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Original Article

Silviculture

Development of Forestry Species Inoculated with *Trichoderma* spp. Fertilized with Rock Phosphate

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ABSTRACT

The study aimed to determine the effects of inoculation of *Trichoderma* spp. on the development and extraction of nutrients from *Eugenia pyriformis* Cambess. (uvaieira) and *Myrcianthes punges* (O.Berg.) (guabiju) subjected to fertilization with rock phosphate. The treatments used at planting were: Control; Trichonat PM®; *Trichoderma* sp. FS1; Rock phosphate; Trichonat PM® + Rock phosphate; and *Trichoderma* sp. FS1 + Rock Phosphate. The following characteristics were evaluated at 540 days after planting: plant height, crown diameter, branch insertion points, biomass, and nutrient content. The uvaieiras inoculated with Trichonat PM® and *Thrichoderma* spp. showed higher vegetative growth and higher values of N, P, K, Ca, Mg and S, respectively. For guabijuzeiros, the treatment with *Trichoderma* spp. presented the highest insertion point of the branches without phosphorus fertilization However, the treatments did not affect nutrient extraction. Thus, *Trichoderma* spp. inoculants have the potential to promote vegetative growth and nutrient extraction in plants.

Keywords: *Eugenia pyriformis*, *Myrcianthes punges*, nutrient extraction.

1. INTRODUCTION

Forest species have a great source of vitamins, antioxidants, and essential oils. Thus, research in these areas has increased to meet the demand from the food, cosmetics and pharmaceutical industries (Franzon et al., 2010; Marin et al., 2008). Management of fruit plants in preservation areas or on small properties can become a practice that combines environmental conservation and generates income to small farmers. Myrtaceae is a species that can be used for restoring vegetation (Lorenzi, 2002; Pirola, 2013).

The *Myrcianthes punges* (O.Berg.) (popularly known in Brazil as *guabijuzeiro*) and the *Eugenia pyriformis* Cambess. (popularly known in Brazil as *uvaieira*) which are native trees to the Southern Region of Brazil are among the plants belonging to the Myrtaceae Family. *Myrcianthes punges* is a perennial forest fruit tree with slow growth and *Eugenia pyriformis* is also a perennial forest fruit tree, but with fast growth and early fructification. Both can be used in forest management systems for reforestation of degraded areas and ciliary recomposition (Carvalho, 2010).

Technical and scientific information regarding nutritional requirements and their reflection on the productivity and quality of *uvaia* and *guabiju* fruits are scarce. The phosphorus nutrient is of paramount importance for fruit species in general, as its deficiency is marked by a delay in plant and root growth, a delay in flowering, inhibition of lateral bud sprouting and reduced quantity of fruits; and consequently, a reduction in productivity (Araújo & Machado, 2006).

According to Lima et al. (2011), phosphorus favors roots and seedlings to develop faster, increases resistance to cold, improves water use efficiency, promotes disease resistance in some plants and increases nutrient absorption. The use of rock phosphate in its natural form is very restricted due to its low solubility, which requires an association of this material with other sources of soluble phosphorus. To increase phosphorus solubilization in natural sources, studies have been conducted with the inoculation of phosphorus solubilizing microorganisms (Novais et al., 2007).

In this context, the use of the *Trichoderma* spp. Fungus is highlighted. It is a natural inhabitant of soils and highly interactive in the root and in the interior of the plants through different action mechanisms such as competition, parasitism, and antibiosis against microorganisms capable of causing disease in plants (Bedendo et al., 2011). This fungus aids in decomposing and mineralizing plant residues, as well as promoting P solubilization that contributes to the availability of this nutrient to the plants, allowing greater and faster absorption (Jesus et al., 2011). With the objective of evaluating the *in vitro* phosphate solubilization potential, Oliveira et al. (2012) used seven isolates of *Trichoderma* spp. and found that all were able to solubilize calcium phosphate in the culture medium.

