Phosphate solubilization, synthesis of Indol Acetic Acid and effect on biomass soybean inoculated with *Pochonia*

Solubilização de Fosfato, síntese de Ácido Indol Acético e efeito na biomassa de soja inoculada com *Pochonia*

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ABSTRACT
The fungus *Pochonia* has been highlighted for its potential as a biological control agent and is very promising as a growth promoter in plants. With this, it is possible to evaluate the phosphate solubilization capacity and the production of indole acetic acid (IAA), in vitro, as well as select strains with potential to promote vegetative growth in soybean crop by *Pochonia* fungus. For phosphate solubilization assay half-strength PD (Potato + dextrose) broth added with K_2HPO_4 and $CaCl_2$ solution was used. For the IAA production assay, the isolates were transferred to erlenmeyer flasks with culture medium in the absence (control) and presence of L-tryptophan. All isolates were able to solubilize phosphate. For the production of IAA, the best results were higher in the presence of the L-tryptophan inducer, and the UFT-PO4 (12.3 μg mL^{-1}) the best isolate. Shoot dry mass, root dry mass, total dry mass and plant height at 45 and 56 DAE (days after emergence) were evaluated in greenhouse conditions. Among the strains, the strain UFT-P05 and UFT-P03 has shown the highest relative efficiency of 77 and 75% respectively. The results of the present work prove the efficiency of the isolates of *Pochonia* spp. as a plant growth promoter.

Keywords: Fungus, Growth Promoter, *Glycine Max* (L.) Merr.

RESUMO
O fungo *Pochonia* tem se destacado por seu potencial como agente de controle biológico, sendo muito promissor como promotor de crescimento em plantas. Com isso, é possível avaliar a capacidade de solubilização de fosfato e a produção de ácido indol acético (IAA), in vitro, bem como selecionar linhagens com potencial para promover o crescimento vegetativo na cultura da soja pelo fungo *Pochonia*. Para o ensaio de solubilização de fosfato, foi usado caldo PD (Batata + dextrose) adicionado de solução com K_2HPO_4 e $CaCl_2$. Para o ensaio de produção de IAA, os isolados foram transferidos

para frascos de erlenmeyer com meio de cultura na ausência (controle) e presença de L-triptofano. Todos os isolados foram capazes de solubilizar o fosfato. Para a produção de IAA, os melhores resultados foram superiores na presença do indutor L-triptofano, sendo o UFT-PO4 (12,3µg mL^{-1}) o melhor isolado. Avaliaram-se a massa seca aérea, massa seca de raiz, massa seca total e altura de planta aos 45 e 56 DAE (dias após a emergência) em casa de vegetação. Os isolados UFT-P05 e UFT-P03 apresentaram eficiência relativa de 77 e 75%, respectivamente. Os resultados do presente trabalho comprovam a eficiência dos isolados de *Pochonia* spp. como promotor de crescimento vegetal.

Palavras-Chave: Fungo, Promotor de Crescimento, *Glycine Max* (L.) Merr.

1 INTRODUCTION

The practice in agriculture has shown that the systematic use of organic fertilizers and biological controls allows the production with better yields, without the need of applying chemical products (Farias et al., 2018). Conservation and management are the most economical way to improve plant nutrition and, therefore, plays an important role in soil and substrate fertility.

The rhizosphere is the contact zone between soil and roots, and shelters a great diversity of microorganisms, where bacteria, fungi, algae, among others are found. Diverse microorganisms participate in plant immunity, aiding their survival, aptitude in defense and nutrition (Kozyrovska, 2013). The solubilization of phosphate by microorganisms is important, since the natural phosphates have the disadvantage of having low solubility and, therefore, are little available to the plants. The production of indole acetic acid (IAA) has also received a lot of attention because of its importance since it is a hormone directly linked to regulation of plant growth. The principal inducer of the IAA production pathway is the amino acid tryptophan (Trp). However, the biosynthesis of this hormone can also occur independently of the precursor Trp (Zhao, 2014; Inácio et al., 2020).

The selection of specific isolates for crops of agricultural interest is fundamental and culminates with the production of inoculants more suitable to the cultivated species.

