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Growth promotion of radish microgreens with *Trichoderma harzianum* inoculation

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ABSTRACT

The fungus *Trichoderma harzianum* is a sustainable and environmentally friendly solution for use in agriculture. Its application stimulates the growth of radish microgreens, a short-cycle crop, standing out as an agricultural practice that meets the growing demand for healthier crops. This study aimed to evaluate the use of *Trichoderma harzianum* on the growth and quality of radish microgreens. The research was conducted in a greenhouse, using a completely randomized experimental design (CRD) with three treatments and eight replications. The treatments included a control, the application of the fungus on the seeds, and the application of the fungus on the substrate. The phytotechnical and post-harvest characteristics were evaluated. The application of the *Trichoderma harzianum* to the substrate resulted in a 19.75% increase in yield, a 19.87% increase in the conversion of seed mass into fresh mass, a 2.94% increase in the shoot dry mass, a 2.31% increase in water content, and a 28.29% increase in hypocotyl length compared to the control. The use of *Trichoderma harzianum* proved to be effective in promoting the growth of radish microgreens.

Keywords: *Raphanus sativus*, Biological, Fungi, Vegetables, Quality.

Promoção de crescimento de microverdes de rabanete com a inoculação de *Trichoderma harzianum*

RESUMO

O fungo *Trichoderma harzianum* é uma solução sustentável e ecologicamente correta para utilização na agricultura. Sua aplicação estimula o crescimento de microverdes de rabanete, uma cultura de ciclo curto, destacando-se como uma prática agrícola que atende à crescente demanda por cultivos mais saudáveis. O objetivo foi avaliar o uso de *Trichoderma harzianum* no crescimento e qualidade dos microverdes de rabanete. A pesquisa foi conduzida em casa de vegetação, utilizando o delineamento experimental inteiramente casualizado (DIC) com 3 tratamentos e 8 repetições. Os tratamentos foram: controle, aplicação do fungo na semente e no substrato. Foram avaliadas as características fitotécnicas e de pós-colheita. A aplicação do fungo *Trichoderma harzianum* no substrato teve incremento de 19,75% na produtividade, aumento de 19,87% na conversão de massa de semente em massa fresca, 2,94% a mais de massa seca da parte aérea, 2,31% de aumento no teor de água e, aumento de 28,29% no comprimento de hipocótilo, em comparação ao controle. O uso do *Trichoderma harzianum* mostrou-se eficaz para promover o crescimento dos microverdes de rabanete.

Palavras-chave: *Raphanus sativus*, Biológico, Fungos, Hortaliças, Qualidade.

1. Introduction

There is a notable demand for healthier foods, including fruits and vegetables (Rouphael et al., 2021). Consumers are increasingly looking for products grown using sustainable techniques that guarantee traceability, transparency, health, and quality (Purquerio et al., 2016, Rouphael et al., 2021, Santos et al., 2022). Changes in eating habits have intensified during the Covid 19 pandemic, with a considerable decrease in the consumption of processed foods and eating out (Galiani et al., 2020). Conversely, there has been a significant increase in the consumption of vegetables attributed to their association with health and disease prevention. This increase reflects consumers' growing awareness of the benefits of these foods (Rouphael et al., 2021).

In response to this growing consumer demand, producers see an opportunity to produce and offer high value-added foods that meet market demands. As a promising option, microgreens are characterized by a short production cycle and a concentrated nutritional profile. These young seedlings offer high nutrients and compounds that benefit health, outperforming conventional vegetables (Galiani et al., 2020). Microgreens are harvested between 7 and 21 days after emergence, while radish microgreens are harvested between 7 and 10 days after emergence, depending on the growing environment (Xiao et al., 2015). Harvesting occurs when the cotyledons have fully developed and before the first true leaf appears, with an average height of 3 to 9 centimeters, varying according to the species grown (Di Giogia et al., 2017, Bhatt & Sharma 2018).

Chemical pesticides are not used when growing microgreens because the grace period is often longer than the crop cycle. As microgreens have a rapid production cycle, any chemical residue can remain on the plant during harvest, compromising food safety (Speer et al., 2020). Therefore, using biological products is essential as it is a safe and effective alternative for controlling pests and diseases, allowing microgreens to grow healthily and free of chemical residues. In addition, growing microgreens using bio inputs promotes environmental sustainability, contributing to a more efficient agricultural system (Speer et al., 2020).