In addition to helping in the solubility of nutrients necessary to plants, the *Trichoderma* genus can promote their growth due the production of Indole-3-acetic acid (IAA) (Hoyos-Carvajal et al., 2009; Carvalho et al., 2008). Fortes et al. (2007) found that the *Trichoderma* isolate (E15) promoted eucalyptus micro-cut rooting (*Eucalyptus* sp.), showing a significant increase in the rooting percentage.

In this sense, the objective of the study was to evaluate the effect of inoculating *Trichoderma* spp. fungus and fertilization with rock phosphate on the initial development and nutrient extraction of *Eugenia pyriformis* and *Myrcianthes punges* plants in field conditions.

2. MATERIAL AND METHODS

2.1. Obtaining seedlings

Myrcianthes punges and *Eugenia pyriformis* seeds were obtained from a forest fragment in the municipality of São José das Palmeiras in the western region of the State of Paraná, Brazil, and seeded in plastic pots measuring 45 x 17 cm and 14 cm in height (two seeds per pot), with MECPLANTA FSC® as substrate registered at MAP PR 0954910001-0 as an "F" class soil conditioner (composition of the product: pine bark, vermiculite, corrective acidity, mineral fertilizers) and ravine soil at the ratio of 1:1, and then kept in a greenhouse under intermittent mist. The seedlings were transferred to polyethylene bags (10 x 20 cm) after 60 days with a capacity of 500 ml of MECPLANTA FSC® substrate and then remained in the greenhouse for another 90 days. The seedlings were planted in the second half of September 2011, into previously prepared pits with 2x2 m spacing and dimensions of 40 x 40 x 40 cm.

2.2. Implemented products

The commercial product Trichonat PM® (Natural Rural S.A., Araraquara-SP) was used as an inoculum source of *Trichoderma* spp. fungi, composed of a powder formulation at a concentration of $6x10^6$ conidia g^{-1}. A sample of *Ficus carica* L. rhizospheric soil containing *Trichoderma* was transferred to Petri dishes containing potato-dextrose-agar (PDA) culture medium to obtain *Trichoderma* spp. FS1 isolates, and the plates were subsequently incubated in a growth chamber (BOD = *bio-oxygen demand*) at 25 °C with a photoperiod of 12 h for 7 days. After this period, 5 mm diameter discs of PDA culture medium containing *Trichoderma* mycelium were added to glass vials containing 5g of sterile parboiled rice, and the vials were placed in a BOD chamber/incubator at 25 °C with a photoperiod of 12 h for 20 days.

The rock phosphate used was Arad origin, which generally has 10 to 12% of P_2O_5 soluble in citric acid and total P_2O_5 contents between 28 and 30%.

2.3. Field experiment

The experiment was carried out in Guarapuava, south-central state of Paraná, Brazil, with geographic coordinates of 25° 23' 36" S, 51° 27' 19" W and 1,120 m altitude. According to Köppen, the region's climate is classified as humid subtropical Cfb with mild summers, frosty winters, and annual rainfall of 1,800-2,000 mm (data provided by the Technological Institute of Meteorological System of Paraná).

The soil is classified as Distroferric Brazilian Oxisol with a typical clayey texture (Santos et al., 2006). Chemical analyses of the 0-20 cm soil layer carried out beforethe experiment presented the following results: pH ($CaCl_2$) = 5.5; H^+ + Al^{3+}=3.62 cmol dm^{-3}; Ca^{2+}=3.3 cmol dm^{-3}; Mg^{2+}= 3.2 cmol dm^{-3}; P (mehlich)= 1.0 mg dm^{-3}; K^+= 0.28 cmol dm^{-3}; S=5.7 mg dm^{-3}; V%= 65%; CEC = 10.4 cmolc dm^{-3}, methodology described by Malavolta et al. (1997).

