Journal of Sustainable Development; Vol. 17, No. 3; 2024
ISSN 1913-9063 E-ISSN 1913-9071
Published by Canadian Center of Science and Education

Growth Promotion of *Raphanus Sativus* L. under Doses of Nitrogen 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.v17n3p39 URL: https://doi.org/10.5539/jsd.v17n3p39

Abstract

Radishes are excellent sources of nutrients, such as antioxidants that benefit the immune system, dietary fibers that aid digestion, and vitamins that help control blood pressure and cardiovascular health. Despite this nutritional composition and rapid vegetative cycle, radishes still need to be consumed more and are understudied in Brazil. Another challenge is the limited knowledge about fungi of the *Trichoderma* spp. genus and their combination with fertilizers. Thus, this study aimed to evaluate the growth promotion of *Raphanus sativus* L. under nitrogen doses combined with applying *Trichoderma* spp. isolates and their promoting effect on radish development. The experimental design used was a randomized complete block design in a 4 x 5 factorial scheme (isolates and doses), with seven replications, conducted in a greenhouse. The *Trichoderma* treatments were T_1 – control without isolates application, T_2 – ESALQ 1306, T_3 – IBLF 006 WP, and T_4 – URM 5911, and the nitrogen doses were 0, 50, 100, 150, 200, and 250 kg ha^{-1} of urea. The use of *Trichoderma* spp. demonstrates a positive effect on radish development, offering possibilities for promotion with an emphasis on lower doses of nitrogen. The ESALQ 1306 isolate (*T. harzianum*), at a dose of approximately 113 kg ha^{-1} of urea, a promotion was of 11% tuberous root diameter, 6% for the tuber root length, 50% for the leaf dry mass, 35% for the tuberous roots fresh mass, and 63% for the tuberous roots dry mass). The *T. harzianum* IBLF 006 WP and *T. asperellum* URM 5911 isolates are not recommended, as they presented an antagonistic effect.

Keywords: biofertilizer, radish, strain, synergism, urea

1. Introduction

Consuming vegetables is paramount for maintaining a healthy diet, as they provide a rich source of vitamins, minerals, fibers, and antioxidants essential for human health. Consequently, their consumption is widely recommended as an effective practice in the prevention and control of non-communicable chronic diseases such as obesity, cancer, diabetes mellitus, and cardiovascular diseases, which represent the leading causes of morbidity and mortality in Brazil (Kwok et al., 2019).

In addition to their nutritional significance, as highlighted by Brazilian Association of Fruit and Fruit Products Exporters - Abrafrutas (2018), vegetable cultivation, known as olericulture, is one of the economic activities that significantly contributes to Brazil's development and income, generating approximately R$ 25 billion and creating about 7 million direct and indirect jobs. This substantial economic impact can be attributed to the fact that the cultivation of these plants typically involves a short cycle, providing a rapid financial return to the producer, which is also correlated with greater care in their handling.

Among vegetables, radish (*Raphanus sativus* L.) stands out, belonging to the Brassicaceae family. It is an ancient vegetable widely consumed and famous worldwide (Ali et al., 2023). Its tuberous roots are edible, exhibiting external color variations ranging from white to pink. The pulp, primarily white, has a characteristic spicy flavor (Silva et al., 2020). Additionally, this species is distinguished by its high nutritional value, being a rich source of vitamins A and C, B-complex vitamins, carbohydrates, proteins, and minerals such as manganese, calcium, phosphorus, and potassium (Shahzadi et al., 2023; Shrestha & Thapa, 2018).

Vegetables generally require macro and micronutrients during development, and improper fertilizer management can directly impact quality and productivity. In radish cultivation, the formation and enlargement of the root demand adequate nutrients, which are significantly influenced by the soil's nutritional status, especially nitrogen (N) (Ding et al., 2023). According to Kumar et al. (2020), nitrogen is a vital plant nutrient, forming protoplasm and proteins, promoting cell division, and initiating meristematic activities when applied in ideal quantities.