Pochonia fungus has been shown to have a potential as a biological control agent that exhibits a multitropical lifestyle (Larriba et al., 2014). An ematophagous fungi present in soil that naturally eliminates plant parasites such as nematodes (Dallemole-Giaretta et al., 2014; Bontempo et al., 2017; Hidalgo-Díaz & Ceiro, 2017). Some studies have shown results suggesting that *Pochonia* isolates may be plant growth promoters in crops of great importance, as reported in studies with barley (Rosso et al., 2014), tomato

and lettuce (Dallemole-Giaretta et al., 2015), besides decreasing flowering time and fruiting time in *Solanum lycopersicum* (Zavala-González et al., 2015), and biomass of bean (Ortega et al., 2019).

A number of studies has reported the expression of plant genes related to biosynthesis and transport of plant growth and defense hormones in response to the endophytic colonization by these fungi (Larriba et al., 2015). There is strong evidence that part of these phytohormone-related effects results in increased root growth. For example, the regulation of genes for auxin biosynthesis increased the root production in barley colonized by *Pochonia chlamydosporia* (Maciá-Vicente et al. 2009).

Thus, *Pochonia* spp. are fungi of major importance in the production of metabolites with application in agriculture. The aim of this study was to evaluate the phosphate solubilization capacity and indole acetic acid (IAA) production in vitro, besides selecting with potential as soybean plant growth promoter in a greenhouse.

2 MATERIALS AND METHODS

Isolation and phenotypic characterization of Pochonia Isolates

The experiments were conducted at the Microbiology Laboratory of the Federal University of Tocantins - Gurupi Campus, Brazil. The inoculum sources of *Pochonia* spp. were isolated from different samples of soils cultivated in regions of the state of Tocantins, Brazilian center-north region.

To obtain the isolates of *Pochonia* samples of 15 g soil (near roots of crops) were collected separately in 150 mL Erlenmeyer flasks containing 90 mL of sterilized water and shaken for 25 minutes on an orbital shaker (Novatécnica®, 100 rpm at 26 ± 2°C). Subsequently, serial dilutions were made up to $1x10^{-3}$, and a 1 mL aliquot of each sample was spread onto Petri dishes containing semi-selective medium, with the aid of a Drigalsky. The plates were stored at 28 °C until the growth of fungal colonies. The colonies of each soil sample were peeled onto plates containing PDA medium (20% Potato, 2% dextrose, 1.5% agar and distilled water, pH 6.8) and incubated in BOD at 29 ± 1°C with photoperiod of 12 hours with and 12 without light on seven days. The fungal colonies were pre-identified, according to the morphological characteristics of the isolate, such as colony color and conidiophore type. Subsequently, the isolates were stored and maintained at 8 °C for further studies (Gonçalves et al., 2007).

Phosphate solubilization

After collection and isolation of *Pochonia* the ability to produce in vitro substances related to plant growth were evaluated, as well as the ability to solubilize insoluble phosphates, according to the methodologies described in Cattelan (1999).

For the in vitro phosphate solubilization assay in liquid medium, the isolates of *Pochonia* were initially cultured on PDA medium (20% Potato, 2% dextrose, 1,5% agar and distilled water pH 6,8) and incubated in BOD at 29 ± 1°C for seven days. In order to determine the potential for solubilization of phosphate in vitro in modified NBRIP medium, the isolates were extracted with Erlenmeyer (250 mL) containing the following ingredients (g L^{-1}): glucose, 10.0; $MgCl_2.6H_2O$, 5.0; $MgSO_4$.$7H_2O$, 0.25; KCl, 0.2; $(NH_4)_2SO_4$, 0.1. 50 ml of K_2HPO_4 (10%) and 100 ml of $CaCl_2$ (10%) were added to the medium to form an insoluble precipitate of calcium phosphate ($CaHPO_4$).

The quantitative estimation of phosphate solubilization was performed in triplicate in a completely randomized design. Afterwards they were cultivated in a stirring table with orbital movement (Novatécnica®, 150 rpm at 28 ± 2 ºC) for eight days. Evaluations were performed at two, four, six and eight days post transfer. For the determination of the soluble phosphorus (P) concentration, the colorimetric method was used, subtracting the soluble P contained in the treatments by the contained in the control sample (culture medium with phosphate and without inoculum).