The experimental design followed randomized blocks according to a 3x2 factorial scheme (inoculants x phosphate fertilization) with six treatments, four replications and an experimental plot constituted by one plant. The treatments consisted of the following applications to the planting pit incorporated into the soil: T1) Control; T2) Trichonat PM® (50g/pit); T3) *Trichoderma* spp. FS1 (50g/pit); T4) Rock phosphate (1kg/pit); T5) Trichonat PM® (50g/pit) + Rock phosphate (1kg/pit) and T6) *Trichoderma* spp. FS1 (50g/pit) + Rock phosphate (1kg/pit). All pits received 5 kg of tanned cattle manure.

2.4. Evaluations

The following characteristics were evaluated at 540 days post planting in July 2013: a) plant height: a measuring tape (cm) was used from the base of the plant stem until the end of the crown; b) canopy diameter: a measuring tape on two equidistant sides (cm) was used, c) insertion point of the branches: a measuring tape from the base of the plant stem to the insertion point of the first branch (cm) was used.

After the measurements, the plants were completely removed from the soil for chemical analysis. The plants were excavated and divided into roots, stems, branches, and leaves. The samples were dried at 70 °C in a forced-circulation oven with air renewal. Fresh and dry mass for all parts were evaluated with a precision digital scale. Then, the samples were ground into fine particles in an electric knife mill with a 2 mm sieve, and stored in a cool place at room temperature until the chemical analyses were carried out.

The macronutrients contents from the shoot and the root of the plants were determined according to the methodology described by Malavolta et al. (1997). The N concentrations in the dry tissues were determined by digestion using sulfuric acid and semi-micro Kjeldahl method. After the nitric-perchloric acid digestion, phosphorus (P) was determined by molecular absorption spectrophotometry, sulfur (S) by barium sulphate turbidimetry, potassium (K) by flame photometry and calcium (Ca) and magnesium (Mg) nutrients by atomic absorption spectrophotometry.

The nutrient content was calculated according to the dry mass and the nutrient content in the plant (= dry mass x nutrient content) (Malavolta et al., 1997).

The results were submitted to analysis of variance and the means were compared by the Tukey test when significant at 5% probability level using the statistical program SISVAR 5.0 (Ferreira, 2011).

3. RESULTS

3.1. Vegetative growth

Regarding the evaluations of the *Eugenia pyriformis* species, there was no interaction for the variables plant height and crown diameter between the factors. However, a significant effect was observed for the inoculum factor, regardless of the phosphate fertilization. The Trichonat PM® treatment had the highest plant height and crown diameter when compared to the control, which was not statistically different from the treatment with *Trichoderma* spp. FS1 (Figures 1A and 1B).

There was an interaction between the factors observed for the biomass and first branch insertion point evaluations in *Eugenia pyriformis* (Figures 1C and D). The treatment with T*richoderma* spp. provided the highest biomass, followed by the Trichonat PM® treatment, which differed from the control treatment in the absence and presence of phosphate fertilization. However, only the Trichonat PM® inoculum presented a statistical difference in the phosphate fertilization, increasing plant biomass by 36%, when compared to the absence of phosphate fertilization (Figure 1C). On the other hand, the *Trichoderma* spp. FS1 inoculum significantly increased the insertion point of the first branches of *Eugenia pyriformis* in the presence of phosphate fertilization, and it was statistically different from the control treatment (Figure 1D).

Guarapuava-PR, 2013.

Regarding the vegetative growth assessments of *Myrcianthes punges*, no significant differences were found for the variables crown diameter, plant height and biomass. For the insertion point of the first branches variable, there was an interaction between the inoculum and fertilization factors. The *Trichoderma* spp. treatment increased the insertion point of the branches by 130% when compared to the control in the absence of fertilization, differing statistically from those with the presence of phosphate fertilization (Figure 2). *Trichoderma* spp. inocula, Guarapuava-PR, 2013.

3.2. Nutrient extraction

A significant interaction was found between the factors of nutrient extraction results in *Eugenia pyriformis* plants (Table 1). In plants fertilized with rock phosphate, the *Trichoderma* spp. FS1 treatment provided the highest content of N, P, K, Ca and Mg in the plants increasing by 160, 150, 133, 210 and 133%, respectively, in relation to the control without inoculation.

