

Bioprospecting of microorganisms with growth-promoting potential in lettuce (*Lactuca sativa*)

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ABSTRACT. Lettuce (*Lactuca sativa* L.) is a widely cultivated vegetable crop whose productivity can be affected by edaphoclimatic factors and the presence of soilborne pathogens. In this context, the use of beneficial microorganisms has emerged as a sustainable alternative to promote plant growth and reduce dependence on chemical inputs. This study aimed to carry out the bioprospecting of microorganisms and evaluate their efficiency in seed inoculation and in the development of curly lettuce (*Lactuca sativa*) var. Crispa. Microorganisms were isolated from soil and root samples collected in different anthropized environments in the municipality of Cristalina, Goiás, Brazil. A fungal isolate belonging to the genus *Trichoderma*, obtained from areas near waterfalls, was selected. Based on this isolate, seed inoculation and substrate application assays were conducted to evaluate germination potential and plant development. Treatments were tested at concentrations of 2.0×10^8 and 4.5×10^9 CFU mL⁻¹, including a commercial isolate of *Trichoderma harzianum* as a standard, as well as a control without application. The experiment was conducted in a completely randomized design with four treatments and ten replicates. No statistically significant differences ($p > 0.05$) were observed among treatments for germination and agronomic traits, such as number of leaves, plant height, and shoot fresh mass. Nevertheless, the *Trichoderma* isolate shows potential for future studies under different conditions and crops, aiming at its use as a sustainable agricultural bioinput.

Key-words: inoculants; growth-promoting microorganisms; bioinputs; *L. sativa*

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INTRODUCTION

Lettuce (*Lactuca sativa* L.) is one of the most widely consumed vegetables in Brazil and worldwide, standing out for its high nutritional value, low caloric content, and wide culinary versatility. Its cultivation plays a strategic role in food security and in the national agricultural economy, being grown in nearly all regions of the country (Venzon & Júnior, 2019). National production reaches approximately 680,000 tons per year (IBGE, 2024), with predominance of curly, crisphead, and loose-leaf varieties. However, crop productivity can be severely affected by biotic and abiotic factors, such as adverse climatic conditions, the occurrence of soilborne pathogens, and low soil fertility (Arruda et al., 2025).

In this context, sustainable agricultural practices have gained prominence as alternatives to conventional management, which relies heavily on chemical inputs. Among these practices, the use of beneficial microorganisms has emerged as a promising strategy to increase crop productivity and resilience while reducing environmental impacts and production costs

(Vásconez et al., 2020). These microorganisms, including bacteria, fungi, and actinomycetes, establish beneficial interactions with plants, contributing to nutrient uptake, biological nitrogen fixation, pathogen suppression, and the stimulation of plant defense systems (Rezende et al., 2021).

In recent years, microbial inoculants have attracted increasing interest in agriculture. These microorganisms act through different mechanisms, such as phytohormone production, phosphate solubilization, and the release of antimicrobial compounds, resulting in improved root development, enhanced nutrient uptake, and increased plant biomass (Gomes et al., 2016). In lettuce, the use of plant growth-promoting rhizobacteria can contribute to the suppression of diseases caused by soilborne pathogens, such as *Pythium* spp. and *Rhizoctonia solani*, as well as to improved plant establishment through the production of auxins, cytokinins, and other bioactive substances (Venceslau et al., 2021).

Despite advances in the use of bioinputs in agriculture, there is still a lack of scientific information regarding the application of beneficial microorganisms

in leafy vegetables, particularly lettuce. Most studies have focused on major crops such as soybean, maize, and wheat, with limited exploration of microbial diversity associated with horticultural species. This knowledge gap highlights the importance of developing research focused on the bioprospecting of new strains adapted to specific edaphoclimatic and physiological conditions, aiming to better understand plant-microorganism interactions and to select isolates with high agronomic potential (Silva, 2022).

