004).

It is very important that the selection of bioinoculants involves native species, since these will likely show better acquisition of nutrients, better development, and greater modulation in the protection against pathogens. In the plants of the Anacafe, Costa Rica, and Marsellesa varieties, the increase in the available phosphorus was up to 1.42 $mg.kg^{-1}$, 2.52 $mg.kg^{-1}$, and 2.28 $mg.kg^{-1}$, respectively. In the case of the controls, the increase was limited and was even exhausted in the plants of the Costa Rica variety. Perea-Rojas *et al*. (2019) under controlled conditions using coffee plants (*C. arabica* var. *garnica*) inoculated with a consortium of arbuscular mycorrhizal fungi, two strains of P-solubilizing fungi (*Aspergillus niger* and *Penicillium brevicompactum*) resulting in an increase of 1.41-3.8 $mg.kg^{-1}$. In another field study in three coffee plantations, Arias *et al*., (2023) in plants (Costa Rica variety) inoculated with a strain of the phosphorus-solubilizing fungus *P. brevicompactum* showed a significant increase in soil soluble phosphorus, and the benefits of this increase were highlighted in coffee bean production.

It is important to emphasize that, even within the same *Trichoderma* species, not all isolates can promote plant growth. In this study, the beneficial effect was reflected in a higher content of soluble phosphorus in the substrate, however under these conditions it was not related to the height of the plants of most coffee varieties. More time will probably be required for this to be reflected in this variable; long-term tests are needed, including studies to analyze the effect of these fungi on plant survival in the field. The data show large differences in phosphorus content between plants inoculated and those not inoculated with *Trichoderma*, which leads us to assume that under optimal conditions of this nutrient the plant will have a better development that will subsequently translate into greater crop productivity and protection against pathogens. In the rhizosphere, extensive communication occurs between plants and their associated microorganisms through the exchange and perception of signals. In this sense, signaling molecules can have a positive or negative effect on the interaction. However, the composition of organic matter and the associated biotic and abiotic environment can affect the activities of *Trichoderma* (Cano, 2015). It is difficult to predict the outcome of interactions between plants and beneficial soil microorganisms, since the response of plants to inoculation varies depending on the functional and biochemical compatibilities of the interaction. Therefore, further research is required in Mexico to corroborate the effectiveness of strains with phosphorus solubilizing potential to develop technological packages for successful application in coffee cultivation. *Trichoderma* strains may be one of the most important alternatives to chemical fertilizers that can have negative effects on human health and the environment. It is recommended that future studies explore the possibility of promoting these isolates as biofertilizers to improve phosphorus nutrition in multiple crops.

Conclusions

Two strains of *T. harzianum* (Th53 y Th48) from coffee plantations are potentially important for plant development, as well as for the solubilization of insoluble phosphorus. These strains favored the availability of phosphorus *in vitro* in the presence of calcium phosphate (Ca_2PO_4) as well as a higher phosphorus content in the substrate of the three coffee varieties (Anacafe, Costa Rica and Marsellesa). For its part, *T. harzianum* (Th48) promoted the height of the Anacafé and Marsellesa varieties. The *T. asperellum* strains (Th40, Th49, and Th57) were the best at solubilizing aluminum phosphate ($AlPO_4$); this was only evaluated under *in vitro* conditions, so it is recommended to continue with the tests in coffee seedlings. It would be interesting to evaluate other coffee variables, as well as more phosphorus-solubilizing strains and evaluate other agronomic variables. It is also recommended to evaluate plant phosphorus acquisition through whole-plant phosphorus analysis. Field survival tests are also necessary to evaluate the activity of these fungi in different environmental conditions. It also deserves further research to evaluate the effect of the application of *Trichoderma* consortia and with consortia of other microorganisms such as mycorrhizae on the mechanisms of phosphor mobilization. The results presented here are a basis for the development of future study aimed at the use of native and properly identified *Trichoderma* strains in coffee farming and influencing the use of biofertilizers as an environmentally friendly strategy that also favors the production of organic coffee since it has a better position in the market and thus favors the economy of coffee producers in the region. It is suggested to continue the exploration of the selection of strains with high potential to solubilize both calcium, aluminum and iron phosphate, continue testing on application methods and promote integration with other sustainable practices.