However, the commercial Trichonat PM® inoculum in the absence of phosphate fertilization had higher values for most of the macronutrients, followed by

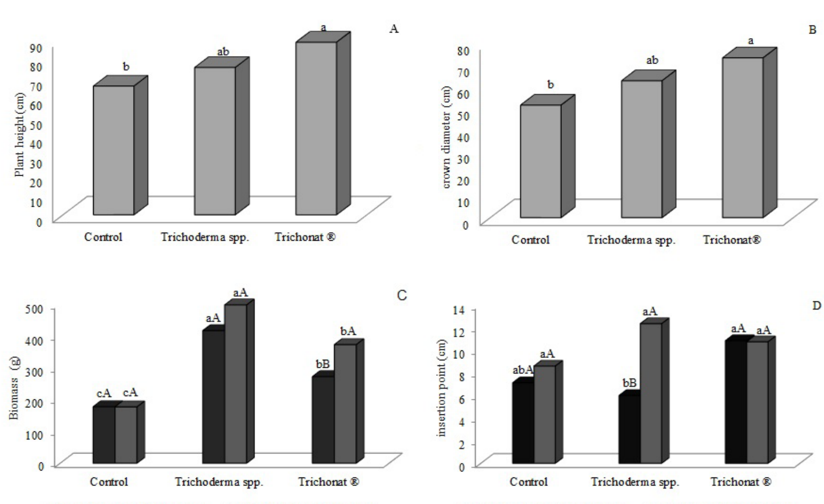

Figure 1. Plant height (A), crown diameter (B), biomass (C) and the insertion point of the first branches (D) *Eugenia pyriformis* subjected to several *Trichoderma* spp. Inocula.

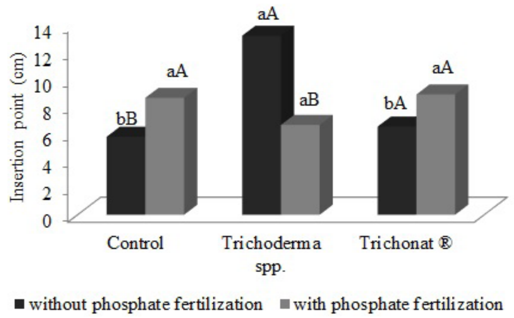

Figure 2. Insertion point of the first branch of *Myrcianthes punges* subjected to several.

Trichoderma spp. FS1, which differed significantly from the control treatment. For the P, K, and Mg elements, the increases were 300, 271 and 150%, respectively, in relation to the control.

For nitrogen, the *Trichoderma* spp. FS1 inoculum had higher extraction. For the sulfur macronutrient, an effect was only observed for the inoculum factor, in which the maximum content was observed in the Trichonat PM® treatment (1.11 g of S plant^{-1}), which did not significantly differ from the *Trichoderma* spp. FS1 treatment (0.67 g of S plant^{-1}).

Table 1. Effect of different *Trichoderma* spp. inocula with or without phosphorus fertilization (phf) on the extraction of *Eugenia pyriformis* nutrients.

