

Growth Promotion of *Raphanus Sativus* L. under Doses of Phosphorus with Application of *Trichoderma* Spp.

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Received: April 5, 2024

Accepted: May 8, 2024

Online Published: May 14, 2024

doi:10.5539/jsd.v17n3p49

URL: <https://doi.org/10.5539/jsd.v17n3p49>

Abstract

This study aimed to evaluate the growth of *Raphanus sativus* L. under six phosphorus doses in combination with three strains of *Trichoderma* spp. The design was a randomized block design with seven replications in a factorial scheme, 4 x 5 (treatments and doses). Treatments related to *Trichoderma* spp. were T₁ – control, without application of *T. harzianum* strain; T₂ – *T. harzianum* ESALQ 1306, T₃ – *T. harzianum* IBLF 006 WP, and T₄ – *T. asperellum* URM 5911, at doses of 0, 40, 80, 120, 160, and 200 kg ha⁻¹ of P₂O₅, in a greenhouse. The evaluated characteristics were tuberous root diameter (RDI), tuberous root length (RLE), relative chlorophyll index (RCI), leaf dry mass (LDM), tuberous root fresh mass (FTRM), and tuberous root dry mass (TRDM). It was observed that there was a significant difference for the different sources of variation evaluated, indicating an interaction between the different isolates and doses. It is concluded that the application of *Trichoderma harzianum* - ESALQ 1306 strain is recommended for radish cultivation, with increases of 13% for tuberous root length, 7% for relative chlorophyll index, 23% for leaf dry mass, 35% for tuberous root fresh mass, and 12% for tuberous root dry mass compared to the control, at a dose of 104 kg ha⁻¹ of P₂O₅, with the possibility of dose reduction and more sustainable management. Additionally, strains *T. harzianum* IBLF 006 WP (T₃) and *T. asperellum* URM 5911 (T₄) are not recommended for radish cultivation.

Keywords: radish, strain, fungus, tuberous root

1. Introduction

The radish (*Raphanus sativus* L.) is a vegetable from the Brassicaceae family. The plant is grown for its tubers and leaves, which can be consumed in various forms, such as raw, canned, dried, or cooked (Rugang et al., 2020; Yu et al. 2020). Over time, the radish crop has gained immense popularity due to the changing dietary habits of individuals who prefer a healthier and more balanced diet, especially among vegans and vegetarians.

A vegetarian diet can be healthy and provide adequate nutrition when planned correctly. This diet may have several health benefits, like preventing obesity, cardiovascular disease, hypertension, type 2 diabetes, and some cancers (Melina et al., 2016). Flavonoids are present in more significant quantities in plants and are responsible for controlling some of these health issues. They are also a significant source of nutrients and antioxidants (Lee; Park, 2017; Mota et al., 2009).

Radishes are a widely cultivated crop due to their small size and rapid growth. They are often grown alongside other crops in intercropping systems, which makes them an essential source of income for farmers and have significant socioeconomic importance (Bonela et al., 2017). However, radishes are nutrient-demanding crops, which can decrease profitability due to the need for higher quantities of inputs, such as mineral fertilizers (Bonfim-Silva et al., 2020).

Among the essential macronutrients, phosphorus (P) is a mobile nutrient that plays several crucial roles in plants, serving as a structural component of nucleic acids, nucleotides, phosphoproteins, phospholipids, and enzymatic cofactors involved in metabolic regulation (Malhotra et al., 2018). This macronutrient is absorbed in small quantities by plants, however, it is one of the most used elements in Brazilian fertilization, as it can interact with highly weathered soils, which are widely found in tropical and subtropical conditions (Vilar; Vilar, 2013). In radish,

it may be associated with the development of the root system, resulting in increased fresh produce yield (Baloch et al., 2014), albeit with elevated costs and low long-term benefits.