For the evaluations, part of the reagent, 0.5 mL of the filtered sample and 5 mL of distilled water were used for each sample. After 20 minutes of reaction the soluble P was quantified in a spectrophotometer at the wavelength of 725 nm of absorbance. The standard curve for quantification of P was made from the potassium phosphate monobasic (KH_2PO_4) and the calculated concentrations in μg mL^{-1}.

Production of indole acetic acid (IAA)

For the production tests of IAA, colonies of *Pochonia* were previously cultured in Petri dishes in PDA culture medium (20% Potato, 2% dextrose, 1,5% agar and distilled water pH 6,8) and incubated in BOD at 29 ± 1°C for seven days. PD medium (20% Potato, 2% dextrose and distilled water, pH 6.4) was used. Isolates were transferred through disks approximately 4-5 mm in diameter containing the fungus mass to Erlenmeyer flasks (250 mL) containing 100 mL with the PD media in the absence (control) and presence of L-tryptophan. The experiment was carried out in a completely randomized design with four replicates. Evaluations were done at 48, 96, 144 and 192 hours after inoculation of

Pochonia being cultivated in a stirring table with orbital movement (Novatécnica®, 100 rpm at 26 ± 2 ºC). Subsequently the cell biomass of the fungus was separated by centrifugation (Excelsa® 4 model 280R, 12,000 rpm for 15 min.). A portion of the Salkowski reagent [$FeCl_3$ 0.5mol L^{-1} + $HClO_4$ (35%)] and two parts of the supernatant obtained from each isolate were used for the colorimetric analysis of IAA. After the qualitative verification of the presence of IAA (pink color after 25 minutes of reaction at 26 ± 2 ºC in the dark), the phytormonium was quantified in spectrophotometer at 530 nm. Concentrations in µg mL^{-1} were calculated from a standard curve with known concentrations of the synthetic form of the hormone (0 to 100 µg mL^{-1}), whose readings were based on the calculation of the concentration of IAA in the samples (Cattelan, 1999).

Greenhouse experiment

The experiments were conducted at the Experimental Station of the Federal University of Tocantins, Gurupi Campus, located at 11°43'45"S and 49°04'07" W at 278 m altitude. Soil samples (surface layer 0- 0,20m) were collected for chemical and physical soil analysis. The soil of the experimental area was classified as a Typical Dystrophic Yellow Red Oxisol with the following chemical characteristics: Ca+Mg 1.11 cmol dm^{3-1}; Ca 0.63 cmol dm^{3-1}; Mg 0.48 cmol dm^{3-1}; Al 0.40 cmol dm^{3-1}; H+Al 2.71 cmol dm^{3-1}; K 0.04 cmol dm^{3-1}; CTC (T) 3.85 cmol dm^{3-1}; SB 1.15 cmol dm^{3-1}; K 14.45 mg dm^{3-1} (ppm); P (Mel) 0.29 mg dm^{3-1} (PP); V 29.77%; M 25.86%; organic matter 2.04% 20.37 g dm^{3-1}; pH $Cacl_2$ 5.58; pH H_2O 5.73 (Embrapa, 2011).

The study was carried out with the inoculation of five isolates of *Pochonia* (UFT-P01, UFT-P02 UFT-P03, UFT-P04 and UFT-P05) in soybean (Glycine max L.) cultivar M8210 IPRO which shows the following phenotypic characteristics: semi-erect size, of 72 cm, flowers of white color, color of the black thread, maturity group 8.2. It is a widely adapted variety, with high productive potential and can be used as 2nd harvest due to its precocity. The seeds were previously treated. The fungicides and insecticides [in g ai (active ingredient) (100 kg of seeds)$^{-1}$] + 2% of water were added on the seeds, shaking until completely covered according to the recommendation for the crop, using a fungicide of contact and systemic class and insecticide of contact and ingestion class of the pyrazole group. Subsequently, the addition of 100 mL for each 50 kg of seeds of the commercial liquid inoculant of *Bradyrhizobium japonicum* (strains SEMEA 5079 and SEMEA 5080) with a minimum concentration of 6 x 10^9 UFC mL^{-1} was added.