Several studies indicate that different doses of nitrogen positively influence various parameters in radish cultivation, such as relative chlorophyll index, nitrogen content, number of leaves, fresh and dry shoot mass, leaf area, length and diameter of the tuberous root, fresh and dry root mass (Caetano et al., 2015; Ribeiro et al., 2019; Silva et al., 2016). On the other hand, excessive application of this nutrient poses considerable challenges for plants, representing toxicity to human health and causing environmental damage, including nitrous oxide emission and groundwater contamination by nitrates (Elhanafi et al., 2019).

Soil fungi belonging to the genus *Trichoderma* are among the most studied and utilized beneficial microorganisms in global agriculture. They are primarily employed as biological agents for pathogen control, seed treatment, growth hormone production, and the development of tolerance/resistance to abiotic stresses, among other applications (Poveda, 2021; Taha et al., 2020). However, their role as promoters in vegetables still needs to be explored, with few reports on the interaction of these fungi with different nutrients and doses, as well as potential incompatibility, which can result in reduced synergism or even antagonism. Nevertheless, producers need to utilize biofertilizers more efficiently through radish cultivation, along with a scarcity of information on potential combinations to reduce chemical fertilization, aiming for a more sustainable and economical management approach.

Therefore, the objective was to evaluate the growth promotion of *Raphanus sativus* L. under nitrogen doses combined with applying *Trichoderma* spp. strains and their promoting effect on crop development.

2. Method

The experiment took place at the University of State of Goiás - UEG, Ipameri Unit, in a greenhouse measuring 30 x 7 x 3.5 m, built with a metal structure, covered with 150-micron diffuse polyethylene, and with 25% shade cloth on the sides.

A randomized complete block design was employed in a 4 x 5 factorial scheme (strains and nitrogen doses) with seven replications. The treatments related to *Trichoderma* spp. were T_1 – control, without strain application, T_2 – ESALQ 1306 (*Trichoderma harzianum* - Trichodermil®), T_3 – IBLF 006 WP (*Trichoderma harzianum* - Ecotrich®), and T_4 – URM 5911 (*Trichoderma asperellum* - Quality WG®), at a dose of 4 ml suspension (2 x 108 conidia pot^{-1}). Regarding nitrogen treatments, doses of 0, 50, 100, 150, 200, and 250 kg ha^{-1} of urea (± 0, 20, 40, 60, 80, and 100 kg of N) were used, along with the application of 300 kg ha^{-1} of single superphosphate (± 120 kg ha^{-1} of P_2O_5) and 100 kg ha^{-1} of potassium chloride (± 60 kg ha^{-1} of K_2O), respectively, based on results obtained by Castro et al. (2016) and Costa et al. (2019).

Soil samples were collected before the experiment's setup, and the analysis results were as follows: pH ($CaCl_2$) - 4.9; organic matter - 24.1 g dm^{-3}; P (resin) - 1.5 mg dm^{-3}; 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, as described by Santos et al., (2018). The soil was previously sieved, mixed with 3.5 g of limestone per kilogram of soil according to soil analysis, and left to rest for 40 days. The pots were irrigated every four days with 80% of the soil's water retention capacity. Subsequently, *Trichoderma* applications were performed in the soil before planting using a manual sprayer (capacity of 550 ml). Initially, five Saxa® cultivar (Isla Company) seeds were sown per pot, and after seven days, thinning was carried out, 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

In the summary of the analysis of variance, significant differences were observed for all variables ($p \leq 0.01$) regarding the different sources of variation, including strain (S), dose (D), and the interaction between these factors (Table 1). Therefore, there may be favorable combinations between strains and doses of nitrogen, which may result in synergism and more excellent promotion in the development of radish.

