Original Research Article

Response of lettuce cultivars to inoculation with *Trichoderma* spp.

ABSTRACT

Fungi of the *Trichoderma* *genus* are present in practically all type of soil and they have the ability to establish a beneficial relationship with plants. In addition to acting as direct biological control agents, they also act as plant growth promoters, in an indirect biological control mechanism way. Because of these, many of products containing *Trichoderma* strains are used to improve the seeds health, providing better development of roots and aerial parts of plants. In view of this fact, a research work was carried out in green house with the aim of evaluating the effect of five *Trichoderma* strains, belonging to the species *T. virens, T. asperellum, T. asperelloides,* and *T. koningiopsis*, in three new crisp lettuce cultivars (BRS Lélia, BRS Leila, and BRS Mediterrânea). A conidial suspension of each of the strains were prepared (1,0 x 10^{7} conidia / mL^{-1}) and applied at the time of sowing the lettuce in pots. The experiment was completely randomized in a factorial 5x3 design (*Trichoderma* spp. x cultivars). Controls treatment consisted of pots containing plants without any of the fungi. All *Trichoderma* strains applied increased fresh mass and length of root, fresh mass of aerial part and lettuce height in comparison to the controls treated just with water. The cultivar BRS Leila showed an increase of 44, 45%, 15% and 23.94% in the variables: fresh root weight, fresh aerial part weight, plant height and root length, with *T. virens*, *T. koningiopsis* and *Trichoderma* sp., respectively. For 'BRS Lélia' the highest values of fresh root weight, fresh weight of aerial part and root length were 30%, 36.71% and 13.33% achieved with *T. asperellum*. *Trichoderma asperelloides* provided 13.72% more in the height than the control. The 'BRS Mediterrânea' showed increments of 75% of fresh root weight, 78.45% of fresh weight of aerial part, 44.37% of height with *T. virens,* and 40.61% of root length with *T. asperelloides*. The strain *T. virens* performed better within all the analyzed variables. The BRS Mediterrânea cultivar showed the best response to inoculations of *Trichoderma* strains, while 'BRS Lélia' showed the lowest results under the conditions tested.

Keywords: Biological control, lettuce genotypes, antagonistic fungi, plant growth promoting, Lactuca sativa, microbiolization.

Comment [L1]: Not in italic

Comment [L2]: greenhouse

Comment [L3]: you refer 4 different increase values for four characteristics, related with three different species of Trichoderma genus – something is missing or not well explained

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1. INTRODUCTION

Lettuce (*Lactuca sativa* L.) is among the most cultivated and consumed leafy vegetables worldwide. Its short cycle allows planting throughout the year in different production and cultivation systems [1,2,3]. However, some aspects influence the development of this vegetable species, from the establishment of the culture to the harvest. For example, good germination generates vigorous seedlings for transplanting and will have an effect on the volume and quality of the product to be marketed. Thus, the proper treatment of seeds before being sown in the substrate is of great importance [4].

Among the products available for use in seed treatment there are several biofungicides and biostimulators based on *Trichoderma*. Species of this important genus contains more than 300 accepted species. Besides, the fungi are considered cosmopolitan inhabiting different ecological niches and they are able to colonize practically all type of soil [5,6]. Several *Trichoderma* species perform antagonism against phytopathogenic agents, especially bacteria and fungi. Its various mechanisms of action have been widely studied in recent decades, supporting the development and use of these products with relative success among food producers on farms [7]. Plant growth promotion is an indirect biological control mechanism, by which the action of these fungi improves germination and seedlings health in lettuce by various paths. It promotes the development of roots, aerial parts and makes plants more resistant to invasion by phytopathogens [8].