For the installation of the experiments, vessels with a capacity of 1.7 L were used,

filled with sieved soil (4 mm mesh). The fertilization was carried out according to the recommendation for the culture and soil chemical analysis. To prepare the inoculum, rice was previously weighed and distilled water added and then transferred to polypropylene bags [(300 g of rice) (bag with 300 ml of distilled water)$^{-1}$] and autoclaved at 121 °C for 1 hour. After the inoculation of the *Pochonia* ssp. strains, disks (6 mm diameter, 6 units) of the *Pochonia*, previously cultivated (28 °C) on PDA agar (20% potato, dextrose 2% and 1,5% agar, distilled water, pH 6.8), were added and inoculated into rice with use of a sterilized tweezer through the opening of the polypropylene bag in a laminar flow chamber.

After the mycelia inoculation, the bags were incubated in a BOD (Biochemical Oxygen Demand) chamber with controlled temperature and light, at 25 °C in the dark for 21 days. Every two days, the substrate containing rice was stirred, to facilitate the gas exchange and the breakdown of the mycelial aggregates for the increase of sporulation. After 21 days of incubation, the substrate with rice was then weighed in a proportion of 30 g per vessel of 1.7 L, and then inoculated to the soil, which remained for seven days submitted to irrigation for colonization in the soil, preceding sowing.

The concentration of *Pochonia* was determined by quantifying the number of conidia. One gram of rice was washed in 10 mL of distilled water and agitated for 1 min. Then the number of conidia was counted in a Neubauer chamber using an optical microscope. For the experiment, 1,0 x 10^8 conidia per gram of colonized rice were used. Rice without *Pochonia* served as the control.

In the experiments, four seeds were sown and at twelve days after sowing (DAS) the thinning was done leaving two plants per vessel. The irrigation was done with the aid of a graduated glass, maintaining the field capacity. For the fertilization the formulated NPK formulation 5-25-15 in the proportion of 520 kg ha^{-1}, equivalent to 2 g per vessel was used. Two evaluations were made in the experiment, the first at 45 days after emergence (DAE) and the second at 56 DAE when the plants showed full flowering. Plant height (H) was evaluated. Afterwards, the root system was separated from the aerial part of the plants and washed in running water to remove the adhered soil and after, the material was placed in oven for drying with forced aeration at 65 °C for 72 hours until reaching constant mass. After dried, the material was weighed in a precision analytical balance (0.001 g) to obtain the shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM). With the biomass data, the relative efficiency of each treatment was determined in relation to the control without inoculation, calculated according to formula:

RE = (SDM inoculated with the isolates / SDM without inoculant) x 100.

Statistics

After verification of the assumptions, the data were submitted to analysis of variance F test and to the clustering test of Ducan averages at 5% probability using the statistical program ASSISTAT 7.6 beta version.

3 RESULTS AND DISCUSSION

Phosphate solubilization

All the isolates showed the ability to solubilize phosphorus by the in vitro calcium phosphate solubilization method performed in the laboratory, even in small quantities, all the isolates acidified the medium (Table 1). Phosphorus readings ranged from 3.48 to 13.42 μg mL^{-1}.

Table 1. Means of concentration of solubilized phosphate (μg mL^{-1}) in NBRIP medium (modified) by *Pochonia* isolates, at different time intervals (days)[1].

Isolates	Evaluation time (hours after transfer)				
	48 h	96 h	144 h	192 h	pH
UFT-P01	4.15 bc	5.39 b	5.90 c	3.64 c	5.4 b
UFT-P02	10.82 a	12.44 a	12.53 a	13.42 a	4.9 a
UFT-P03	4.26 bc	5.77 b	3.93 d	3.48 c	5.5 b
UFT-P04	6.29 b	6.30 b	9.07 b	10.39 b	4.8 a
UFT-P05	5.42 b	3.98 c	4.06 d	4.66 c	5.4 b
Control	0.28 d	0.40 d	0,39 e	0.41 d	5.9 c
C.V.(%) [2]	8.5	12.1	11.7	12.0	

[1]Averages followed by the same lowercase letter in the column do not differ from each other by the Duncan test at 5% significance. [2]Coefficient of Variation.