According to Rakshit et al. (2015), *Trichoderma* spp. not only can promote plant growth and induce tolerance to abiotic stresses, mainly through interactions with roots. These interactions result in the production of various proteins that plant receptors can recognize. The host regulates these associations, and when there is no favorable association, detrimental effects on development can occur, as evidenced by treatments T_3 (IBLF 006 WP) and T_4 (URM 5911), as shown in Figure 1. In summary, *Trichoderma* spp. secretes a variety of soluble proteins, including enzymes and others, playing essential roles in its ability to survive in the rhizosphere ecosystem, although the combination is not always successful (Mendoza-Mendoza et al., 2018).

A synergistic effect was observed in the diameter of the tuberous root, where the application of *Trichoderma harzianum* (strain ESALQ 1306), treatment T_2, at a dose of 113 kg ha^{-1} of urea (45.2 kg ha^{-1} of N), resulted in better root development (41.5 mm), with an increase of 13%, compared to the control treatment – T_1 (Figure 1). A quadratic performance was also observed in treatment T_2, where the minor diameters were found at lower doses of N, with more excellent promotion achieved at intermediate doses and reduction at higher doses. These results were also observed in lettuce and arugula, as reported by Visconti et al. (2020), who indicated little difference in productive yield with the application of *T. harzianum* or *T. virens* strains in these species. However, it is necessary to monitor nitrogen levels in the soil, as its scarcity may not promote crop development. At the same time, excess can be detrimental, resulting in economic waste, low yield, and environmental degradation.

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 nitrogen doses with the application of *Trichoderma* spp. Ipameri, GO, 2024

SV	DF	TRD	RLE	RCI	LFM	FTRM	TRDM
Strain (S)	3	41.07**	191.61**	144.73**	0.84**	429.86**	43.54**
Dose (D)	4	157.50**	510.21**	235.04**	1.05**	1080.87**	92.50**
S x D	12	57.72**	135.39**	124.50**	0.7**	610.80**	19.20**
Block	6	5.32	48.73	8.84	0.22	236.01	8.82
Error	72	8.43	14.63	9.35	0.06	30.27	2.20
CV (%)		8.04	8.72	9.12	15.97	16.32	15.72

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

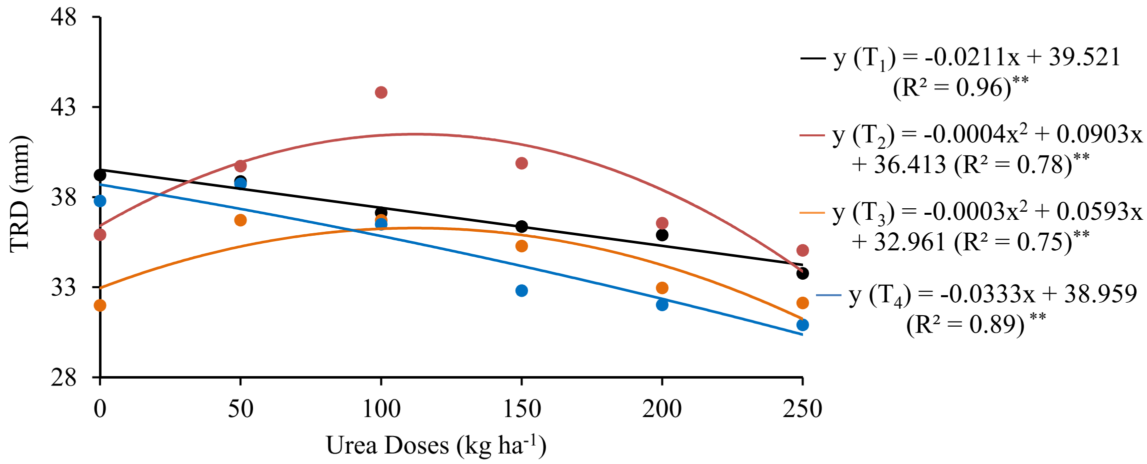
The authors Silva et al. (2016) conducted tests with doses of 0, 100, 200, 300, and 400 kg ha^{-1} of nitrogen using fertigation, while Shrestha and Thapa (2018) evaluated doses of 0, 30, 60, 90, 120, and 150 kg ha^{-1} of N. Both studies did not identify significant differences among the tested doses for the root diameter in radish cultivation. However, it is suggested that the strain ESALQ 1306, in particular, promoted an increase in this variable, thus demonstrating a better interaction with radish roots.

