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Trichoderma spp. isolates with potential of phosphate solubilization and growth promotion in cherry tomato¹

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ABSTRACT

Trichoderma fungi are considered relevant plant growth promoters for increasing the efficiency in the use of nutrients, as well as acting as biological control agents. This study aimed to select *Trichoderma* spp. isolates with potential for phosphate solubilization and their application as growth promoters in interaction with homeopathic preparations, in cherry tomato. Among 16 *Trichoderma* spp. isolates obtained from soils of organic tomato growing areas tested *in vitro*, together with a commercial product (Trichodermil®), two of them showed the ability for indole-3-acetic acid production and phosphate solubilization. The *Trichoderma* “R” had the highest mycelial growth speed index and presented twice as much spores than the commercial product. An *in vivo* experiment was also conducted in a greenhouse, in order to observe the potential of *Trichoderma* spp. isolates and homeopathic preparations on the cherry tomato growth promotion, using a randomized block experimental design, in a 4 x 3 factorial arrangement, with three *Trichoderma* isolates and two homeopathic preparations (*Phosphorus* 6CH and *Carbo vegetabilis* 6CH) + treatment without homeopathic preparation. The leaf area and dry mass of leaves and roots were determined. It was possible to observe that the isolate “R”, identified as *Trichoderma asperellum*, was effective in the cherry tomato growth promotion, while the homeopathic preparations applied did not show any effect.

KEYWORDS: *Solanum lycopersicum* L.; phosphorus; phytohormones; homeopathy.

RESUMO

Isolados de *Trichoderma* spp. com potencial de solubilização de fosfato e promoção de crescimento em tomateiro cereja

Fungos do gênero *Trichoderma* são importantes promotores de crescimento vegetal, por aumentarem a eficiência na utilização de nutrientes e atuarem como agentes de biocontrole. Objetivou-se a seleção de isolados de *Trichoderma* spp. com potencial de solubilização de fosfato e sua aplicação como promotores de crescimento em interação com preparados homeopáticos, em tomateiro cereja. Dentre 16 isolados de *Trichoderma* spp. obtidos em solos de áreas de produção orgânica de tomateiro testados *in vitro*, em conjunto com um produto comercial (Trichodermil®), dois apresentaram produção de ácido indolacético e solubilização de fosfato. O *Trichoderma* “R” foi o isolado de maior índice de velocidade de crescimento micelial, com duas vezes mais esporos do que o produto comercial. Um experimento *in vivo* também foi conduzido em estufa, para verificar o potencial de isolados de *Trichoderma* spp. e preparados homeopáticos no crescimento de tomateiro cereja, utilizando-se delineamento experimental em blocos casualizados, em esquema fatorial 4 x 3, com três isolados de *Trichoderma* e dois preparados homeopáticos (*Phosphorus* 6CH e *Carbo vegetabilis* 6CH) + tratamento sem homeopatia. Avaliou-se a área foliar e a massa seca das folhas e das raízes. Verificou-se que o isolado “R”, identificado como *Trichoderma asperellum*, foi eficiente na promoção de crescimento do tomateiro cereja, ao passo que os preparados homeopáticos aplicados não apresentaram efeito.

PALAVRAS-CHAVE: *Solanum lycopersicum* L.; fósforo; fitohormônios; homeopatia.

INTRODUCTION

Trichoderma is a biocontrol agent used to improve the resistance to diseases that may also stimulate plant growth (Oliveira et al. 2016, Roese et al. 2017). Growth promotion mechanisms in plants are associated with microorganisms such as

fungi and bacteria, which are capable of solubilizing phosphates through their metabolism, producing phytohormones or fixing nitrogen (Péres-Montañón et al. 2014).

Fungi of the *Trichoderma* genus are among the most studied microorganisms, due to their ability to colonize the rhizosphere and different plant organs,

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promoting beneficial effects on the plant development (Carvalho et al. 2011). These fungi promote growth in plants, since some species have the ability to produce phytohormones (Carvalho et al. 2011) and solubilize phosphorus (Oliveira et al. 2012).