Treatment	N (g plant $^{-1}$)		Mean
	Without phf	With phf	
Control	1.2 bA*	2.0 bA	1.6
Trichoderma spp.FS1	3.9 aB	5.2 aA	3.7
Trichonat PM ®	2.3 bA	3.2 bA	3.6
Mean	2.5	3.5	CV= 22.7
Treatment	**P (g plant $^{-1}$)**		**Mean**
	Without phf	**With phf**	
Control	0.1 cB	0.2 cA	0.2
Trichoderma spp.FS1	0.2 bB	0.5 aA	0.4
Trichonat PM ®	0.4 aA	0.3 bA	0.4
Mean	0.2	0.4	CV= 17.0
Treatment	**K (g plant $^{-1}$)**		**Mean**
	Without phf	**With phf**	
Control	0.7 cB	1.5 cA	1.1
Trichoderma spp.FS1	1.5 bB	3.5 aA	2.5
Trichonat PM ®	2.6 aA	2.7 bA	2.6
Mean	1.6	2.5	CV= 13.2
Treatment	**Ca (g plant $^{-1}$)**		**Mean**
	Without phf	**With phf**	
Control	0.5^{ns}	1.0	0.8
Trichoderma spp.FS1	1.5	3.1	2.3
Trichonat PM ®	2.5	1.9	2.2
Mean	1.5	2.0	CV=14.0
Treatment	**Mg (g plant $^{-1}$)**		**Mean**
	Without phf	**With phf**	
Control	0.2cB	0.3 cA	0.2
Trichoderma spp.FS1	0.3 bB	0.7 aA	0.5
Trichonat PM ®	0.5 aA	0.4 bB	0.5
Mean	0.3	0.5	CV= 14.4
Treatment	**S (g plant $^{-1}$)**		**Mean**
	Without phf	**With phf**	
Control	0.4^{ns}	0.3	0.4 b
Trichoderma spp.FS1	0.5	0.8	0.7 ab
Trichonat PM ®	1.5	0.8	1.1 a
Mean	0.8	0.6	CV= 69.4

*Means followed by the same lowercase letter in the column and the same capital letter on the line do not differ from one another according to the Tukey test ($p < 0.05$). phf = phosphate fertilization (1 kg plant $^{-1}$ of ARAD rock phosphate); ^{ns} = Not significant.

Table 2. Effect of different *Trichoderma* spp. inocula with or without phosphorus fertilization (phf) on the extraction of *Myrcianthes punges* nutrients.

N (g plant $^{-1}$)			
Treatment	**Without phf**	**With phf**	**Mean**
Control	3.23 ^{ns}	4.7	3.97
Trichoderma spp.FS1	6.12	3.9	4.99
Trichonat PM ®	3.4	3.6	3.49
Mean	4.25	4.05	CV= 49.92
P (g plant $^{-1}$)			
Treatment	**Without phf**	**With phf**	**Mean**
Control	0.28 ^{ns}	0.37	0.32
Trichoderma spp.FS1	0.53	0.31	0.42
Trichonat PM ®	0.44	0.27	0.35
Mean	0.41	0.31	CV= 50.40
K (g plant $^{-1}$)			
Treatment	**Without phf**	**With phf**	**Mean**
Control	3.6 ^{ns}	5.8	4.68
Trichoderma spp.FS1	6.6	4.53	5.56
Trichonat PM ®	5.04	4.1	4.57
Mean	5.07	4.8	CV= 51.32
S (g plant $^{-1}$)			
Treatment	**Without phf**	**With phf**	**Mean**
Control	0.33 ^{ns}	0.79	0.56
Trichoderma spp.FS1	0.54	0.38	0.46
Trichonat PM ®	0.38	0.3	0.34
Mean	0.42	0.49	CV= 81.65
Ca (g plant $^{-1}$)			
Treatment	**Without phf**	**With phf**	**Mean**
Control	4.1 ^{ns}	6.4	5.24
Trichoderma spp.FS1	7.3	5	6.11
Trichonat PM ®	5.81	5.3	5.56
Mean	5.72	5.53	CV= 48.15
Mg (g plant $^{-1}$)			
Treatment	**Without phf**	**With phf**	**Mean**
Control	0.43 ^{ns}	0.58	0.5
Trichoderma spp.FS1	0.74	0.51	0.63
Trichonat PM ®	0.72	0.61	0.66
Mean	0.63	0.57	CV= 47.05

^{ns} = not significant according to the Tukey probability test ($p < 0.05$), phf = phosphate fertilization; (1 kg plant $^{-1}$ of ARAD rock phosphate).

Evaluation of nutrient extraction from *Myrcianthes punges* plants found no significant differences for the elements N, P, K S, Ca and Mg (Table 2).