Thus, the use of plant biostimulants such as beneficial microorganisms, for example, the fungus *Trichoderma* spp., aims to increase nutrient and water absorption and productivity under a sustainable approach to ensure yield stability, with reduced fertilizer usage (Fiorentino et al., 2018), while also possibly improving the rhizosphere. Some strains of *Trichoderma* species produce secondary metabolites similar to hormones, with the exudation of molecules with auxin-like activity, small peptides, or volatile organic compounds in the rhizosphere, which are also known to have plant growth-promoting effects (Sofo et al., 2012; Pelagio-Flores et al., 2017).

This study aimed to evaluate the growth of *Raphanus sativus* L. under six phosphorus doses combined with three strains of *Trichoderma* spp.

2. Method

The experiment was conducted at the Universidade Estadual do Goiás - UEG, Ipameri Unit, in a greenhouse measuring 30 x 7 x 3,5 m, constructed with a metal structure, covered with 150-micron diffused light polyethylene, and with 25% shade cloth on the sides.

The experimental design was a randomized complete block design in a 4 x 5 factorial scheme (treatments and doses) with seven replications. Treatments related to *Trichoderma* spp. were T₁ – control, without application of *T. harzianum* strain; T₂ – *T. harzianum* ESALQ 1306, T₃ – *T. harzianum* IBLF 006 WP, and T₄ – (*T. asperellum* URM 5911), at a dose of 4 ml suspension (2 x 10⁸ conidia pot⁻¹), using a manual sprayer (capacity - 550 ml).

The doses used were 100 kg ha⁻¹ of urea (45% of N), in addition to the application of 0, 40, 80, 120, 160, and 200 kg ha⁻¹ of P₂O₅ (single superphosphate – 18% of P₂O₅) and 75 kg ha⁻¹ of potassium chloride (60% - K₂O), respectively, based on results obtained by Castro et al. (2016), Costa et al. (2019), and Oliveira et al. (2010). Soil samples were taken before the experiment setup, and the soil analysis results were: pH (CaCl₂) – 4,9; organic matter – 24,1 g dm⁻³; P – 1,5 mg dm⁻³ (resin); H+Al – 30,3 mmol_c dm⁻³; K – 4,1 mmol_c dm⁻³; Ca - 18,2 mmol_c dm⁻³; Mg – 7,5 mmol_c dm⁻³; and CEC – 53,6 mmol_c dm⁻³.

The sampling unit consisted of eight-liter pots filled with Dystrophic Red Latosol soil (Oxisols), with a clayey texture (Santos et al., 2018), which was previously sieved, mixed with 3,5 g of limestone per kilogram of soil, according to soil analysis, and stored for 40 days. The pots were irrigated to 80% of soil water holding capacity every four days. Subsequently, the respective doses were applied, *Trichoderma* strains were applied to the soil, and then five seeds of the Saxa[®] cultivar (Isla Company) were planted per pot. After seven days, thinning was performed, leaving only one plant per pot.

The evaluated characteristics included the tuberous root diameter (TRD) and length (TRL), measured using a digital caliper at the middle region of the root, with measurements given in millimeters. The relative chlorophyll index (RCI) was determined using the Clorofilog Falker[®] device on two leaves per plot, with an average generated for each plot expressed in g chlorophyll. Leaf dry mass (LDM) was obtained by placing fresh leaves in kraft paper bags, drying them in a forced ventilation oven at 65°C for 72 hours, and then weighing them, with values expressed in grams per plant. Tuberous root fresh mass (TRFM) was measured by weighing roots on a precision scale after removal from the soil and washing, expressed in grams per plant. Tuberous root dry mass (TRDM) was determined by placing roots in kraft paper bags, drying them in a forced ventilation oven at 65°C for 72 hours, and then weighing them, with values expressed in grams per plant. These evaluations were conducted 35 days after sowing.

The data were first subjected to tests of normality and homogeneity of variance, and then analysis of variance and regression were performed using the SISVAR computer program (Ferreira, 2011).