Phosphorus is an essential element to the metabolism and development of plants from their initial stage of life. It plays a key role in the root system growth of plants. This element is also one of the nutrients needed to promote energy transfer among cells, in the photosynthesis process (Magdoff & Van Es 2009, Ribas et al. 2016).

Indole-3-acetic acid (IAA) is a phytohormone which belongs to the class of the auxins. It acts as a growth regulator in plants, promoting growth by stimulating mitosis, elongating existing cells or even increasing the number of plant cells. There are several microorganisms, such as *Trichoderma* spp., capable of interacting with plants and synthesizing IAA through its precursor L-tryptophan (Ribas et al. 2016).

Homeopathy is the science of the similarity principle, which consists in administering to ill individuals substances that cause similar symptoms in healthy individuals (Teixeira 2012). Among numerous substances that may be employed, chosen by similarity, *Carbo vegetabilis* and *Phosphorus* stand out, the first made from charred plant material from trees and the second one from organic phosphorus salts (phosphates) (Casali et al. 2009). *Carbo vegetabilis* has an extensive effectiveness of action, being used in agricultural homeopathy for seed dormancy break or to recover plants that suffered with frost and other factors relating to environmental stress (Pinto et al. 2014). *Phosphorus* is a homeopathic treatment used in plants suffering with excessive transpiration, due to excessive heat, or even plants suffering with nutritional deficiencies and growth problems (Santos et al. 2011). It is a homeopathic preparation used to stimulate the development of tissues, buds and leaves (Toledo et al. 2015, Gonçalves et al. 2016) and may have an influence in the phosphorus absorption by plants. The 6CH dinamization was studied for application in plants by several researchers, showing positive effects (Rossi et al. 2006, Modolon et al. 2012, Toledo et al. 2015, Gonçalves et al. 2016, Mioranza et al. 2017).

Tomato (*Solanum lycopersicum* L.) (Syn.: *Lycopersicon esculentum* Mill.), a plant from the

Solanaceae family, is one of the most consumed vegetables in Brazil (Embrapa 2006). Among vegetables, it has one of the largest cultivated areas in the world (Carvalho et al. 2014). Studies have sought alternatives to pest and disease control (Modolon et al. 2012), crop management (Takahashi & Cardoso 2015) and physiological disorders related to nutrition and edaphoclimatic factors (Loos et al. 2008, Costa et al. 2011). Cherry tomatoes, if compared to table tomatoes (salad, Italian or kaki), usually have a greater resistance to pests and diseases, having possibly suffered a natural selection pressure for hardness (Maciel & Silva 2008).

Thus, this study aimed to select *Trichoderma* spp. isolates with a phosphate solubilization potential and use them as growth promoters in interaction with homeopathic substances, in cherry tomato.

MATERIAL AND METHODS

The experiments were performed *in vitro*, from January to June 2015. Soil samples were collected up to 0.20 m of depth, with a Dutch auger, in the rhizosphere of tomatoes, in organic tomato producing farms in Piracicaba, São Paulo state, Brazil, and stored in plastic bags.

For the *Trichoderma* spp. isolation, 10 g of each soil sample were mixed with 90 mL of sterile distilled water, in a 200 mL Erlenmeyer flask, and kept in a shaker incubator, for homogenization, for 3 hours. After this period, dilutions were performed in series with samples of up to 10^{-3} , and 2 mL of this solution were applied using a modified Martin medium (Martin 1950).

The microbial growth on the plates was monitored. Successive subcultures in culture medium formulated with potato-dextrose-agar (PDA) were made to obtain seemingly pure cultures. *Trichoderma* spp. isolates were identified using an optical microscope, from monosporic cultures obtained from a small fragment of culture medium colonized in 10 mL of sterile H_2O , stirred and diluted up to 10^{-4} . The conidia-diluted suspensions were applied in a PDA medium and stored in a BOD incubator, for a period of 2 days. Subsequently, isolated young colonies of *Trichoderma* were transferred to new Petri dishes with PDA. At the end of this procedure, 16 isolates were obtained directly from soil samples and one isolate was obtained from Trichodermil® (*Trichoderma harzianum*, Strain ESALQ 1306).