The application of *Trichoderma harzianum* is a promising alternative for growing high-value-added vegetables. Besides controlling pests and diseases more naturally, this fungus benefits plants by promoting healthy plant growth, strengthening the root system, increasing resistance to environmental stresses, and improving nutrient absorption. In addition, due to the symbiosis between microorganisms and plants, plant hormones induce physiological growth responses when the interaction is established (Neelipally et al., 2020). By opting for biological products such as *Trichoderma harzianum*, microgreen producers guarantee food safety for consumers and contribute to environmental sustainability by offering a more responsible way of producing high-quality food (Shi et al., 2016).

Radish (*Raphanus sativus*) microgreens have a versatile nature and fast growth rate and are recognized for their high concentration of nutrients in a small portion, making them an ideal option for the application of *Trichoderma harzianum* to enhance nutritional quality and plant growth (Poveda et al., 2020). In addition, radish is a crop easily grown in different environmental conditions and substrates. With their growing popularity due to their association with healthy eating, the study of radish microgreens has positive practical implications for producing nutritious foods with increased biochemical and nutritional potential (Pescarini et al., 2023).

This study aimed to evaluate the use of *Trichoderma harzianum* to stimulate growth and improve the biochemical quality of radish microgreens (*Raphanus sativus*, cultivar Indra) when applied to the seeds and the growing substrate.

2. Material and Methods

The research was conducted in a greenhouse at the University of São Paulo, campus ESALQ, in Piracicaba, SP (22º42'30"S and 47º38'01"W) and was conducted in a completely randomized design (CRD) with three treatments and eight replications. The treatments were as follows: T1, without the application of *Trichoderma harzianum* to the seed and substrate (control); T2, with the application of *Trichoderma harzianum* to the radish microgreen seeds; and T3, with the application of *Trichoderma harzianum* to the substrate. Each tray used for growing radish microgreens was 16 cm long and 10.5 cm wide, totaling 0.0168 m², and represented one replication.

A total of 24 trays were used in the experiment. Radish seeds from the cultivar Indra were used in the experiment. The seeds are from the company Isla® (Porto Alegre, RS, Brazil), which has an exclusive line for growing microgreens free from chemical pesticides. The sowing density used to grow the radish microgreens was 300 g/m², totaling 5.04 g of seeds per tray.

Carolina Soil® (Carolina Soil 35 H) substrate was used to grow the microgreens. The substrate contained the following nutrients (values in ppm): phosphorus 145.41, potassium 222.5, calcium 43.6, magnesium 62, sulfur 75.37, sodium 51.75, copper 0.18, iron 0.3, manganese 0.45, zinc 0.25, ammoniacal nitrogen 108.65, and boron 50.01. Other characteristics of the substrate included humidity (65 ºC) of 31.37%, organic matter of 52%, ash of 48%, organic carbon of 26%, pH 6, electrical conductivity (EC) of 1.65 mS cm^{-1}, and density of 0.3 g/cm³.

Treatment (T2) was inoculated with *Trichoderma harzianum* strain BK Th001 via seed, which consisted of 0.00302 grams of Natucontrol® (Biotrop, Vinhedo, SP, Brazil) powder diluted in 1 ml of water. The seeds were submerged in the solution for 20 minutes before being sown with $1x10^7$ CFU/g, 1.15% w/w. The basis for calculating the proportion is 60g of the product/100kg of microgreen seed, according to the manufacturer's recommendation. The seeds were sown manually, spreading them evenly over the growing substrate. To inoculate the *Trichoderma harzianum* into the substrate (T3), a solution containing one gram of the Natucontrol® product diluted in one liter of water was added to 1 kg of the substrate, which was left to stand for 20 minutes before sowing.

After sowing, the trays were kept in a controlled environment, with stable climatic conditions (temperature of 23 °C and relative humidity of 70%-75%) and no light to ensure uniform germination and adequate expansion of the hypocotyl. The data was recorded using a datalogger (Onset, H21 USB, Bourne, USA) and stored for later analysis. The software HOBOware v. 3.7.18 (Onset, Bourne, USA) was used for processing. After the first 48 hours of sowing, the substrate was watered using a spray bottle. At the end of this period, the trays were transferred to the greenhouse and placed on the germination table for the microgreens to grow.