In the first evaluation with 48 h, the UFT-P02 isolate was superior, presenting 10.82 μg mL^{-1}, followed by the other isolates that had variations from 4.14 to 6.29 μg mL^{-1}. For the 96 h evaluation, the UFT-P02 isolates were superior to the others (12.44 μg mL^{-1}), followed by the isolates UFT-P01, UFT-P03 and UFT-P04, with 5.39; 5.77 and 6.30 μg mL^{-1}, respectively (Table 1).

In the last evaluations (144 and 192 h), the best isolates in the general mean were the isolates UFT-P02 and UFT-P04, 12.53 and 9.07 μg mL^{-1} in 144 h, and 13.42 and 10.39 μg mL^{-1} in 192 h, respectively. At a general average, it is observed that UFT-P02 and UFT-P04 isolates were the best for phosphate solubilization at all evaluations.

Production of indole-acetic acid (IAA)

All isolates showed the ability to synthesize IAA, even in small amounts, both in the presence and absence of L-tryptophan (Table 2). In the absence of the inducer, the synthesis of IAA ranged from 0.77 to 7.45 µg mL^{-1}.

Table 2. Production of IAA (µg mL^{-1}) by *Pochonia* isolates in PD medium in the absence (A) and presence (P) of L-tryptophan[1].

Isolates	Evaluation time (hours after transfer)					
	48 hours			96 hours		
	A	P	Mean	A	P	Mean
Control	0.60 cA	0.72 bcA	0.66 c	0.71 bcA	0.77 cA	0.74 c
UFT-P01	0.77 bcB	0.99 bA	0.88 b	0.63 cB	0.94 cA	0.79 c
UFT-P02	0.90 bA	0.94 bA	0.92 b	0.83 bA	0.88 cA	0.86 c
UFT-P03	0.80 bA	0.96 bA	0.88 b	0.71 bcA	0.91 cA	0.81 c
UFT-P04	5.65 aA	6.45 aA	6.05 a	5.09 aB	8.01 aA	6.55 a
UFT-P05	0.84 bA	1.04 bA	0.94 b	0.80 bB	1.43 bA	1.12 b
Mean	1.59 B	1.85 A	-	1.46 B	2.16 A	-
C.V.(%) [2]	7.6	7.6	7.6	8.8	8.8	8.8
	144 hours			192 hours		
Control	0.51 bB	0.70 bcA	0.61 bc	0.71 cA	0.75 cA	0.73 c
UFT-P01	0.41 bcA	0.51 cA	0.46 c	0.45 dB	0.71 cA	0.58 c
UFT-P02	0.59 bA	0.67 bcA	0.63 bc	1.10 bA	1.51 bA	1.31 a
UFT-P03	0.63 bA	0.88 bA	0.76 b	0.68 cB	1.44 bA	1.06 ab
UFT-P04	5.66 aB	9.44 aA	7.55 a	7.45 aB	12.30 aA	9.88 a
UFT-P05	0.59 bB	1.00 bA	0.80 b	0.77 cB	1.34 bA	1.06 ab
Mean	1.40 B	2.2 A	-	2.18 B	3.01 A	-
C.V.(%) [2]	8.1	8.1	8.1	8.6	8.6	8.6

[1]Averages followed by the same lower case letter in the column and upper case in the row do not differ by Duncan's test at 5% significance. [2]Coefficient of Variation.

In the first 48 and 96 h of incubation, UFT-P04 (5.65 and 5.09 µg mL^{-1}) and UFT-P02 (0.9 and 0.83 µg mL^{-1}) isolates were better. In the evaluation of 144 and 196 h, the UFT-P04 isolate maintained the best results, increasing production to 5.66 µg mL^{-1} in 144 h and maintaining the value in 196 h (7.45 µg mL^{-1}) (Table 2).