Isolates were identified as A, B, C, D, E, F, G, H, I, J, L, M (*Trichoderma*®), N, O, P, Q and R.

Trichoderma spp. isolates were assessed for their ability to produce IAA (Gordon & Weber 1951). The samples were filtered to separate the mycelium from the potato-dextrose (PD). Then, 0.5 mL of filtered broth was pipetted into Eppendorf tubes (2 mL) and 0.5 mL of Sawkolski reagent was added. The samples were shaken for 20 minutes, to verify the discoloration in the mixture, which indicates the production of IAA. Subsequently, isolates that showed a positive staining for IAA production were subjected to a culture medium sample collection at 24, 48, 72, 96 and 144 hours, for a better study of the behavior and potential quantification of IAA production over time. Quantitation was performed by spectrophotometry, with absorbance readings at 530 nm (Carvalho-Filho et al. 2008). This experiment was conducted with three replicates for each sample collection time from each isolate producing IAA and one control sample.

To evaluate the phosphate solubilization capacity of the *Trichoderma* spp. isolates, an experiment was conducted with three replications for each sample and the control (without *Trichoderma* spp.). The methodology was based on Murphy & Riley (1962), whose insoluble phosphate source is the tri-calcium phosphate (Ca_3HPO_4). The isolates highlighted due to the phosphate solubilizing capacity were subjected one more time to a solubilization test. Collections of the phosphatic culture medium were made with *Trichoderma* spp. samples at 48, 96 and 144 hours, for a better observation of the phosphate solubilization potential behavior over time. Means, in each time, were compared by the Tukey test at 5 %, using the Sisvar 5.3 software (Ferreira 2011).

To compare the growth characteristics of *Trichoderma* spp. isolates, 5 mm fragments of colonies of each isolate were transferred to Petri dishes with PDA culture medium. The plates were kept in a BOD incubator chamber at 27 °C, during a 12-hour photoperiod. From the first day, on a daily basis, measurements of mycelium development were made, using digital calipers in an orthogonal position (Poltronieri et al. 2013). The experiment was designed with four replications for each isolate. The data were submitted to a calculation of the mycelial growth speed index (MGSI), according to the following equation: $\text{MGSI} = \Sigma(D - D_p)/N$, where D is the current average diameter of the colony, D_p

the average diameter of the colony from the previous day and N the number of days after inoculation. After five days of the isolates development, an evaluation of the conidia production and germination was performed. The conidia production was performed with six replicates of each isolate. For this purpose, 10 mL of autoclaved distilled water were added to scrape the spores, using a glass rod. The suspension was filtered using a sterile gauze to remove mycelial fragments. Subsequently, the determination of the conidium concentration was performed on each treatment, using a Neubauer chamber and an optical microscope (Dias et al. 2005). Microscope slides were prepared with a water-agar solution film (1 %), to determine the conidia germination. The conidia solution of *Trichoderma* “F”, “R” and *Trichoderma*® was pipetted on these slides, at a concentration of 2×10^5 . The slides were kept in a BOD incubator at 27 °C, for 12 hours. With an optical microscope, the counting of 300 conidia was performed in each blade, registering the number of germinated spores after this period (Dias et al. 2005).