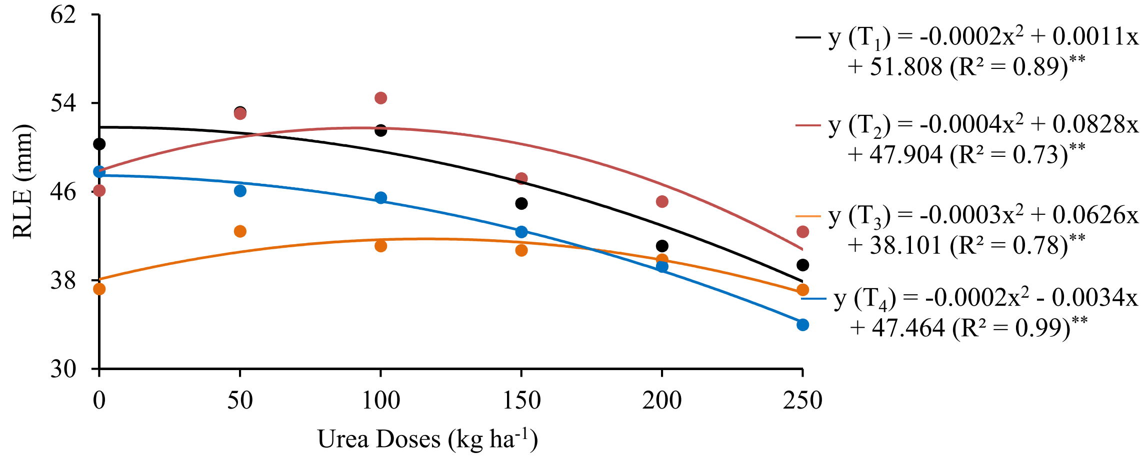
The greatest length of the tuberous root was equally observed with the strain ESALQ 1306 (T_2), with an average value of 52.2 mm at a dose of approximately 104 kg ha^{-1} of urea (42 kg ha^{-1} of N). This length increased until reaching the maximum point and gradually decreased (Figure 1). Similarly to the diameter, the results confirm the synergistic relationship between the strain and the improvement in the quality of the tuberous root, the latter being an essential requirement for producers as it accelerates sales and reduces shelf time, enabling better profitability. However, according to the results presented by Pant and Oli (2021), under this condition, it would be necessary to apply 217 kg ha^{-1} of urea to achieve the desired productivity, which represents twice the application observed with the ESALQ 1306 strain, resulting in increased production costs.

The relative chlorophyll index indirectly expresses the photosynthetic rate of plants, correlating mainly with nutrients, especially nitrogen, present in the plant. This condition was verified and confirmed by the values observed for this variable, which showed higher indices with increasing doses (Figure 1). However, none of the strains used showed superior performance to the control (T_1), which remained practically stable across doses, ranging from 37 to 42 grams of chlorophyll, with the highest rate between 200 and 250 kg ha^{-1} of urea.

According to Domínguez et al. (2016), combining with the fungus should increase the photosynthetic rate and elevate the values compared to the control (T_1). This occurs due to the negative regulation of defense genes and the positive regulation of carbon and nitrogen metabolism genes, accompanied by increased plant growth, carbon levels, and internal nitrogen. However, with greater sensitivity and upon detection of fungi, this defense reaction against the fungus is similar to a reaction against a pathogenic infection, which may affect the quantity of this nutrient in the plant.