In this context, the bioprospecting of beneficial microorganisms emerges as a strategic tool for sustainable agriculture, enabling the identification and use of organisms capable of promoting plant growth and improving the efficiency of production systems (Morandi et al. 2024). The exploration of new microbial strains represents a significant step toward biotechnological solutions that combine productivity and sustainability, especially in crops of socioeconomic importance such as lettuce (Melo et al. 2024). Therefore, this study aimed to carry out the bioprospecting of microorganisms and evaluate their efficiency in seed inoculation and in the development of curly lettuce var. *Crispa*.

MATERIALS AND METHODS

The study was conducted under laboratory and greenhouse conditions at the Instituto Federal Goiano, Campus Cristalina (16°20' S, 46°48' W), from August 2024 to August 2025. Soil and plant root samples were collected from anthropized areas in the municipality of Cristalina, GO, with the aim of bioprospecting efficient microorganisms, which were subsequently evaluated for their ability to promote lettuce germination and development.

Soil and root sampling

To select microorganisms of agricultural interest, four sampling campaigns were carried out between May and November 2024 in anthropized soils and plant roots. Soil samples were classified as Red Latosol and Fluvic Cambisol, collected from agricultural areas and environments near waterfalls, respectively, in Cristalina, Goiás, Brazil. Root samples were collected from species such as pitaya (*Hylocereus* spp.), horseweed (*Conyza* spp.), and orchids (*Phalaenopsis* spp.).

All samples were placed in sterile plastic bags and transported to the Bioinputs Transfer Unit Laboratory at IF Goiano - Cristalina Campus, where they were processed for isolation and subsequent characterization of beneficial microorganisms.

Isolation of microorganisms

The isolation of microorganisms present in soil samples was performed according to the method described by Clark (1965). Briefly, 25 g of soil were added to 225 mL of sterile distilled water to obtain an initial suspension. Serial dilutions were then prepared at a 1:10 ratio up to 10^{-6} . Aliquots of 1 mL from each dilution were inoculated into ten Petri dishes containing Nutrient Agar (NA) and Potato Dextrose Agar (PDA) culture media. The plates were incubated in a BOD incubator at 30°C for bacteria and 28°C for fungi, for up to 10 days (Figure 2).

The isolation of microorganisms associated with plant roots was carried out after surface cleaning and fragmentation of root tissues, which were placed onto Petri dishes containing PDA and Tryptic Soy Agar (TSA) media. This procedure aimed to promote the growth of biofilm-forming microorganisms on the root surface. The plates were incubated under the same temperature and time conditions used for the soil samples (Figure 1).

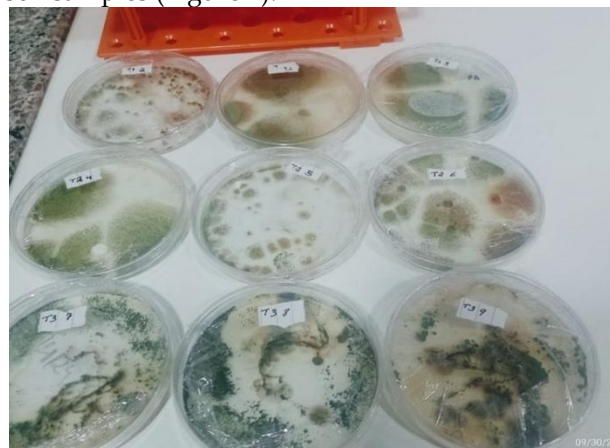


Figure 1. Microorganisms isolated from soil and plant root samples collected in anthropized environments.

After the incubation period, colonies were screened based on their morphological characteristics (color, shape, elevation, margin, and texture). Selected colonies were purified and subcultured onto Potato Dextrose Agar (PDA) and incubated at 29 °C for seven days. Subsequently, the plates were stored under refrigeration at 4 °C until the identification and characterization. All procedures were carried out in triplicate to ensure the reproducibility and reliability of the results.

Identification

The identification of selected microorganisms was performed through morphological analysis of structures observed under a light microscope. For bacteria, Gram staining was used to differentiate between Gram-positive and Gram-negative groups based on cell wall characteristics.

Filamentous fungi were isolated and identified using the microculture technique, which allows the observation of the arrangement and morphology of reproductive structures, including spore production and organization (Figure 2). This method enabled the preliminary characterization of isolates based on their morphological and structural features.