For species identification of the *Trichoderma* isolates “F” and “R”, DNA was extracted from mycelium. DNA extraction was performed with the Reliaprep gDNA Tissue kit (Promega), according to the protocol indicated by the manufacturer, and the obtained gDNA was quantified by UV-Vis spectrophotometry, in the Nanodrop equipment. The non-coded Internal Transcribed Spacer (ITS) region of the ribosomal RNA (rRNA) gene cluster was amplified by PCR. For the amplification, a pair of degenerate primers (White et al. 1990, Toju et al. 2012) designed to cover the ITS1-ITS4 region, ranging in size 500-750 bp, between the small and large units (SSU and LSU, respectively) of the rRNA, were used. The amplified fragment was sequenced on an ABI 3500 Genetic Analyzer (Applied Biosystems) with primers forward (ITS1) and reverse (ITS4), using the Kit BigDye® Terminator v3.1 Cycle Sequencing.

An *in vivo* experiment was conducted in a greenhouse (175 m²), in Pirassununga, São Paulo state, Brazil, from July to August 2015, to observe the potential of the *Trichoderma* spp. isolates and the homeopathic preparations for growth promotion of cherry tomato. The experimental design was randomized blocks, in a 4 x 3 factorial arrangement, with three *Trichoderma* isolates (“F”, “R” and *Trichoderma*®) + absence of *Trichoderma* and two homeopathic preparations (*Phosphorus* 6CH and

Carbo vegetabilis 6CH) + treatment without the homeopathic preparation, to which alcohol at 30 % was applied and used as a vehicle of dynamized dilutions, totaling 12 treatments, with five replications.

After liming, the chemical and particle size characterization was performed according to Embrapa (1997): pH (CaCl_2) = 6.1; Ca = 45 mmol dm^{-3} ; Mg = 7.0 mmol dm^{-3} ; P (Resin) = 3.0 mg dm^{-3} ; K = 0.50 mmol dm^{-3} ; S = 12.0 mg dm^{-3} ; organic matter = 12.0 g dm^{-3} ; V = 76.1 %; B = 0.16 mg dm^{-3} ; Cu = 0.50 mg dm^{-3} ; Fe = 7.0 mg dm^{-3} ; Mn = 2.50 mg dm^{-3} ; Zn = 0.50 mg dm^{-3} ; clay = 341 g dm^{-3} ; sand = 640 g dm^{-3} ; silt = 19 g dm^{-3} .

Seedlings of wild cherry tomatoes (access 21 of the Instituto Agronômico - IAC) (Azevedo Filho & Melo 2001) were produced in expanded polystyrene trays with a sterilized substrate. The cherry tomato seedlings were transplanted to 2.5 dm^3 plastic pots at 30 days after sowing. Each pot constituted an experimental unit. The initial fertilization was made with 15 g dm^{-3} of organic compound and phosphorus (P), at a dose of 200 mg dm^{-3} of P_2O_5 , in the form of Bayóvar natural reactive phosphate (14.5 P_2O_5 , citric acid and 28.6 % of total P_2O_5). It also contains 32 % of calcium (Ca). Potassium was provided in coverage at a dose of 100 mg dm^{-3} , in the form of potassium sulphate, subdivided twice, at 15 and 22 days after transplanting (DAT). The inoculation of *Trichoderma* spp. was performed by adding 150 mL of conidia solution (1×10^7 conidia mL^{-1}) immediately after transplanting.

The *Carbo vegetabilis* 5CH and *Phosphorus* 5CH homeopathic preparations were purchased from a homeopathic pharmacy. The dynamization 6CH was issued in deionized water, at the treatments application (Brasil 2011). The homeopathic treatments were diluted at 0.1 % in water and 50 mL were applied weekly on the soil in each plot.

At 45 DAT, tomato plants were collected. The shoot was separated from the root portion and the leaves were weighed using a precision scale. The leaf area was determined in a leaf area meter Li-Cor, LI-3100C. Subsequently, all the material was dried in a forced ventilation oven at 65 °C, until constant weight.

The data were submitted to analysis of variance (Anova). Sporulation data were transformed to square root of 'X', to normalize the data. Means were compared by the Tukey or Scott-Knott test at 5 %, using the Sisvar 5.3 software (Ferreira 2011).