Observations revealed that the dry leaf mass did not follow a similar pattern to the chlorophyll index, indicating that the strains more significantly stimulated the aboveground development of radishes, except for treatment T_4 (URM 5911), which was superior only at initial doses (0 to 100 kg ha^{-1} of urea) compared to the control (T_1). The larger leaf area likely did not result in a photosynthetic rate, which was unexpected. As highlighted by Haque, Ilias, and Molla (2012), in studies with mustard and tomato, the application of half the recommended nitrogen fertilization, combined with different strains of *T. harzianum*, resulted in the same number of leaves, similar to plants fertilized only with mineral fertilizers. This beneficial effect also extended to other variables, including productivity, resulting in a significant increase in yield. Therefore, the *Trichoderma* population may have increased, making nutrients available and promoting more significant growth and development of the leaves, which is also reflected in the roots (Figure 2).





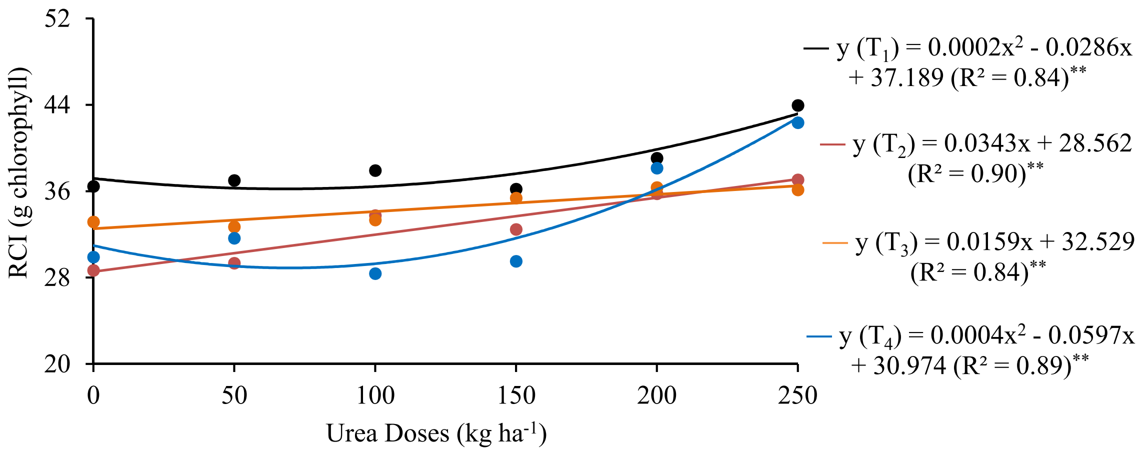
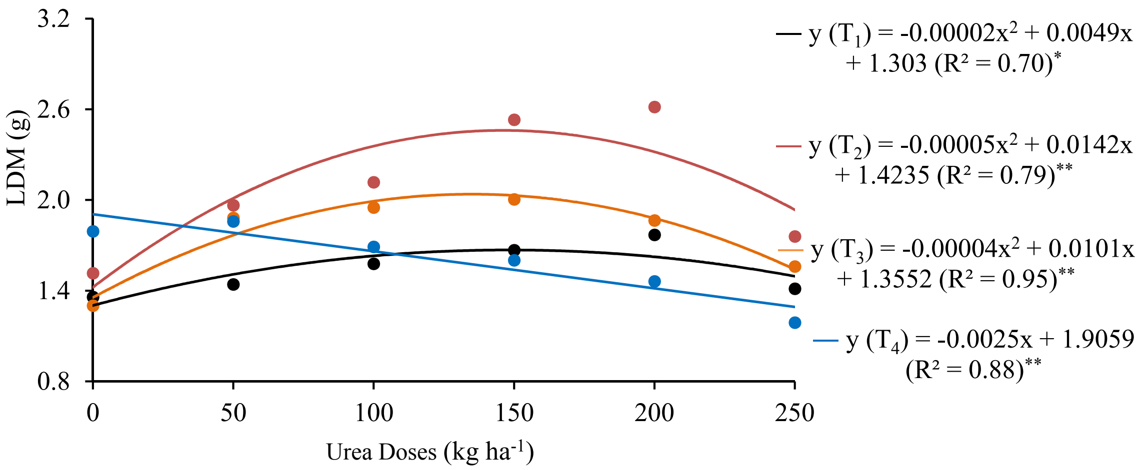