3. Results and Discussion

Significant differences were observed for the various sources of variation evaluated, indicating an interaction between different strains and doses (Table 1). The coefficients of variation ranged from 2.78 to 11.09%, with the lowest value corresponding to the relative chlorophyll index and the highest value to tuberous root dry mass. Bonela et al. (2017) also detected significant differences in total plant weight, number of leaves per plant, average plant length, average tuberous root diameter, tuberous root fresh mass, and tuberous root dry mass in assessing radish root yield and quality in subsequent lettuce cultivation produced with organic fertilization.

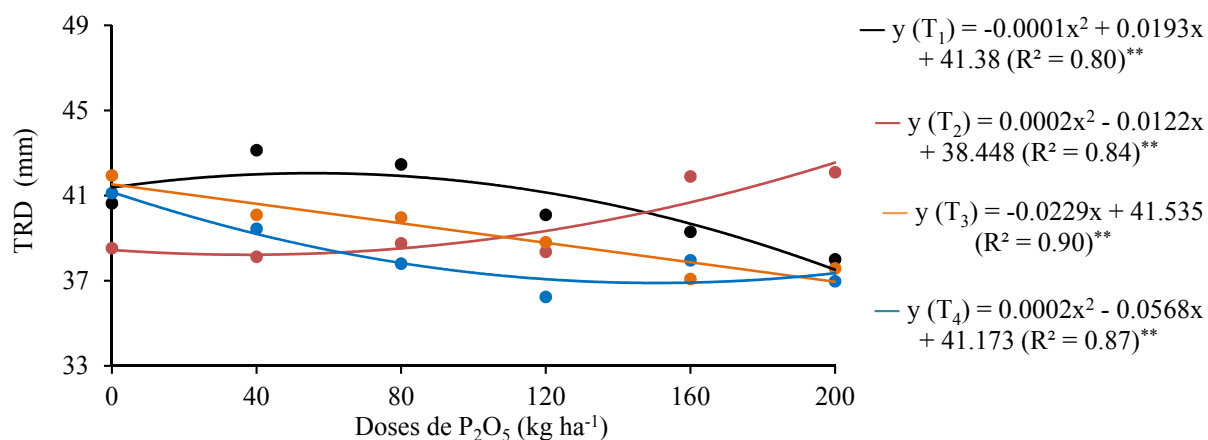
Table 1. Summary of the analysis of variance of the variables tuber root diameter (TRD), tuber root length (TRL), relative chlorophyll index (RCI), leaf dry mass (LDM), tuberous root fresh mass (TRFM), and tuber root dry mass (TRDM), under phosphorus doses with the application of *Trichoderma* spp. Ipameri, GO, 2024

FV	GL	TRD	TRL	RCI	LDM	TRFM	TRDM
Cepa (C)	3	100,20**	327,23**	123,42**	1,34**	538,51**	3,81**
Dose (D)	5	37,72**	161,98**	10,15**	0,34**	280,79**	0,91**
C x D	15	38,31**	81,11**	28,39**	0,95**	170,42**	1,19**
Bloco	6	1,54	8,20	3,28	0,05	42,33	0,17
Erro	90	2,61	3,95	1,28	0,03	9,82	0,04
CV (%)		4,08	4,13	2,78	11,09	9,29	9,67

* and ** - significant at 5 and 1% probability levels by the F-test, respectively; CV (%) – coefficient of variation.

The diameter and length of the tuberous root are essential in product selection and end-consumer evaluation, as they correlate with product quality and shelf life. However, it was observed that, for the diameter of the tuberous root, the application of *Trichoderma* spp. did not show a positive effect for most treatments, which in treatments T₃ (*T. harzianum* IBLF 006) and T₄ (*T. asperellum* URM 5911), the largest dimension was found in the absence of phosphate fertilizer (0 kg ha⁻¹), with values of 41.5 and 41.2 mm, respectively (Figure 1). However, for the T₂ treatment, the *T. harzianum* ESALQ 1306 strain promoted increases above the control at higher doses, approximately 160 kg ha⁻¹, with promotion of 4.2 mm, between doses. As for length, the T₃ treatment obtained a similar result to the control and, for T₄ treatment, the opposite to the diameter, with an increase between doses but with low promotion. It is noteworthy that at the 108 kg ha⁻¹ dose, the T₂ treatment was the one that showed the greatest promotion (50 mm), but with a diameter of 39 mm, obtaining a shape that is more oval than rounded, preferred by the Brazilian market.