In the presence of L-tryptophan, there is an increase in the synthesis of indole acetic acid, varying from 0.94 to 6.45 µg mL^{-1}. At 48 h incubation, the best result was observed with UFT-P04 isolate (6.45 µg mL^{-1}), followed by isolates UFT-P05, UFT-P01 and UFT-P03 and UFT-P02, obtaining 1.04; 0.99; 0.96 and 0.94 µg mL^{-1}, respectively. The best results were observed with the isolates UFT-P04 and UFT-P05, obtaining respective values of 8.01 and 1.43 µg mL^{-1} in 96 h; 9.44 and 1.00 µg mL^{-1}in 144 h; and 12.30 and 1.34 µg mL^{-1}in 192 h of incubation (Table 2).

In a general average, UFT-P04 isolates were the best for the production of IAA in

the first hours of incubation, 6.05, 6.55 and 7.55 µg mL^{-1} in 48, 96 and 144 h, respectively. At 192 h of incubation, on average the best isolates in the general mean were UFT-P04 and UFT-P02, with 9.88 and 1.31 µg mL^{-1}, respectively. It is also important to highlight that the isolates presented more satisfactory results than those obtained with the control, both in the presence and absence of L-tryptophan (Table 2).

Greenhouse experiment

In the greenhouse experiment, it was evaluated the growth promotion of soybean cultivation in vessels with strains of *Pochonia*, in which some isolates showed a greater capacity to promote plant growth and increase the dry mass of the crop according to the evaluations (Tables 3).

Table 3. Mean values of plant height (H), shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM) of soybean (M8210 IPRO) inoculated with Pochonia isolates under greenhouse conditions[1].

Isolates	H (cm)	SDM (g $pots^{-1}$)	RDM (g $pots^{-1}$)	TDM (g $pots^{-1}$)
		45 DAE		
UFT-P01	19.0 ab	1.67 a	1.24 a	2.91 a
UFT-P02	18.0 b	1.61 a	1.17 a	2.78 a
UFT-P03	15.3 c	1.71 a	1.10 a	2.81 a
UFT-P04	20.3 a	1.63 a	1.19 a	2.82 a
UFT-P05	19.0 ab	1.94 a	1.20 a	3.14 a
Control	10.7 d	1.46 a	0.93 a	2.39 a
CV (%)[2]	6.8	18.3	24.4	14.5
		56 DAE		
UFT-P01	27.3 a	2.29 b	3.26 a	5.55 a
UFT-P02	26.3 a	2.78 ab	3.20 a	5.98 a
UFT-P03	26.7 a	3.72 a	3.41 a	7.13 a
UFT-P04	26.3 a	2.97 ab	3.47 a	6.44 a
UFT-P05	26.3 a	3.76 a	3.92 a	7.68 a
Control	17.0 b	2.13 b	3.14 a	5.27 a
CV (%)[2]	7.8	24.9	33.0	26.6

[1]Averages followed by the same letter do not differ statistically from each other by Duncan's Test at5% probability level.[2] Coefficient of Variation.