Figure 1. Tuber root diameter (TRD), tuber root length (RLE), and relative chlorophyll index (RCI), as a function of different nitrogen doses (0, 50, 100, 150, 200, and 250 kg ha^{-1} of urea), with the application of *Trichoderma* spp. (T_1 – control, without application of *T. harzianum*; T_2 – *T. harzianum* ESALQ 1306, T_3 – *T. harzianum* IBLF 006 WP, and T_4 – *T. asperellum* URM 5911)

Contreras-Cornejo et al. (2009) evaluated the response of Arabidopsis seedlings to inoculation with *T. atroviride* (also known as *T. harzianum*) and *T. virens*. They observed that fungal inoculation stimulated lateral root formation and increased aboveground biomass production, resulting in a beneficial effect on plant growth and development. Additionally, they highlighted the role of auxin signaling in promoting plant growth by *T. virens*, suggesting that the hormone may be the leading promoter in other species.

Azarmi, Hajieghrari and Giglou (2011) found that seed inoculation rather than soil inoculation proved more suitable for promoting tomato growth, resulting in a more significant benefit in increased leaf number and leaf area. This method also led to better fresh and dry leaf and root mass performance, with the *T. harzianum* T447 strain standing out. Results obtained with this strain also showed increased nutrient content, such as calcium, phosphorus, sodium, and potassium, both in leaves and roots, particularly at early stages (45 days). According to findings by Molla et al. (2012), inoculation with *T. harzianum* T22, combined with 50% of the recommended fertilization for tomatoes, increased flower and fruit numbers, reducing costs without compromising quality. Thus, fungi of the *Trichoderma* spp. genus play a vital role in soil nutrient cycling, mobilizing and absorbing nutrients, which can lead to cost reduction through reduced application and greater phyto-stimulation (KASHYAP et al., 2017).