RESULTS AND DISCUSSION

Among the 17 *Trichoderma* spp. isolates studied, the differences which were found in 48 hours for the IAA production were obtained from the *Trichoderma* isolates "F" and "G", when compared to the control, by the colorimetric test (Figure 1). The remaining isolates did not show differences from the control.

By assessing the behavior of the IAA concentration, it was possible to observe that the *Trichoderma* "F" and "G" isolates had a higher IAA production after 48 hours, with 42 $\mu\text{g L}^{-1}$ and 34 $\mu\text{g L}^{-1}$ of IAA concentration, respectively. In the subsequent samplings at 72, 96 and 144 hours, there was a decrease at the IAA concentration in the culture medium, reaching 0 $\mu\text{g L}^{-1}$ (Figure 2).

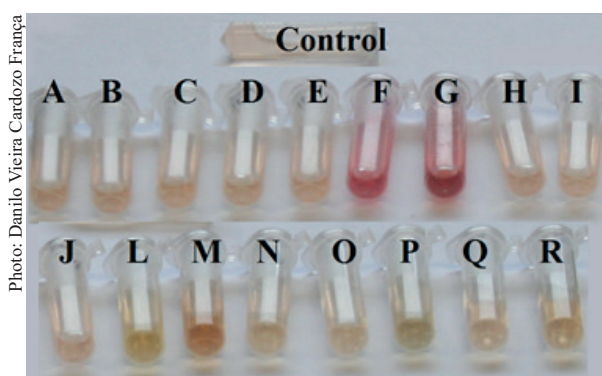


Figure 1. Comparison of *Trichoderma* spp. isolates by the Sakowski colorimetric test.

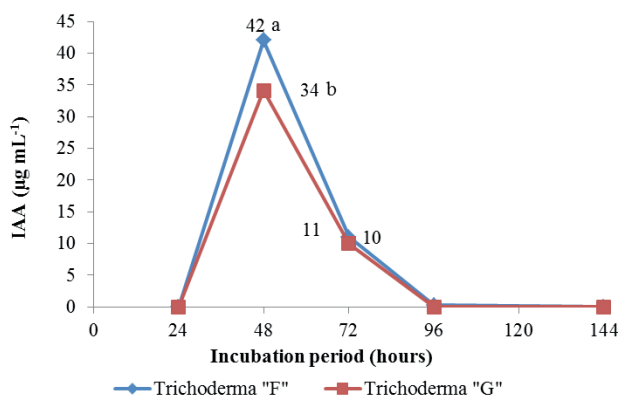


Figure 2. Concentration of indole-3-acetic acid (IAA) of *Trichoderma* spp. in potato-dextrose culture medium, with the addition of L-tryptophan over time. Means in 48 hours followed by different letters differ from each other by the Tukey test at 5 %.

Studies conducted by Carvalho-Filho et al. (2008) showed that *Trichoderma* spp. isolates have detectable levels of IAA concentrations after 7 days of development in a PD culture medium. Oliveira et al. (2012) studied the production of IAA by different *Trichoderma* spp. isolates, using L-tryptophan over time, and found that the isolates were effective in the production of IAA with the addition of the precursor L-tryptophan in the PD medium culture, finding IAA concentrations above the ones in the present study, after the fourth day of development of the isolates in a PD medium. The IAA concentration identified in our study, with *Trichoderma* “F” and “G” isolates, indicated that, within 48 hours, there is a peak in the IAA production, with higher values than those found in other periods, differing from the production behavior observed by Carvalho-Filho et al. (2008) and Oliveira et al. (2012). IAA biosynthesis is tryptophan dependent (Ribas et al. 2016). *Trichoderma* spp. isolates were able to solubilize phosphate in a NBRIP medium, ranging 7.06-11.10 $\mu\text{g mL}^{-1}$ (Figure 3).