4. DISCUSSION

Both species showed greater vegetative development when using *Trichoderma* spp. as verified by increased plant height, canopy diameter, biomass or the insertion point height of the branches. Similarly, Maciel et al. (2012) also found that *Eucalyptus saligna* seedlings showed higher height when planted in substrate with the commercial product Trichodel® [composed of selected strains of the *Trichoderma* spp. fungus (1x10^9 of viable cells per mL)]. In sour passion fruit (*Passiflora edulis* var. *flavicarpa* Degener), effects of *Trichoderma* sp. inoculation (native TCN-014) have been also verified, in which larger stem length, stem diameter, true leaves, root length and total dry weight were observed two months after seedling establishment compared to

TCC-005, the commercial strain of *Trichoderma harzianum* (Cubillos-Hinojosa et al., 2009).

These results indicate that the *Trichoderma* spp. FS1 and the Trichonat PM® directly promote plant growth through the production of hormones (auxins, cytokinins, and ethylene) or by supplying the nutrient requirements for solubilization (Machado et al., 2011; Oliveira et al., 2012). The production of Indole-3-acetic acid in eucalyptus mini-cuttings treated with *Trichoderma* CEN 262 isolate increased on 136%, 136% and 43% of the shoot dry mass, root dry mass and mean height of the plants, respectively, compared to untreated plant cuttings (Carvalho et al., 2008),

In this study, *Eugenia pyriformis* plants notably showed higher nutrient extraction when inoculated with *Trichoderma* spp. for the elements nitrogen, phosphorus, potassium and magnesium. Similarly, Machado et al. (2011) found an increase in the mineral nitrogen absorbed by *Avena strigosa* Schreb (black oat) in plants inoculated with *Trichoderma harzianum* and nitrogen fertilization. In another study, the phosphorus content in coffee leaves was higher when submitted to Organic CS® organic soil conditioner (composed of millet colonized by the *Trichoderma asperellum* fungus at a concentration of $1x10^8$ CFU/g) (Jesus et al., 2011).

According to the results, a difference in the efficiency of *Trichoderma* sp. FS1 and Trichonat PM® was observed, which according to Ethur (2006), may be related to the ability of each isolate to produce secondary metabolites and to compete with other organisms in the rhizosphere environment of the plant.

The results show the potential of using *Trichoderma* sp. FS1 isolates and Trichonat PM® to promote vegetative growth in *Eugenia pyriformis* regardless of phosphate fertilization. Isolates of *Trichoderma* sp. FS1 increased biomass and nutrient content of plants in the presence of phosphate fertilizers, while Trichonat PM® provided higher nutrient extraction in non-fertilized plants.

Myrcianthes punges is a slow-growing fruit tree, and it has not been clearly benefited by localized fertilization with rock phosphate or by the inoculation of *Trichoderma* spp. in the first years of development. Moreover, different types of management may favor or inhibit the establishment of microbial groups in the rhizosphere (Cardoso et al., 1992). Similar results were observed by Macedo & Teixeira (2012) when evaluating the effect of liming and phosphate fertilization on the growth of *Eugenia stipitata* McVaugh seedlings (popularly known as *araçá–boi*), finding that high doses of phosphorus in the substrate affected the nutrient availability, hindering the vegetative development of this plant.

Based on the results, *Tichoderma* spp. (a natural and highly interactive soil resident in the root and interior of plants) promoted vegetative growth and increased nutrient content in *Eugenia pyriformis*. However, it is important to continue studies for a longer period of time to better elucidate the results in *Myrcianthes punges*.

5. CONCLUSION

The best results in growth and nutrient extraction were found when using Trichonat PM® without fertilization, and *Trichoderma* sp. FS1 with phosphate fertilization in *Eugenia pyriformis* plants.

In *Myrcianthes punges* (O.Berg.), the T*richoderma* sp. FS1 promoted a greater insertion point of the first branches However, the treatments did not influence nutrient extraction by the plants.

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