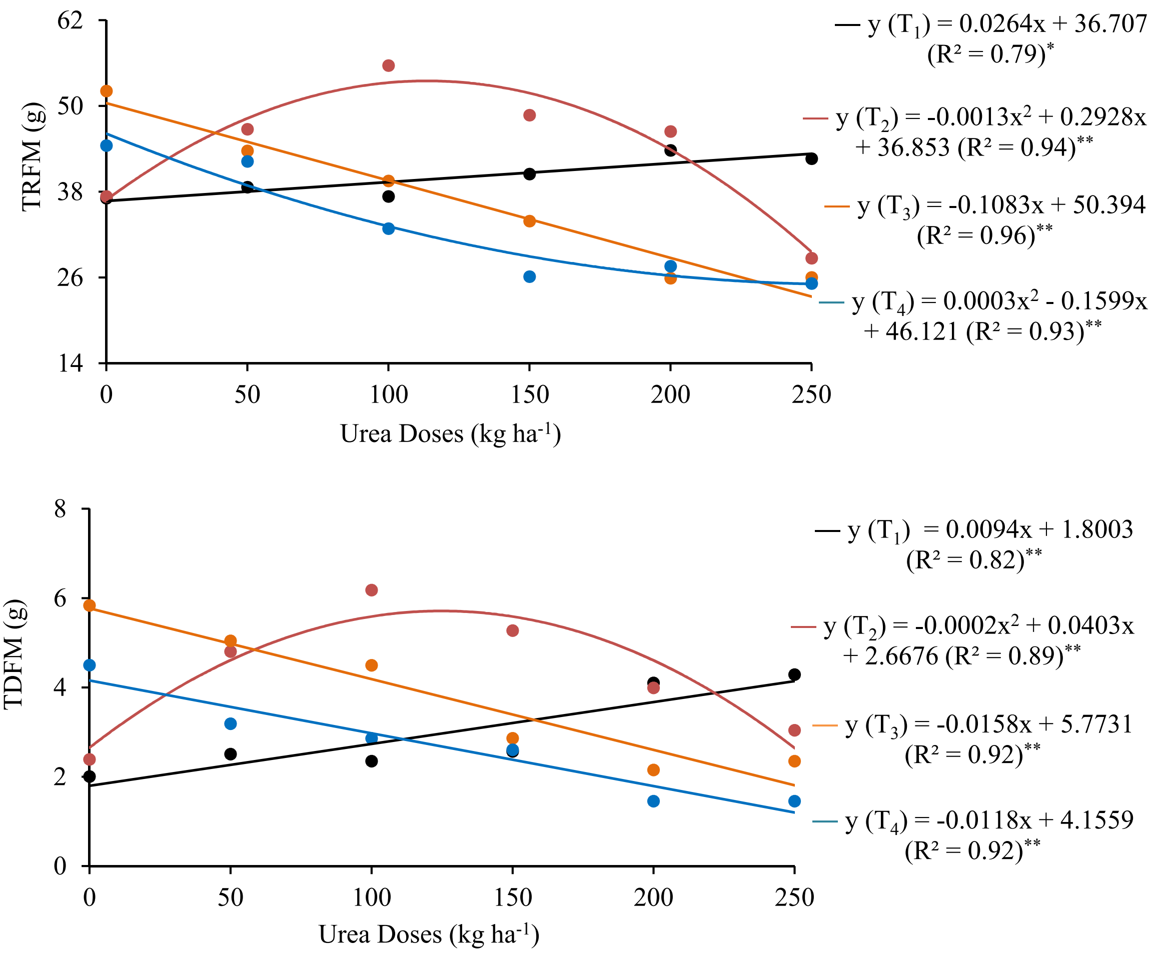


Figure 2. Leaf dry mass (LDM), tuberous roots fresh mass (TRFM), and tuberous roots dry mass (TRDM), as a function of different nitrogen doses (0, 50, 100, 150, 200, and 250 kg ha^{-1} of urea), with the application of *Trichoderma* spp. (T_1 - control, without application of *T. harzianum*; T_2 - *T. harzianum* ESALQ 1306, T_3 - *T. harzianum* IBLF 006 WP, and T_4 - *T. asperellum* URM 5911)

A greater fresh mass of tuberous root (53.3 g) was obtained with the use of the ESALQ 1306 strain (T_2), combined with a dose of approximately 113 kg ha^{-1} of urea, representing a 25.5% increase compared to T_1, without application (Figure 2). Similarly, for the dry mass of the tuberous root, the dose was 101 kg ha^{-1} of urea with the same strain T_2. The advantage observed when no fertilizer was applied in treatments T_3 (IBLF 006 WP) and T_4 (URM 5911) for the masses is worth noting. Thus, the effect on radish development was confirmed, evidencing a synergistic relationship of promotion that benefits both the fungus population and the growth and development of the plant. On the other hand, strain T_4, using *Trichoderma asperellum* (URM 5911), showed antagonistic effects on different variables, except for the relative chlorophyll index. There may be a correlation between the fungus species, increasing doses of nitrogen, and its negative, usually linear, effect on the characteristics (Figures 1 and 2).

In tests conducted in pots, Lijung, Chaurtsuen and Chongho (2011) found that the *Trichoderma* strain 1295-22 significantly increased the absorption of potassium, calcium, magnesium, and manganese in radish plants compared to untreated plants grown in acidic soil. However, the R42 strain demonstrated a significant increase in nitrogen absorption by radish plants.

Lijung et al. (2011) did not observe any significant effect of *Trichoderma* strains on plant growth or nutrient absorption in alkaline soil. Therefore, these results suggest that the effectiveness of *Trichoderma* strains may depend on the complex interaction of soil characteristics, fertilization regime, and the cultivar used to achieve more satisfactory results with a better cost-benefit ratio.