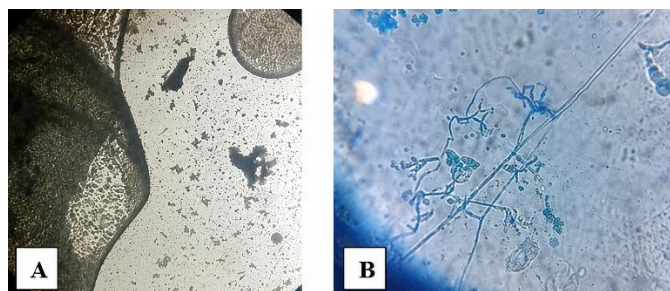


Figure 2. Morphological structures of filamentous fungi (spores and mycelia) (A). Morphological characteristics of the fungus belonging to the genus *Trichoderma* (B).

Multiplication of *Trichoderma* sp.

The fungal isolate belonging to the genus *Trichoderma*, obtained during the bioprospecting process, was multiplied on rice grains according to the methodology described by Aguilera et al. (2020), aiming to produce inoculum for germination and lettuce development assays. After the fungal growth period, the colonized rice was ground, and conidial viability was determined, showing 92% viability, which indicates a high germination capacity and potential for application in subsequent experiments.

Lettuce germination and development after seed treatment

In this assay, different doses of *Trichoderma* spp. were tested for their effects on seed germination and agronomic traits of curly lettuce (*Lactuca sativa*) var. Crispa. For inoculation, 100 g of lettuce seeds were previously disinfected with 98.2% ethanol, 2% sodium hypochlorite, and sterile distilled water for 1 minute. Subsequently, the seeds were immersed for five minutes in 100 mL of suspensions containing 5 and 10 g of *Trichoderma* sp., at concentrations of 2.0×10^8 and 4.5×10^9 CFU mL⁻¹.

The fungal conidial concentration was previously determined by counting colony-forming units (CFU) from a fungal suspension prepared in sterile distilled water with 0.05% Tween 80. As a standard for comparison, a commercial isolate of *Trichoderma harzianum* was tested at a concentration of 1.0×10^8 CFU mL⁻¹ at a dose of 10 g. In the control treatment, no seed inoculation was performed.

After inoculation and a drying period of 30 minutes, ten treated seeds were sown in 500 mL plastic pots containing 100 g of commercial substrate (Carolina

Soil Ltda.). The pots were maintained under greenhouse conditions and irrigated daily. Twelve days after sowing, evaluations of seedling germination percentage were initiated, as well as the following agronomic traits throughout the crop cycle: shoot fresh mass (FM), number of leaves (NL), plant height (PH), and stem diameter (SD).

To assess agronomic traits, seedlings were thinned to maintain only one plant per pot. The experiment followed a completely randomized design with four treatments and ten replicates. Each experimental unit consisted of one pot containing a single lettuce plant.

Soil application

In this assay, the same doses and concentrations of *Trichoderma* isolates used in the seed inoculation test were applied via substrate to evaluate lettuce germination and development of *L. sativa* var. Crispa. For this purpose, fungal suspensions were applied directly to the substrate after seed sowing, using a volume of 10 mL per pot. Two applications were performed: the first immediately after sowing and the second 15 days after planting. Plants received the same management practices adopted in the previous assay, and the same variables were evaluated.

The experimental design was completely randomized (CRD), with ten replicates per treatment. Each experimental unit consisted of one pot containing a single lettuce plant. The data obtained were subjected to analysis of variance (ANOVA), and when significant differences were detected by the F-test, treatment means were compared using Tukey's test at the 5% probability level. Statistical analyses were performed using SISVAR software (Ferreira, 2011).

RESULTS AND DISCUSSION

Bioprospected microorganisms

During the bioprospecting stages conducted in different environments in the region of Cristalina, Goiás, Brazil, a fungal isolate belonging to the genus *Trichoderma* was obtained from soil samples collected in humid environments near waterfalls. The isolate exhibited rapid mycelial growth, intense green coloration, and high conidial production. According to Park et al. (2005), these characteristics are classical morphological markers of the genus, associated with its high capacity for colonization and sporulation. Similarly, Kamala et al. (2015) also reported this pattern for *Trichoderma* isolates, corroborating the findings of the present study and supporting the identification based on morphological traits.