The isolates “F”, “G” and “R” had the highest values for phosphate solubilization. Then, Trichodermil®, “B” and “H” stood out. Regarding the pH, it was observed that “F”, “G” and Trichodermil® changed differently the medium, in relation to the other isolates, after 96 hours, acidifying the medium from 5 to 5.2 (Figure 3). It is interesting to note that the isolate “R” differed from the others, with a pH of 5.6, i.e., phosphate solubilization was not

only influenced by reducing the pH. Ribas et al. (2016) studied the *in vitro* potential for phosphate solubilization by *Trichoderma* spp. and concluded that the isolates showed potential for solubilization, not only acidification of the culture medium, but also the production of acid and alkaline phosphatases.

The isolate “R” differed within the first 48 hours from the isolates “F” and “G”, with higher soluble phosphate concentrations (9.01 $\mu\text{g L}^{-1}$), and kept values above 10 $\mu\text{g L}^{-1}$ up to the sixth day (144 hours) of incubation (Figure 4). All the other isolates showed a peak of solubilization at 96 hours. There was no difference regarding soluble phosphorus among the isolates at 96 and 144 hours.

Oliveira et al. (2012), evaluating the calcium phosphate solubilization capacity by *Trichoderma* spp. isolates in a NBRIP culture medium, at different time intervals, found, in the fourth and sixth days, that the isolates showed a higher solubilizing capacity than the ones from the previous days. After the sixth day, a significant reduction of solubilized phosphate concentration in the NBRIP medium was observed, showing that it may have been also used by fungi for their own development. Kapri & Tewari (2010) also observed a gradual increase in the phosphorus concentration over the period between 24 hours and 96 hours of incubation. The authors subsequently reported a reduction of soluble phosphate concentrations in the culture medium. Oliveira et al. (2012) found a significant

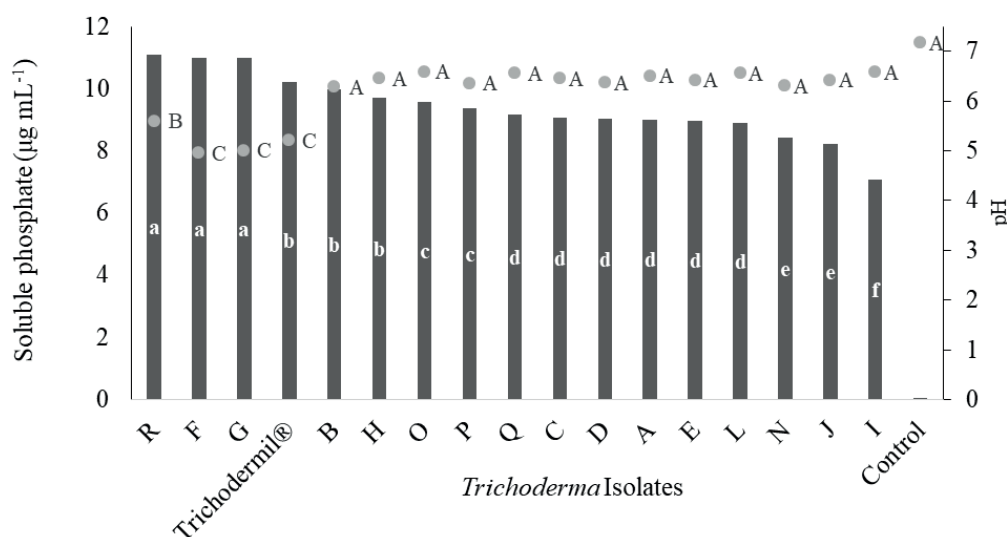


Figure 3. Average of solubilized phosphate concentration (bars) and pH (dots), regarding *Trichoderma* spp. isolates, after 96 hours of development in a NBRIP culture medium. Means followed by different letters, lowercase for soluble phosphorus and uppercase for pH, differ by the Scott-Knott test at 5 %.

reduction in the pH of the culture medium using phosphate-solubilizing *Trichoderma* sp. isolates, also associating this phosphate solubilization efficiency to this factor.