Phosphorus is a nutrient of great importance for developing tuberous roots and is found in low levels in Brazilian soils. However, even with application, it can be adsorbed by the soil and become unavailable to the plant (Luz et al., 2013). Avalhaes et al. (2009), who evaluated beetroot culture under controlled conditions and with phosphorus application, noted positive results up to a dose of 316 mg kg dm⁻³ of P (632 kg ha⁻¹), for variables such as plant height, leaf area, root diameter, tuberous root fresh mass and dry mass of the aerial part, a value well above in Dystrophic Red Latosol soil, however, with lower organic matter and pH.



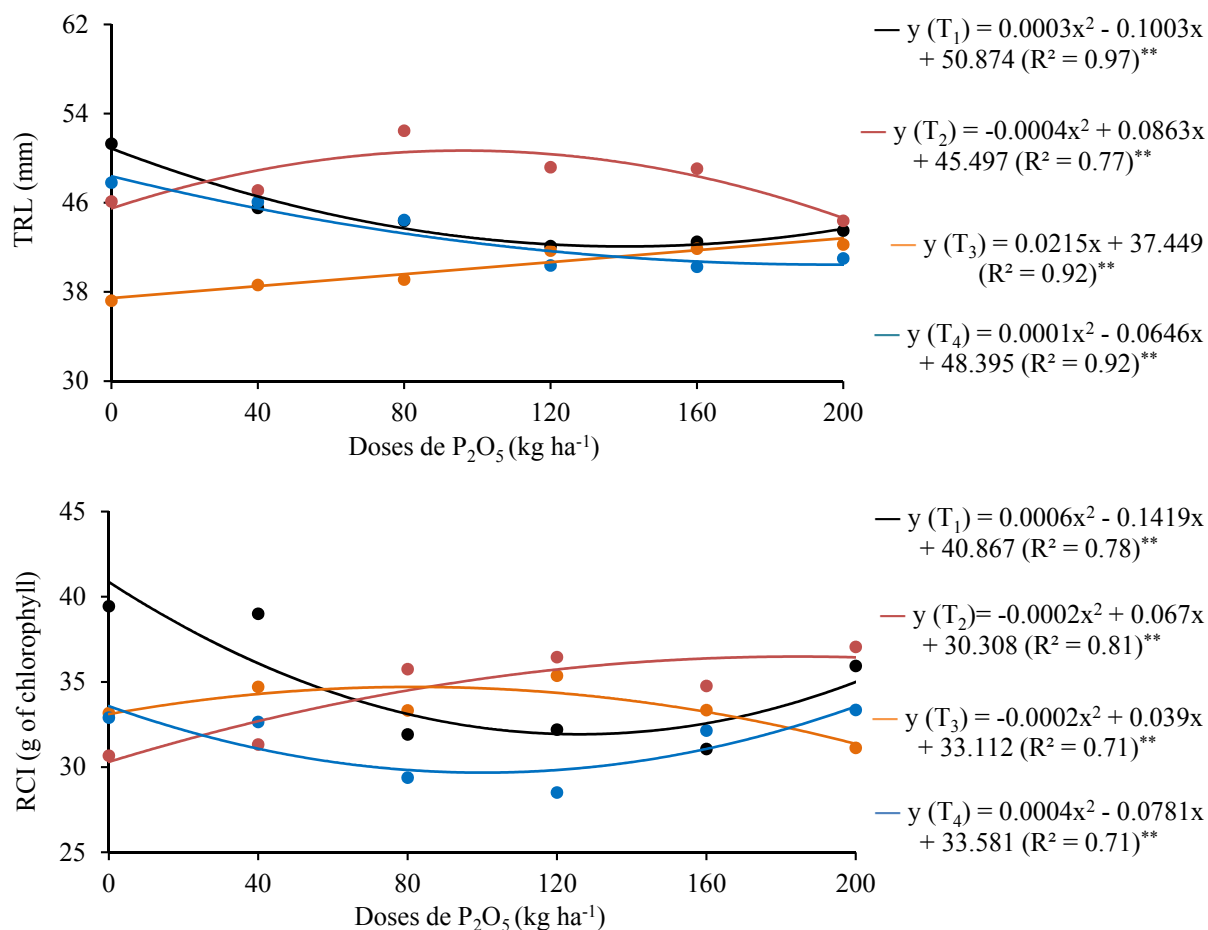


Figure 1. Tuber root diameter (TRD), tuber root length (TRL), and relative chlorophyll index (RCI), as a function of different phosphorus doses (0, 40, 80, 120, 160, and 200 kg ha⁻¹ of P₂O₅), with the application of *Trichoderma* spp. (T₁ – control, without application of *T. harzianum*; T₂ – *T. harzianum* ESALQ 1306, T₃ – *T. harzianum* IBLF 006 WP, and T₄ – *T. asperellum* URM 5911)

With the objective of evaluating the influence of phosphorus fertilization on radish cultivation under controlled conditions, Melo et al. (2021) found that treatments containing nitrogen, potassium and twice the recommended phosphorus were the ones with the greatest number of leaves, the greatest root diameter and length, and also the highest chlorophyll content. However, when the dose of phosphorus is quadrupled there is a reduction of 9.5% and when it is increased eight times this reduction is 15.5%, indicating the effect of increased fertilization on the photosynthetic rate. It was observed in the experiment, that treatments with application of *Trichoderma* spp. they have greater stability in their photosynthetic rate and this can be a benefit or a form of protection against excess, which can cause damage to the plant (Figure 1). The T₂ treatment, in addition to greater stability, also presents higher chlorophyll values, in doses above 70 kg ha⁻¹ P₂O₅, enabling greater production of photoassimilates and greater capacity to generate an increase in yield. According to the studies of Malavolta (2006) and Silva et al. (2020), phosphorus is associated with plant architecture and root growth, directly affecting the development of the aerial part; thus, the root development of radish largely depends on the adequate availability of this nutrient.