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Literature cited

Arias, R. M., & Heredia, G. (2014). Fungal diversity in coffee plantation systems and in a tropical montane cloud forest in Veracruz, Mexico. *Agroforestry systems*, *88*, 921-933. https://doi.org/10.1007/s10457-014-9736-z

Arias, R. M., Heredia, G., Perea-Rojas, Y. del C., de la Cruz, E.Y., & García G. K. Y. (2023). Selection and characterization of phosphate-solubilizing fungi and their effects on coffee plantations. *Plants, 12*(19)*,* 3395. https://doi.org/10.3390/plants12193395

Arias, R. M., Juárez, G.A., Heredia, G., & de la Cruz, E. Y. (2022). Capacidad fosfato solubilizadora de hongos rizosféricos provenientes de cafetales de Jilotepec, Veracruz. *Alianzas y Tendencias BUAP, 7*(27), 6986. http://doi.org/10.5281/zenodo.7094878

Bader, A. N., Salerno, G. L., Covacevich, F., & Consolo, V. F. (2020). Native *Trichoderma harzianum* strains from Argentina produce indole-3 acetic acid and phosphorus solubilization, promote growth and control wilt disease on tomato (*Solanum lycopersicum* L.). *Journal of King Saud University Science, 32*(1), 867–873. https://doi.org/10.1016/j.jksus.2019.04.002

Bononi, L., Chiaramonte, J. B., Pansa, C. C., Moitinho, M. A., & Melo, I. S. (2020). Phosphorus-solubilizing *Trichoderma* spp. from Amazon soils improve soybean plant growth. *Scientific Reports*, *10*(1), 2858. https://doi.org/10.1038/s41598-020-59793-8

Bray, R. H., & Kurtz. L. T. (1945). Determination of total, organic and available forms of phosphorus in soil. *Soil Science, 59,* 39-45. DOI:10.1097/00010694-194501000-00006

Cano, M. 2015. Interacción de microorganismos benéficos en plantas: Micorrizas, *Trichoderma* spp. y *Pseudomonas* spp. una revisión. *Revista U.D.C.A Actualidad & Divulgación Científica, 14*(2), 15-31.

Chagas, L. F. B., De Castro, H. G., Colonia, B. S. O., De Carvalho Filho, M. R., Miller, L. D. O., & Chagas, A. F. J. (2016). Efficiency of *Trichoderma* spp. as a growth promoter of cowpea (*Vigna unguiculata*) and analysis of phosphate solubilization and indole acetic acid synthesis. *Brazilian Journal of Botany*, *39*(2), 437-445. https://doi.org/10.1007/s40415-015-0247-6

Clesceri, S., Greenberg, A., & Trusell, R. (1992). Métodos normalizados para el análisis de aguas potables y residuales. 17th ed. Madrid: Ediciones Díaz de Santos.

Galeano, R. M. S., Ribeiro, J. V. S., Silva, S. M., de Oliveira-Simas, A. L., de Alencar-Guimarães, N. C., Masui, D. C., Corrêa, B. O., Giannesi, G. C., Ferreira de Lima, S., da Silva-Brasil, M., & Zanoelo, F. F. (2024). New strains of *Trichoderma* with potential for biocontrol and plant growth promotion improve early soybean growth and development. *Journal Plant Growth Regulation, 43*, 4099–4119. https://doi.org/10.1007/s00344-024-11374-z

Gams, W., & Bissett, J. (2002). Morphology and identification of *Trichoderma Vol 1. Basic biology, taxonomy and genetics*. In P. C. Kubicek & G. E. Harman (Eds.), *Trichoderma and Gliocladium* (pp. 3-31). Taylor and Francis.