The isolate “R” had the highest mycelial growth speed index. However, it did not differ from Trichodermil®. The *Trichoderma* “F” had a growth speed 40.7 % lower than the *Trichoderma* “R” (Table 1).

It was possible to observe that the isolate “R” stood out, presenting approximately 2.5 times more spores than the isolate “F” and Trichodermil®.

The isolates “F” and “R” were selected for further characterization, because of their growth promoting action in cherry tomato. After DNA extraction and sequencing of the non-coded Internal Transcribed Spacer (ITS) of the ribosomal RNA

(rRNA) gene cluster, the *Trichoderma* spp. isolates “F” and “R” were identified as *Trichoderma atroviride* and *Trichoderma asperellum*, respectively.

Regarding germination, it was observed that the isolate “R” had the lowest conidia percentage after 12 hours of incubation (41.09 %), differing from the isolate “F” (64.00 %) and Trichodermil® (55.42 %).

The capacity of the *Trichoderma* spp. (“F”, Trichodermil® and “R”) isolates was evaluated in interaction with homeopathic treatments (*Carbo vegetabilis* 6CH and *Phosphorus* 6CH) as growth promoters in cherry tomato. The homeopathic preparations had no effect. The effect of the homeopathic preparation is variable according to the dynamization used. Mioranza et al. (2017) studied the control of *Meloidogyne incognita* in tomato plants with highly diluted solutions of *Thuya occidentalis* (6, 12, 24, 50, 100, 200 and 400CH) and just *T. occidentalis* 100CH showed a potential for the control of *M. incognita*. Toledo et al. (2015) evaluated the action of the homeopathic drugs *Propolis*, *Sulphur* and *Ferrum sulphuricum*, at the dynamizations 6, 12, 30 and 60CH, on the control of *Alternaria solani* and growth of tomato plants. *Sulphur* at 12 and 30CH, *Ferrum sulphuricum* at 6, 12 and 30CH and *Propolis* at all dilutions reduced the area under the disease progress curve by 17-49 %. *Propolis* at 30CH increased the fresh mass of shoots by 35 % and the dry mass of roots by 38 %.

The isolate “R”, when compared with the control, influenced the parameters leaf number, total leaf area, leaf dry mass and shoot dry mass. They were higher than the control and did not differ from Trichodermil® (Table 2).

Concerning the total leaf area, the isolate “R” showed a higher value (272.72 cm²), differing by 11 %, on average, from other treatments. Regarding the leaf number, leaf dry mass and shoot dry mass, the isolates “R” and “F” were similar to Trichodermil®

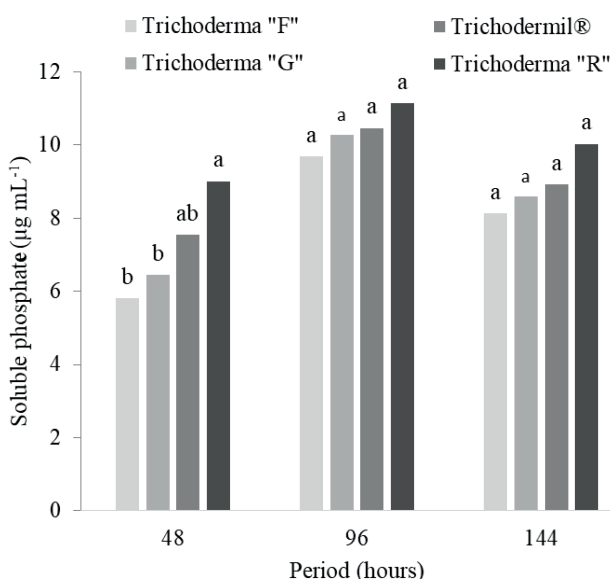


Figure 4. Average of phosphate concentration ($\mu\text{g mL}^{-1}$) solubilized by *Trichoderma* spp. isolates at 48, 96 and 144 hours of development in a NBRIP culture medium. Means followed by different letters at each time differ from each other by the Tukey test at 5 %.