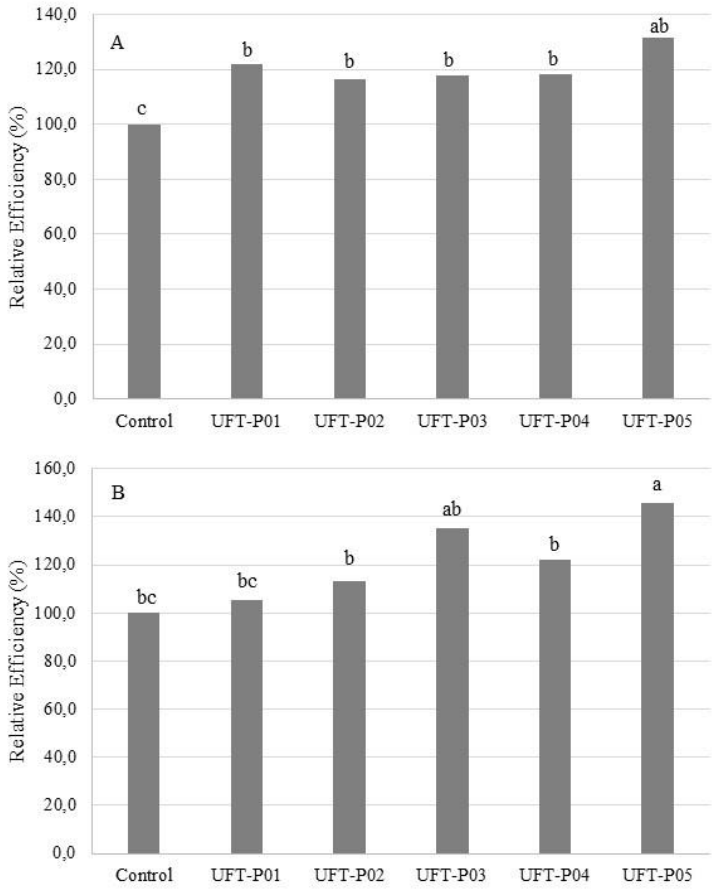
In the first evaluation at 45 DAE, for the parameter height all the treatments in which the isolates of *Pochonia* ($p<0.05$) increased the height of the soybean plants in relation to control (Table 3). The isolates with the highest values were: UFT-P04 with the highest value (20.3 cm), the isolates UFT-P01 and UFT-P05 that did not differ statistically from the first (19.0 cm), the control, without inoculation of fungus, obtained height of (10.7 cm). With these values, the increment obtained by the isolates of *Pochonia* was approximately 90% higher in comparison of the UFT-P04 isolate with the control (Table

3). For the other characteristics: Shoot dry mass (SDM), dry mass the root (RDM) and total dry mass (TDM), there was no variation between treatments (Table 3). For the second evaluation at 56 DAE, for the height parameter, all the treatments inoculated with *Pochonia* sign a statistical difference of the control, but among the treatments inoculated with *Pochonia* there was no statistical difference (Table 3).

For the SDM parameter, there were differences between some isolates in relation to the control, with strains UFTP-05 and UFT-P03 with the highest values (3.76 and 3.72 g pots^{-1}, respectively). For RDM and TDM, the treatments presented no significant difference ($p<0.05$) in relation to the control (Table 3).

Regarding the relative efficiency of the treatments at 45 DAE, all the isolates presented a relative efficiency superior to the control (Figure 1), with emphasis on the isolate UFTP-05 that presented relative efficiency (RE) greater than 30%.

Fig1. Relative efficiency of soybean plants (M8210 IPRO) at 45 (A) and 56 (B) DAE inoculated with *Pochonia* in relation to the control without inoculation.



Averages followed by the same letter do not differ statistically from each other by Duncan's Test at5% probability level.

The results of the second evaluation showed that all the isolates had an increase

in relative efficiency. In addition, all treatments with *Pochonia* inoculation were superior to the control, especially the isolates UFT-P05 and UFT-P03 that had the highest values (77% and 75%).

The *Pochonia* isolates presented capacity for phosphate solubilization, as shown in Table 1. In a study of solubilization by microorganisms, both, fungi and bacteria isolated from the rhizosphere of pigeon pea (*Cajanus cajan* L. Millsp.), it was observed that most of the fungi lowered the pH of the medium from 6.5 to values between 2.0 and 4.0, while for the bacteria the variation was 4.0 and 6.5. This caused higher solubilized phosphate averages to be observed with fungi (122 mg L^{-1}) than with bacteria (15 mg L^{-1}). In addition, the authors state that solubilized phosphate content depends on the type of species and the type of soil in which they were isolated (Souchie & Abboud, 2007). In the present work it can also be observed in the *Pochonia* isolates where a higher pH decrease occurred in the isolates with greater capacity to solubilize phosphate (Table 1).

The genes present in several species of *Pochonia* are related to their endophytic behavior, also comprising the production of hydrolytic enzymes, transporters, proteases, chitinases and a large number of secondary metabolites, aiding in the phosphate solubilization and production of IAA (Larriba et al., 2014). In this interaction between the plant and microorganism, the fungus can increase the tolerance of the culture to the biotic and abiotic stresses, besides promoting the growth of the plants (Manzanilla-López et al., 2013), as note by Dallemole-Giaretta et al. (2015), were the isolates Pc-3, Pc-10 and Pc-19 of P. *chlamydosporia* promote the growth of tomato and lettuce seedlings. This fungus colonizes the roots of both plant species and produces hyphae and chlamydospores in the rhizoplane of tomato plants.