The research conducted by Samolski et al. (2012) highlighted that fungal adherence and enhanced cellular protection play a crucial role in lateral root growth and root hair elongation, influencing root architecture modification. This alteration increases total surface area, facilitating nutrient absorption and translocation to the plant's aerial part. This process, in turn, contributes to increased plant biomass through efficient nutrient utilization. Additionally, a significant water accumulation was observed in *Trichoderma* spp. treatments, particularly at initial doses, which is vital for the fresh mass of the tuberous root intended for commercialization. This aspect is directly related to the quality of radishes, as confirmed by the dry mass of the tuberous root, even if sold in dehydrated form (Figure 2).

Haque, Ilias, and Molla (2012) reported that the efficient use of compost enriched with *Trichoderma* can result in increased yield, reduced use of nitrogen fertilizers, decreased presence of soil pathogens, and improved soil health. The ESALQ 1306 isolate (T_2), combined with a dose of 113 kg ha^{-1} of urea, leads to a reduction of approximately 45% in nitrogen fertilization, aligning with more sustainable practices (Figure 1 and 2). According to the results presented by Molla et al. (2012), this condition is feasible and more advisable than conventional farming. Furthermore, based on the nitrogen prices in urea in the state of Goiás, a ton would cost around 2,250.00 Brazilian reais, decreasing by R$ 62.00 reais per hectare, without considering other indirect benefits.

The treatment T_2, at a dose of approximately 113 kg ha^{-1} of urea, showed greater promotion in different variables, with the exception of relative chlorophyll index, which the control remained stable and presented higher values than the treatments with Trichoderma spp. (Figure 1 and 2). Therefore, a promotion was of 11% tuberous root diameter (TRD), 6% for the tuber root length (TRL), 50% for the leaf dry mass (LDM), 35% for the tuberous roots fresh mass (TRFM), and 63% for the tuberous roots dry mass (TRFM), compared to the control (T_1). These results demonstrate the potential of the technique for producers and enable greater crop yields. The *T. harzianum* IBLF 006 WP (T_3) and *T. asperellum* URM 5911 (T_4) isolates are not recommended, as they presented an antagonistic effect, when compared to the control. This condition may have occurred due to competition with other organisms, population increase of other organisms that are not beneficial with the application of isolates or even competition for nutrients destined for plants, however, the condition most reported by several researchers is the population increase of organisms not beneficial to in the rhizosphere.

Biofertilizers contribute to the efficient absorption of nitrogen by the roots of radish plants, reducing losses through leaching and/or volatilization and minimizing groundwater and surface water pollution. In conclusion, soil microbial processes are highly complex and warrant further exploration through research and analysis of the interactions involved. However, it is essential to note that there is a need for more studies addressing the importance of combining fungi and doses, especially in vegetable crops widely consumed worldwide.

4. Conclusion

The use of *Trichoderma* spp. demonstrates a positive effect on radish development, offering possibilities for promotion with an emphasis on lower doses of nitrogen.

The ESALQ 1306 isolate (*T. harzianum*), at a dose of approximately 113 kg ha^{-1} of urea, a promotion was of 11% tuberous root diameter, 6% for the tuber root length, 50% for the leaf dry mass, 35% for the tuberous roots fresh mass, and 63% for the tuberous roots dry mass.

The *T. harzianum* IBLF 006 WP and *T. asperellum* URM 5911 isolates are not recommended, as they presented an antagonistic effect.

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Acknowledgments

The authors thank the Universidade Estadual de Goiás for the financial support provided through Call Notice Nº. 21/2022 - Pro-Programs.

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Bruna Stefhane Santos Reis, Cíntia da Silva de Oliveira, Mariéla Do Amaral Silva were responsible for data collection.

Mariana Pina da Silva Berti and Katiane Santiago Silva 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.