The natural occurrence of *Trichoderma* in humid and anthropized environments, such as waterfall

margins and agricultural soils, has been reported in several studies (Schuster & Schmoll, 2010, Oliveira et al. 2022, Alencar et al. 2023), indicating that these environments may act as natural reservoirs of strains adapted to variable environmental conditions and potentially beneficial to plant development.

On the other hand, no bacterial strains with plant growth-promoting characteristics were identified in the analyzed samples. This absence may be related to high microbial competition in the environment or to soil physicochemical conditions that may have favored fungal development over bacterial populations. Studies such as those by Compant et al. (2005) and Silva et al. (2022) also report that the recovery of plant growth-promoting rhizobacteria may be influenced by edaphic, seasonal, and methodological factors, reinforcing the need to expand isolation and screening strategies in future microbial prospecting studies.

Germination of lettuce seeds inoculated with *Trichoderma*

In the evaluation of germination of seeds inoculated with the bioprospected *Trichoderma* sp.

isolate and the commercial *T. harzianum* isolate, no statistically significant differences were observed among treatments (Table 1). Similarly, seed inoculation with different doses of the isolates did not significantly affect the initial agronomic traits of the plants, including mean number of leaves, plant height, and shoot fresh mass (Table 2).

Table 1. Germination percentage of curly lettuce (*L. Sativa*) var. Crispa seeds after inoculation with different doses of *Trichoderma* spp. isolates

Treatments	Germination (%) ¹
Control	10.91 a
Seed treatment with 10 g of commercial <i>Trichoderma</i>	11.95 a
Seed treatment with 5 g of <i>Trichoderma</i>	17.14 a
Seed treatment with 10 g of <i>Trichoderma</i>	12.85 a
CV (%)	19.49

¹ Means followed by the same letter do not differ statistically according to Tukey's test at 5% probability.

Table 2. Agronomic traits of curly lettuce (*L. sativa*) var. Crispa plants after seed inoculation with different doses of *Trichoderma* spp. Isolates

Treatments	Number of leaves ¹	Plant height (cm) ¹	Fresh mass (g) ¹
Control	9.30 a	7.00 a	14.28 a
Seed treatment with 10 g of commercial <i>Trichoderma</i>	8.10 a	6.05 a	8.71 a
Seed treatment with 5 g of <i>Trichoderma</i>	7.80 a	5.95 a	11.16 a
Seed treatment with 10 g of <i>Trichoderma</i>	6.20 a	6.10 a	9.56 a
CV (%)	27.7	19.10	20.19

¹ Means followed by the same letter do not differ statistically according to Tukey's test at 5% probability

These findings indicate that, under the experimental conditions adopted, seed inoculation with *Trichoderma* did not promote changes in the initial growth of lettuce. These results are consistent with those reported by Meyer et al. (2019) and Ribeiro et al. (2023), who also did not observe significant effects of seed inoculation with *Trichoderma* during the early stages of plant development, suggesting that the benefits of this microorganism may not be expressed during the initial phases of the crop cycle. In contrast, Gutiérrez-Chávez et al. (2025) demonstrated that the positive effects of *Trichoderma* tend to be more pronounced in later stages of lettuce development, resulting in increased biomass and plant growth.

The absence of effects observed in the initial assays may also be associated with the application method and inoculum dose, as studies have shown that the effectiveness of *Trichoderma* depends on the number of viable conidia in contact with the radicle and the time required for substrate colonization (Bashan et al. 2010, Compant et al. 2005). In some cases,

native isolates require adjustments in formulation or specific substrate conditions to express their growth-promoting potential (Druzhinina et al. 2011, Alencar et al. 2023).

The literature indicates that the growth-promoting effects of *Trichoderma* are often expressed at more advanced stages of the crop cycle or under biotic or abiotic stress conditions, when the fungus can stimulate root growth, enhance nutrient uptake, and provide protection against pathogens (Compant et al. 2005, Chagas et al. 2017, Meyer et al. 2019).