Table 1. Mycelial growth speed index, sporulation and germination of *Trichoderma* isolates.

Treatment	Mycelial growth speed index	Sporulation	Germination
	mm day ⁻¹	$\times 10^7$ spores mL ⁻¹	%
<i>Trichoderma</i> “F”	36.38 b*	0.795 b	64.00 a
<i>Trichoderma</i> “R”	51.17 a	2.489 a	41.09 b
Trichodermil®	47.62 a	0.969 b	55.42 a
CV (%)	6.06	17.29	12.46

* Means followed by different letters differ from each other by the Tukey test ($p \leq 0.05$).

Table 2. Leaf number, total leaf area, leaf dry mass and shoot dry mass of cherry tomato in initial culture with *Trichoderma* spp. isolates.

Treatment	Leaf number	Total leaf area	Leaf dry mass	Shoot dry mass
	Leaves plant ⁻¹	cm ²	g	
Control	12.67 b*	238.18 b	1.93 b	4.12 b
<i>Trichoderma</i> “F”	12.33 b	244.39 b	2.04 ab	4.22 b
Trichodermil®	13.07 ab	248.18 b	2.07 ab	4.36 ab
<i>Trichoderma</i> “R”	13.53 a	272.72 a	2.25 a	4.59 a
CV (%)	9.41	7.95	11.19	8.31

* Means followed by different letters differ from each other by the Tukey test ($p \leq 0.05$).

(Table 2), however, just “R” was higher than the control.

A study conducted by Aguiar et al. (2013) demonstrated that the presence of *Trichoderma* spp. allowed a greater accumulation of shoot fresh mass in bean plants. Such result corroborates that observed by Harman (2000), using the T-22 strain of *T. harzianum* with corn, soy and red pepper, favoring the growth promotion of plants and fruit yield. The growth promotion in plants by applying *Trichoderma* spp. was also observed by Silva et al. (2011), who found that isolates from different *Trichoderma* species and Trichodermil® were efficient in the growth promotion of cucumber plants, also inducing resistance to diseases. The results by these authors also indicated that Trichodermil® showed positive results, superior than most of the studied isolates.

The weekly application of the homeopathic preparations *Carbo vegetabilis* 6CH and *Phosphorus* 6CH did not promote the cherry tomato growth. Bastide (2004) reports that the effectiveness of the application of homeopathic treatments is based on three main factors: the matrix of information or substance to be dynamized (the information must “make sense” to the recipient); the mediator of the information, i.e., the homeopathic solution and its preparation (the medium of preparation and dynamization); and the recipient of the information, in this case the soil-plant system. Any problems in one or more of the factors may compromise the success of the treatment. Probably, in the case of this experiment, the main detected effect was the application of the *Trichoderma* isolate “R”, which solubilized the natural phosphate, providing a proper amount of it to the tomato development.

In a study by Rossi et al. (2006), applying *Carbo vegetabilis* 6CH in lettuce, it was noted that this treatment depressed the root dry mass, if

compared to the control plants. Santos et al. (2011), using a treatment with *Phosphorus* in different dynamizations, found that Brazilian lavender plants are sensitive to *Phosphorus* homeopathy and that, among dynamizations, the 21CH stimulated better the seed germination and plant growth.

CONCLUSIONS

1. *Trichoderma* spp. isolates showed an ability for the production of indole-3-acetic acid (isolates “F” and “G”) and solubilization of phosphate;
2. The *Trichoderma asperellum* “R” had the highest mycelial growth speed index, similarly to Trichodermil®. The isolate “R” presented approximately 2.5 times more spores than the isolate “F” (*Trichoderma atroviride*) and Trichodermil®, and the isolate “R” had the lowest percentage of conidia germination;
3. The homeopathic preparations did not affect the development of cherry tomato shoots;
4. The *Trichoderma* “R” isolate showed promising results in the promotion of tomato growth, similarly to Trichodermil®.

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