As for the ability to synthesize IAA, all the isolates in the studies presented results, but were higher in the presence of L-tryptophan added to the culture medium (Table 2). A microorganism has several routes for the synthesis of IAA, and can choose one according to the environment. Different varieties of bacteria have the capacity to produce this auxin, for more than one route, most of them with the use of tryptophan (Goswami et al., 2015). Thus, this precursor has been added to the culture medium with the aim of promote synthesis increase.

Oliveira et al. (2012) in a study of the potential of IAA production by *Trichoderma* spp. in the presence and absence of L-tryptophan, observed significantly higher results with the use of L-tryptophan, reaching values of 19.9 µg mL^{-1} on the sixth day of evaluation. However, the authors observed that in the absence of the inducer the isolates

were also able to produce IAA. Kuss et al. (2007) observed similar results in the study with microorganisms associated to rice roots without the use of the tryptophan inducer, in which IAA was produced by all isolates, varying between 2.79 and 13.47 µg mL^{-1}.

Microorganisms can stimulate the growth of plants directly, through the production of plant hormones, solubilization of phosphorus, acceleration of the mineralization process and synthesis of siderophores; as well as indirectly through the induction of systemic resistance, production of antibiotics and antagonism to pathogens.

It has been documented that the fungus *Pochonia chlamydosporia*, a biological control agent for nematodes and promotes plant growth, increases the content of nutrients in plants not only by the production of plant hormone such as auxin, and also by making phosphorus available through the production of organic acids and phosphatase enzymes that act in the solubilization of phosphate, making this nutrient available to plants (Gouveia, 2018).

As for the selection of the isolates in a greenhouse, the promotion of plant growth is directly linked to the rhizosphere, which is the contact zone between soil and roots. The fungi have the capacity to solubilize phosphate and to produce metabolites directly related to the growth of the plant and to synthesize secondary metabolites reducing the activities of pathogens (Larriba et al., 2014; Manzanilla-López et al., 2013). This fact is observed in the various agronomic characteristics evaluated, such as plant height and shoot dry mass (Table 3).

According to Ortega et al. (2019), the strain IMI SD 187 of *Pochonia chlamydosporia* var. catenulate (Goddard) Zare & Gamsincreased the in vitro germination parameters of the bean seeds, with a significant effect on hypocotyl length and fresh plant weight. High root colonization was achieved with two applications of the fungus, the endophytic root colonization was 16.67 % in 30 days and seed germination was advanced in two days. There was a tendency to increase the parameters number of trifoliate leaves, shoot length, fresh and dry plant weight, and fresh root weight, in different treatments with the fungus compared with the control treatment.

The relative efficiency of the isolates found in the experiments (Figure 1) can be explained by the interactions between plant growth promoted by fungi and induction of systemic resistance to the disease (Khan et al., 2015).

The results of the present work showed the ability of *Pochonia* in the synthesis of AIA and phosphate solubilization, and as a promoter of soybean plant growth. Future research is needed on different soybean cultivars and different applications of the fungus

to ensure protection in field conditions, with a view to promoting better plant growth, given its potential as a phosphate solubilizer and IAA synthesis, as well as its impact on increasing productivity and crop productivity.

4 CONCLUSIONS

Pochonia isolates showed phosphate solubilization capacity, with a solids content of 13.42 μg mL^{-1} (UFT-P02).

Regarding the production of indole acetic acid, the best results were obtained in the presence of the L-tryptophan inducer, and the best isolates observed were UFT-P04 and UFT-P02.

In the greenhouse experiment, strains UFT-P01, UFT-P04 and UFTP-05 were highlighted for the height parameter in the evaluation of 46 DAE. At 50 DAE, it was observed that UFT-P02, UFT-P03, UFT-P04 and UFT-P05 showed the best results in relation to shoot dry matter. In addition, strains UFT-P03 and UFT-P05 were the ones that presented better relative efficiency of the isolates.

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