Geissert, D., & Ibañez, A. (2008). Calidad y ambiente físico-químico de los suelos. In R. Manson, V. Hernández-Ortiz, S. Gallina & K. Mehltreter (Eds.), *Agrosistemas cafetaleros de Veracruz: Biodiversidad, Manejo y Conservación* (pp. 213-221). Instituto de Ecología, A.C. (INECOL) e Instituto Nacional de Ecología (INE-SEMARNAT).

Hernández-Leal, T. I., Carrión, G., & Heredia, G. (2011). *In vitro* phosphate solubilization by a strain of *Paecilomyces lilacinus* (Thom) Samson. *Agrociencia, 45*(8), 881-892. https://www.scielo.org.mx/scielo.php?pid=S1405-31952011000800003&script=sci_abstract&tlng=en

Huerta, P. G., & Holguín, M. (2019). La microbiota del cafetal, modulador del daño producido por insectos y enfermedades. In B. E. Bello, L. Soto Pinto, P. G. Huerta, R. J. Gómez (Eds.), *Caminar el cafetal: perspectivas socioambientales del café y su gente* (pp. 49-64). El Colegio de la Frontera Sur. https://biblioteca.ecosur.mx/cgi-bin/koha/opac-retrieve-file.pl?id=29766d1b642f4ea743b565b20e921ff6

Kaissoumi, H. E. L., Berbera, F., Mouden, N., OuazzaniChandi, A., Ouazzani, T. A., Selmaoui, K., Benkirane, R., & Douira, A. (2024). Tomato growth promotion by *Trichoderma asperellum* laboratory-made bioproduct. In M. Azrour, J. Mabrouki & A. Guezzaz. (Eds.), *Sustainable and Green Technologies for Water and Environmental Management* (pp. 161-171). World Sustainability Series. Springer, Cham. https://doi.org/10.1007/978-3-031-52419-6_13

Kribel, S., Qostal, S., Ouazzani-Touhami, A., Selmaoui, K., Chliyeh, M., Benkirane, R., Achbani, E. H., & Douira, A. (2019). Qualitative and quantitative estimation of the ability of *Trichoderma* spp. Moroccan isolates to solubilize tricalcium phosphate. *Plant Cell Biotechnology and Molecular Biology, 20* (7-8), 275-284. DOI: 10.1556/038.54.2019.016

Li, R. X., Cai, F., Pang, G., Shen, Q. S., Li, R., & Chen, W. (2015). Solubilisation of phosphate and micronutrients by *Trichoderma harzianum* and its relationship with the promotion of tomato plant growth. *PLoS One, 10*(6)*,* e0130081. https://doi.org/10.1371/journal.pone.0130081

Liebersbach, H., Steingrobe, B., & Claassen, N. (2004). Roots regulate ion transport in the rhizosphere to counteract reduced mobility in dry soil. *Plant and Soil*, *260*, 79-88. https://doi.org/10.1023/B:PLSO.0000030191.92338.6a

Menezes-Blackburn, D., Paredes, C., Zhang, H., Giles, C. D., Darch, T., Stutter, M., George, T. S., Shand, C., Lumsdon, D., Cooper, P., Wendler, R., Brown, L., Blackwell, M., Wearing, C., & Haygarth, P. M. (2016). Organic acids regulation of chemical–microbial phosphorus transformations in soils. *Environmental Science & Technology Journal, 50*(21), 11521–11531. 10.1021/acs.est.6b03017 https://pubs.acs.org/doi/10.1021/acs.est.6b03017

Mulatu, A., Megersa, N., Abena, T., Kanagarajan, S., Liu, Q., Tenkegna, T. A., & Vetukuri, R. R. (2022). Biodiversity of the genus *Trichoderma* in the rhizosphere of coffee (*Coffea arabica*) plants in Ethiopia and their potential use in biocontrol of coffee wilt disease. *Crops*, *2*(2), 120-141. https://doi.org/10.3390/crops2020010

National Center for Biotechnology Information (NCBI). (2024). *Trichoderma* Internal Transcribed Spacer RNA (ITS), Fungal Internal Transcribed Spacer RNA (ITS) RefSeq Targeted Loci Project. Available online: https://www.ncbi.nlm.nih.gov/nuccore/?term=Trichoderma+its+type (Consulted january 2024).