Irrigation during the cycle was conducted manually at the base of the hypocotyl according to the irrigation needs of each treatment using a spray bottle to protect the cotyledonary leaves, thus avoiding the appearance of other fungi. The radish was harvested 7 days after sowing (DAS) using clean scissors, cutting at the base of the seedlings to take advantage of the fully developed and expanded hypocotyls and cotyledonary leaves (Wieth 2018).

At the end of the experiment, the following variables were measured: a) shoot fresh mass yield (FM) ($kg.m^{-2}$); b) shoot dry mass yield (DM) (kg. m^{-2}); c) leaf area using ImageJ software (cm^2); d) hypocotyl length (cm); e) RGB index of the hypocotyls (R red, G green, and B blue), extracted using ImageJ software, which processed the images of three seedlings per repetition obtained by a scanner (Flatbed Color Image Scanner FCIS); f) conversion of seed mass sown into fresh mass (FM/MSE), by dividing the microgreen fresh mass by the seed mass sown; g) water content (WC), obtained from the ratio between FM and DM of each treatment, h) root dry mass yield (RDM) (kg. m^{-2}), and i) microgreens yield ($kg.m^{-2}$).

The biochemical and physicochemical analyses were conducted on the radish microgreen samples as follows: the samples were wrapped in aluminum foil and frozen quickly in liquid nitrogen, then kept in a freezer at -20 °C. The samples were then dried in a freeze dryer (LIOTOP, L101) at a temperature of -60 °C until they reached a pressure of less than 31 µHg.

The pH was determined in 100 mL of aqueous solution (method from the Adolfo Lutz Institute, 2008). Soluble solids (°Brix) were evaluated using a pocket refractometer (Pocket Refractometer, PAL-1, Atago). The pigments chlorophyll *a* and *b* and total chlorophylls and carotenoids (methods described by Sims & Gamon, 2002; Wu et al., 2008) were assessed with readings taken on a spectrophotometer at wavelengths of 660 nm, 640 nm, and 470 nm for chlorophyll *a*, chlorophyll *b*, and carotenoids, respectively. All analyses were conducted in triplicate to ensure the accuracy of the results.

After collecting the data, exploratory analyses were conducted to check the normality of the residuals (Shapiro Wilk) and the homogeneity of variance test (Bartlett). For the statistical analysis of the results obtained in the different evaluations, analyses of variance were conducted using the Tukey test. The means of the treatments were compared with each other using the Tukey test at 5% probability, using the RStudio® program (R CORE TEAM 2016).

3. Results and Discussion

The forms of treatment using *Trichoderma harzianum* showed different results in the variables evaluated in the study. The treatment using the fungus in the substrate proved to be statistically superior to the treatment on the seeds and the control in the variables of conversion of seed mass into fresh mass (FM/MSE), fresh mass (FM), dry mass (DM), and water content (WC). Inoculation of *Trichoderma harzianum* in the substrate was more efficient in increasing yield than inoculation of the same in the seed and the control treatment. (Table 1).

Table 1. Conversion of seed mass into fresh mass (FM/MSE), fresh mass yield (FM), and dry mass yield (DM) of the shoot of the microgreens ($kg.m^{-2}$), water content (WC) (%), and root dry mass yield (RDM) ($kg.m^{-2}$).

Treatments	FM/MSE	FM	DM	WC	RDM
control	8.10b	2.43b	0.34b	85.79b	1.19ab
seed	8.01b	2.40b	0.34b	85.67b	1.05b
substrate	9.71a	2.91a	0.35a	87.78a	1.27a
cv (%)	5.66	5.66	2.47	0.75	10.6
dms	0.61	1.84	0.01	0.82	0.15

Means followed by equal letters in the column do not differ by the Tukey test at 5% significance.

For shoot fresh mass yield (FM), it is possible to see an increase of 19.75% in yield when comparing the treatment in the substrate with the control treatment. McGehee et al. (2018) obtained the same result, in which the treatment with bio fungicides resulted in greater plant height of the Brassica genus compared to the control. This increase in yield observed in the study was due to the beneficial relationship between the plant and the microorganism, which contributed to greater absorption of nutrients and production of exudates and plant hormones such as auxins and gibberellins (McGehee et al., 2018).