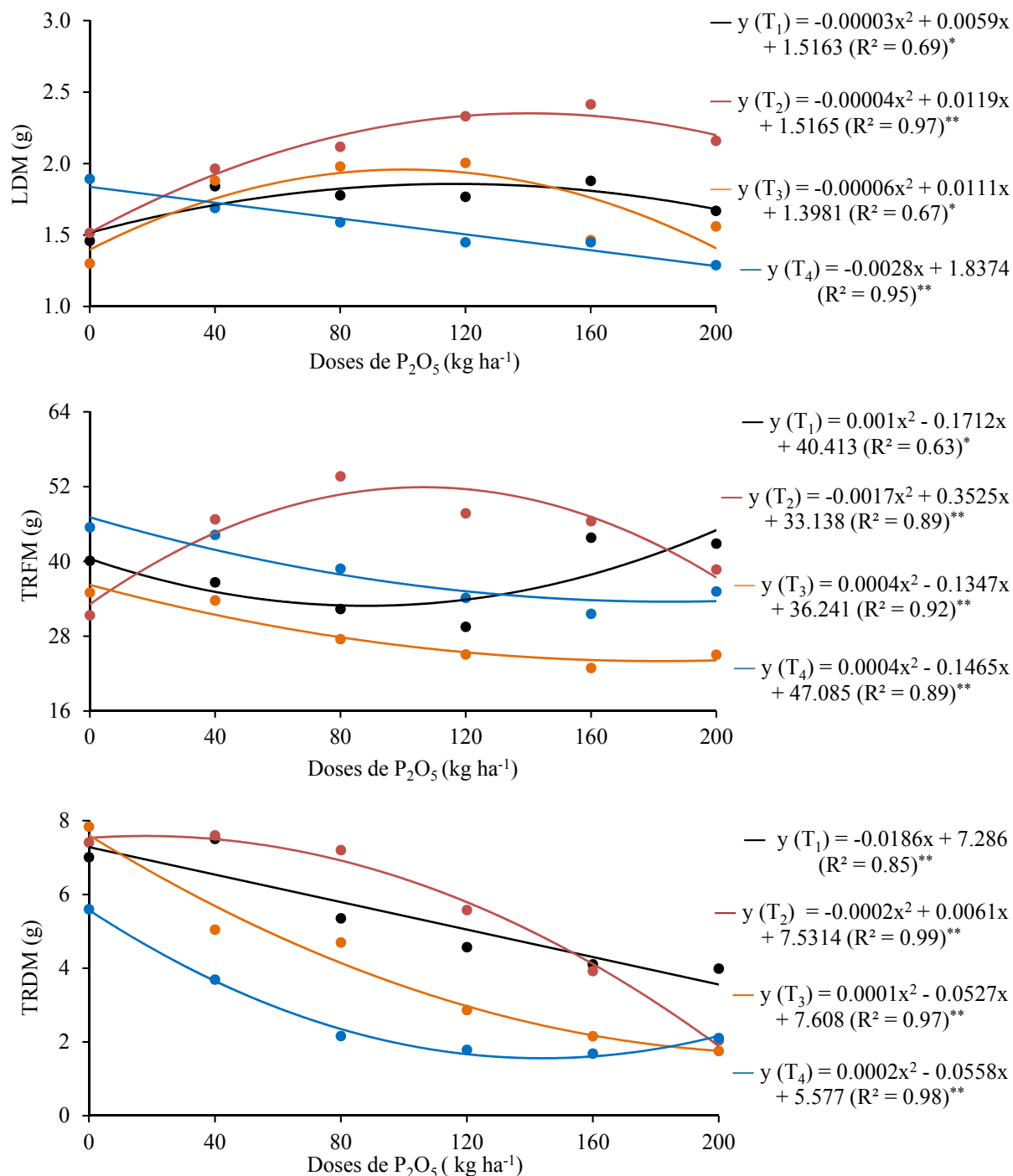


Figure 2. Leaf dry mass (LDM), tuberous roots fresh mass (TRFM), and tuberous roots dry mass (TRDM), as a function of different phosphorus doses (0, 40, 80, 120, 160, and 200 kg ha⁻¹ of P₂O₅), with the application of *Trichoderma* spp (T₁ - control, without application of *T. harzianum*; T₂ - *T. harzianum* ESALQ 1306, T₃ - *T. harzianum* IBLF 006 WP, and T₄ - *T. asperellum* URM 5911)

Leaves play an essential role in the establishment and development of the crop, as their analysis can predict yield, as they are responsible for photosynthesis, and smaller leaves typically result in smaller diameters (Babar et al., 2021). The treatment T₂, for the leaf dry mass variable, was again the one that showed the greatest promotion in radish (Figure 2). It can be seen that above the 80 kg ha⁻¹ dose, the performance of the treatments resembles the behavior of the photosynthetic rate and the promotion of the isolate *T. harzianum* ESALQ 1306 is so great, when compared to the control, that at the maximum point (149 kg ha⁻¹), it is 34% greater in dry mass. Nunes et al. (2014)

found high values for the dry mass of leaves, with maximum productivity being obtained at a dose of 285 mg dm⁻³ of P₂O₅ (570 kg ha⁻¹), this value being almost twice as high as the T₂ treatment mentioned above, which makes it possible to affirm the improvement of the rhizosphere and possibly the greater absorption of phosphorus by plants, when combined with this isolate.

Regarding the variable fresh mass of the tuberous root, the ESALQ 1306 (T₂) strain stands out once again, reaching 51.4 grams, at a dose of approximately 104 kg ha⁻¹ of P₂O₅ (Figure 2). This variable is of great importance for commerce, since the majority of product sales are made fresh. This characteristic is significantly influenced not only by size, but also by weight and, therefore, tubers with greater water content and greater dry mass are the most suitable for producers. The T₄ treatment (*T. asperellum* URM 5911) also performed better than the control, at lower doses and, at approximately 120 kg ha⁻¹, the control becomes the better treatment. Despite this performance, it was noted that this condition is related to a greater quantity of water, based on the dry mass variable and that this product may have a shorter shelf life due to this condition and is not recommended for the producer.