The results obtained in the present study are consistent with those reported by Santos Neto et al. (2024), who also did not observe significant differences when testing different doses of *Trichoderma asperellum* (0, 10, 50, 100, and 200 mg L⁻¹ of substrate) on the emergence of curly lettuce seedlings (cv. Emilia). Similarly, Ethur et al. (2006), using a formulation based on *Trichoderma* spp., found that the use of this microorganism did not influence the emergence of forage radish seedlings.

Substrate Application

In the assays in which *Trichoderma* isolates were applied via substrate, no significant differences were observed among treatments regarding seedling germination, shoot fresh mass, number of leaves, plant height, and stem diameter of lettuce plants (Tables 3 and 4).

The absence of significant effects observed in the substrate application assay may be related to the specific interaction among the fungal strain, the host plant, and the environmental conditions of the experiment. Studies indicate that the beneficial effects of *Trichoderma* are more evident under biotic or abiotic stress conditions, or at more advanced stages of plant development, when greater root system colonization occurs and competition with other soil microorganisms

is intensified (Meyer et al., 2019; Amira et al., 2021).

Table 3. Germination percentage of curly lettuce (*Lactuca sativa*) var. Crispa seeds as affected by substrate application of different doses of *Trichoderma* spp. isolates.

Treatments	Germination (%) ¹
Control	10.14 a
Substrate application with 10 g of commercial <i>Trichoderma</i>	12.85 a
Substrate application with 5 g of <i>Trichoderma</i>	8.57 a
Substrate application with 10 g of <i>Trichoderma</i>	4.28 a
CV (%)	9.49

¹ Means followed by the same letter do not differ statistically according to Tukey's test at 5% probability.

Table 4. Agronomic traits of lettuce plants as affected by soil application of different doses of *Trichoderma* isolates.

Treatments	Number of leaves ¹	Plant height (cm) ¹	Fresh mass (g) ¹
Control	9.30 a	8.55 a	14.28 a
Substrate application with 5 g of <i>Trichoderma</i>	9.50 a	7.95 a	13.89 a
Substrate application with 10 g of <i>Trichoderma</i>	9.20 a	7.00 a	12.61 a
Substrate application with 10 g of commercial <i>Trichoderma</i>	8.20 a	6.80 a	11.72 a
C.V. (%)	17.16	22.89	13.15

¹ Means followed by the same letter do not differ statistically according to Tukey's test at 5% probability.

Silva et al. (2011) and Vinale et al. (2008) reported that *Trichoderma* in vegetable crops exerts more pronounced effects in cultivation systems where there is microbial competition in the soil or the presence of phytopathogenic agents, promoting root growth, increasing nutrient uptake, and enhancing disease resistance.

Therefore, the integrated results from bioprospecting, germination, and soil application assays indicate that, although the *Trichoderma* isolate obtained exhibited promising morphological characteristics and potential for use as a bioinoculant, its effect on germination and early growth of lettuce was not observed under the evaluated experimental conditions, reinforcing the need for further studies under different cultivation conditions and at more advanced stages of plant development.

CONCLUSION

Bioprospecting of microorganisms in different environments of Cristalina, GO, enabled the isolation of a fungal strain belonging to the genus *Trichoderma*, with morphological characteristics compatible with potential agricultural bioinoculants. No bacterial strains with plant growth-promoting properties were identified in the analyzed samples.

Seed inoculation and substrate application assays demonstrated that, under the adopted experimental

conditions, *Trichoderma* isolates, both bioprospected and commercial, did not promote significant effects on lettuce germination and development, including number of leaves, plant height, and shoot fresh mass.

Despite the absence of statistically significant differences in the evaluated parameters, the bioprospected *Trichoderma* isolate shows relevant potential for application in other crops, especially under conditions in which its growth-promoting or biocontrol effects may be more pronounced. Therefore, further investigations are recommended, including evaluations in different plant species, at more advanced developmental stages, under stress conditions, and involving different inoculant formulation strategies, aiming to consolidate its agronomic potential.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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