Regarding the conversion of seed dry mass into fresh mass (FM/MSE), the treatment in the substrate recorded an increase of 19.87% compared to the control. Increased fresh mass production and better performance in converting seed mass into plant mass are crucial factors in boosting the yield of microgreens, as they are consumed and marketed fresh (Murphy & Pill 2010), and seeds are considered one of the biggest costs related to production.

The root dry mass yield showed no statistical difference between the control and the substrate treatment. This result is in agreement with the study by Domingues et al. (2021), in which inoculation of the substrate with *Trichoderma atroviride* in the lettuce cultivar Mediterrânea showed no significant differences between the treatment and the control in terms of root dry weight. On the other hand, compared to the seed treatment, there was a 20.95% increase in root production with the substrate treatment.

Concerning the variable shoot dry mass yield (DM), the results were statistically significant when comparing the substrate treatment with the seed treatment and the control, with 2.94% more shoot dry mass yield in the radish microgreens. This index is important for quantifying the accumulated nutrients and carbon fixation during the crop cycle, which are key factors for the final quality of the microgreens.

Poveda et al. (2020) explain that mass accumulation and root growth are related to the ability of different strains of *Trichoderma* spp. to balance the availability of phytohormones and solubilize nutrients for plants, regulating the levels of indole acetic acid (IAA), gibberellic acid, and ethylene, and controlling their growth due to the direct availability of nutrients and the greater absorption area by the plant root system, which contributes to mass accumulation.

Concerning water content (WC), the substrate treatment showed statistically significant differences compared to the seed treatment and the control, which showed no differences. Water content is directly related to fresh and dry mass (Guimarães & Stone, 2008), as shown in Table 1, with higher values in the substrate treatment. As a result, the water content in this treatment was higher than the others, attributed to the higher yield observed in the substrate treatment with *Trichoderma harzianum* inoculation. The hypocotyl leaf area and RGB index variables showed no statistically significant differences between the treatments. (Table 2).

Figure 1. Representation of radish microgreens with qualitative gains (right) and physiological and biochemical explanations of microgreen-*Trichoderma* interaction (left). Source: Alasse Oliveira (2024).

Table 2. Microgreen leaf area (LA) (cm²), hypocotyl length (HL) (cm), and colorimetry R (Red), G (Green), and B (Blue).

Treatments	LA	HL	R	G	B
Control	1.83a	4.10b	144.87a	130.12a	133.87a
Seed	1.88a	4.84ab	146.25a	129.75a	132.87a
Substrate	1.97a	5.26a	144.50a	130.75a	136.37a
CV (%)	27.44	16.95	5.34	16.02	17.95
DMS	0.65	0.98	9.78	26.29	10.60

Means followed by equal letters in the column do not differ by the Tukey test at 5% significance.

Therefore, it is impossible to state that the substrate and seed treatment influences the coloration of the hypocotyl and the size of the leaves under the conditions of this study. However, hypocotyl length increased by 28.29% and 18.14% with the substrate and seed treatments, respectively, compared to the control treatment, according to Table 2. In addition, the radish microgreens showed no significant influence from the fungus or the application method in the biochemical and physicochemical analyses.

The different treatments did not alter the chlorophyll *a*, *b*, and total contents, total soluble solids (Brix), and pH. The effect of seed inoculation with *Trichoderma* is dependent on the cultivar and plant species used, the growing medium, and the form of inoculation, making further studies necessary to understand the appropriate dose to be used for each cultivar of interest as it is in McGehee et al. (2018). It is, therefore, essential to conduct further studies to determine the correct dose and the best form of inoculation according to each species and cultivar.

4. Conclusions

Trichoderma harzianum effectively increased the fresh mass yield of radish microgreens only when applied to the substrate. Under these experimental conditions, it was impossible to observe an increase in the quality of biochemical and physicochemical attributes with the inoculation of the microorganism. Although it failed to affect all the variables analyzed, such as root dry mass yield, the treatment resulted in greater fresh mass and length of the hypocotyl. These results highlight the potential of the fungus *Trichoderma harzianum* as a promising tool for improving the phytotechnical performance of microgreens.

Authors' Contribution

The authors' contributions to this scientific article were as follows: João Zuppardo has been responsible for experimenting. Simone da Costa Mello has conceived the project and acted as a general reviewer. Alasse Oliveira, Isabela Scavacini, and Walleska Torsian has contributed to data analysis and scientific writing.

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