Zeb et al. (2016) studied the influence of phosphorus on radish cultivation and found that phosphate fertilizer had a significant impact on the number of leaves, fresh mass of leaves, root length, maximum root weight, root diameter, productivity per plot and total productivity, with the use of 60 kg ha⁻¹ of P (330 kg ha⁻¹ of single superphosphate), however, in soil and region of Pakistan, very different from Brazilian soil. According to the values paid in the current market, considering the value of R\$ 1,336.00 per ton of single superphosphate (US\$ 426.95), the cost of phosphorus fertilizer would be around 440 reais (US\$ 86.78). According to the values verified in this experiment, the application value would be 104 kg ha⁻¹ of P₂O₅ (578 kg ha⁻¹ of supersimples – 772 reais), in combination with the isolate that has a value of 180 reais (1 L ha⁻¹), totaling 952 reais per hectare. The use of the control treatment (T₁) has the highest productivity at 200 kg ha⁻¹ (1,111 kg ha⁻¹ of supersimple), which represents a cost of 1,485 reais (US\$ 292.86), even so, the producer will have a reduction in costs of approximately 533 reais ha⁻¹ (US\$ 105.13), demonstrating the potential of the technique and the possibility of more sustainable management that could bring a series of other benefits in the long term.

The ESALQ 1306 isolate (*T. harzianum* – T₂), at a dose of approximately 104 kg ha⁻¹ of P₂O₅, showed the greatest benefits in most variables, with the exception of tuberous root diameter (Figure 1 and 2). Therefore, a promotion of 13% for the tuber root length (TRL), 7% for the relative chlorophyll index (RCI), 23% for the leaf dry mass (LDM), 35% for the tuberous roots fresh mass (TRFM), and 12% for the tuberous roots dry mass (TRFM), compared to the control (T₁). This condition would be advantageous to increase crop yield and profitability for producers. The *T. harzianum* IBLF 006 WP (T₃) and *T. asperellum* URM 5911 (T₄) isolates are not recommended, as they presented an antagonistic effect, when compared to the control, for all variables with the exception of the relative chlorophyll index (RCI). The antagonistic effect may have occurred due to competition with other organisms and even competition for the nutrient intended for plants, however, the most commonly reported condition is the population increase of other non-beneficial organisms in the region close to the rhizosphere.

4. Conclusion

The use of *Trichoderma* spp. demonstrates a positive effect on radish development and with a more evident effect at lower doses of phosphorus.

The application of the *Trichoderma harzianum* - ESALQ 1306 isolate is recommended for radish cultivation, as the ability to promote the 13% for tuberous root length, 7% for relative chlorophyll index, 23% for leaf dry mass, 35% for tuberous root fresh mass, and 12% for tuberous root dry mass compared to the control, at a dose of 104 kg ha⁻¹ of P₂O₅, with the possibility of dose reduction and more sustainable management., at a dose of 104 kg ha⁻¹ of P₂O₅, offering more significant profitability potential to producers.

Applications of the *T. harzianum* IBLF 006 WP and *T. asperellum* URM 5911 strains are not recommended for radish cultivation.

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Acknowledgments

The authors thank the Universidade Estadual de Goiás for the financial support provided through Call Notice N°. 21/2022 - Pro-Programs.

Authors contributions

Gustavo Henrique Oliveira, Letícia Silva e Carvalho, Rikelme Matheus Dos Santos Relvas were responsible for data collection.

Mariana Pina da Silva Berti and Cleiton Gredson Sabin Benett were responsible revising.

Cecília Leão Pereira Resende and Fabricio Rodrigues drafted the manuscript and responsible for study design and revising.

All authors read and approved the final manuscript.

Funding

Not applicable.

Competing interests

Not applicable.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and peer review

Not commissioned; externally double-blind peer reviewed.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

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