Oliveros-Bastidas, A. D. J., Macías, F. A., Fernández, C. C., Marín, D., & Molinillo, J. M. (2009). Exudados de la raíz y su relevancia actual en las interacciones alelopáticas. *Química Nova*, *32*, 198-213. https://doi.org/10.1590/S0100-40422009000100035

Osorno, L., & Osorio, N. (2014). Effect of carbon and nitrogen source and concentration on rock phosphate dissolution induced by fungi. *Journal of Applied Biotechnology, 2*(2), 32-42. 10.5296/jab.v2i2.5475 https://www.macrothink.org/journal/index.php/jab/article/view/5475

Perea-Rojas, Y. C., Arias, R. M., Medel, Ortiz, R., Trejo, A. D., Heredia, G., & Rodríguez, Y. Y. (2019). Effects of native arbuscular mycorrhizal and phosphate-solubilizing fungi on coffee plants. *Agroforestry Systems, 93,* 961–972. https://doi.org/10.1007/s10457-018-0190-1

Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil in con-nection with vital activity of some microbial species. *Mikrobiologiya, 17,* 362-70.

Prasad, A., Dixit, M., Meena, S. K., & Kumar, A. (2023). Qualitative and quantitative estimation for phosphate solubilizing ability of *Trichoderma* isolates: A natural soil health enhancer. *Materials Today: Proceedings, 81*, 360-366. https://doi.org/10.1016/j.matpr.2021.03.305

Sadeghian, S., Alarcon, V. F., Díaz-Poveda, V., Lince-Salazar, L. A., & Rey-Sandoval, J. C. (2019). Fertilidad del suelo y manejo de la nutrición. En Centro Nacional de Investigaciones de Café (Ed.), *Aplicación de ciencia tecnología e innovación en el cultivo del café ajustado a las condiciones particulares del Huila* (pp. 80–105). Cenicafé. https://doi.org/10.38141/10791/0005_4

Souchie, E., Azcón, R., Barea, J., Silva, E., Saggin-Júnior, O. (2010). Enhancement of clover growth by inoculation of P-solubilizing fungi and arbuscular mycorrhizal fungi. *Anais da Academia Brasileira de Ciências, 82*(3), 771-777. https://www.scielo.br/j/aabc/a/88xv7fcjktmPnkshbm6xGJF/?lang=en

Tandon, A., Fatima, T., Shukla, D., Tripathi, P., Srivastava, S., & Singh, P. C. (2020). Phosphate solubilization by *Trichoderma koningiopsis* (NBRI-PR5) under abiotic stress conditions. *Journal of King Saud University, 32*(1)*,* 791-798. https://doi.org/10.1016/j.jksus.2019.02.001

White, T. J., Bruns, T., Lee, S., & Taylor, J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D.H. Gelfand, J. J. Sninsky & T. J. White (Eds), *PCR protocols: a guide to methods and applications* (pp. 315-322). Academic Press.

Yang, T., Li, L., Wang, B., Tian, J., Shi, F., Zhang, S., & Wu, Z. (2022). Isolation, mutagenesis, and organic acid secretion of a highly efficient phosphate-solubilizing fungus. *Frontiers in Microbiology, 13*, 793122. https://doi.org/10.3389/fmicb.2022.793122

Zhang, Y., Chen, F. S., Wu, X. Q., Luan, F. G., Zhang, L. P., Fang, X. M., Wan, S. Z., Hu, X. F., & Ye, J. R. (2018). Isolation and characterization of two phosphate-solubilizing fungi from rhizosphere soil of moso bamboo and their functional capacities when exposed to different phosphorus sources and pH environments. *PLoS One, 13(7),* e0199625. https://doi.org/10.1371/journal.pone.0199625

Zin, N. A., & Badaluddin, N. A. (2020). Biological functions of *Trichoderma* spp. for agriculture applications. *Annals of Agricultural Sciences*, *65*(2), 168-178. https://doi.org/10.1016/j.aoas.2020.09.003