Numerous research papers report positive effects of *Trichoderma* application as a plant growth promoter in different plant species cultivated [9,10,11,12]. Strains of this fungus are able to establish interactions with plant roots, enabling an increase in quality and quantity of biomass, through its different mechanisms at the same time. Among these are the synthesis of growth-stimulating substances, such as phytohormones and the ability to solubilize nutrients present on soil and make them available to be absorbed by the roots of plants, while reducing costs on mineral and organic fertilizers [5]. Given the above, the purpose of work was to analyze the effect of *Trichoderma* spp. applied in the planting furrow, using distinct lettuce cultivars.

In view of this fact, a research work was carried out in green house with the aim of evaluating the effect of five *Trichoderma* strains, belonging to the species *T. virens, T. asperellum, T. asperelloides,* and *T. koningiopsis*, in three new crisp lettuce cultivars (BRS Lélia, BRS Leila, and BRS Mediterrânea).

Comment [L6]: These are 4 identified different species; in your results, and only there you refer also Trichoderma sp.-which species is not identified. So this sentence doesn't seem correct to me.

2. MATERIAL AND METHODS

2.1 Lettuce cultivars and *Trichoderma* strains used

The experiments were conducted under greenhouse conditions with five strains of *Trichoderma* spp. (*Trichoderma* sp., *T. virens*, *T. asperellum*, *T. koningiopsis*, and *T. asperelloides*) and three crisp varietal type lettuce ('BRS Leila', 'BRS Lélia' and 'BRS Mediterrânea'). The fungi strains were multiplied in petri dishes (90 x 15 mm) containing Potato Dextrose Agar (PDA) in a BOD incubator (Nova Técnica®) at 25±1 ºC for seven days. After this period, the spores were collected in sterilized distilled water, by scraping the surface of the medium colonized to obtain the full suspension. Fungal suspensions were homogenized by stirring in a magnetic stirrer (Vortex type). An aliquot of 200 μL of this suspension was used to fill the Neubauer chamber and count the spores in each sample.

Comment [L7]: Petri

2.2 *In vivo* growth promotion tests

Suspensions of *Trichoderma* spp. was evaluation as described in the previous paragraph and spore concentrations adjusted to 1×10^7 conidia per mL^{-1}. The lettuce seeds were disinfected by immersing them in 70% ethanol for 1 minute, followed by a solution 2%

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sodium hypochlorite for 1 minute, then, twice rinsed in a distilled and autoclaved water. Pots with a 1 L capacity were filled with autoclaved substrate (BioPlant Plus®) and three lettuce seeds were sown per pot. Then, the substrate was inoculated with 2 mL of suspension representing each treatment. The pots containing lettuce plants with water were used as control. The pots were randomly distributed on the benches of the greenhouse. The irrigation was daily and was not used any fertilizer. In order to keep one plant per pot, the thinning were conducted after germination of the seeds. The plant height, root length, fresh mass of root and aerial part were checked and measured with a millimeter ruler and weighed a precision scale after 30 days inoculation of *Trichoderma* spp. [11].

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2.3 Statistical analysis

This trial was designed with 15 treatments, consisting of three cultivars, five strains of *Trichoderma* spp. Five repetitions were performed for each treatment. The arrangement adopted was in a 3 x 5 factorial randomly distributed. The values of root and fresh mass of aerial part, plant height, root length were subjected to analysis of variance (ANOVA) and the means were compared by the T ukey test at 5% probability 5%, using the R *software* [13].

3. RESULTS AND DISCUSSION

After 30 days of inoculation, positives differences in root fresh mass, fresh mass of aerial part, plant height and root length were variables according to cultivar, as well as in relation to *Trichoderma* strains. The best performance in terms of fresh root mass in cultivar BRS Leila was verified with *T. virens*, whose average value obtained of 11.97 g meant an increase of 44% in relation to the control (Table 1). This treatment differed from the control, as well as from the treatment with *Trichoderma* sp. For the cultivar BRS Lélia, there was no statistical difference among species used, whose averages ranged from 7.43 to 9.92 g. Also, no differences were observed in relation to the control treatment. As for the cultivar BRS Mediterrânea, once again the treatment with *T. virens* was higher, with a mean value of 13.28 g, although it did not differ statistically from *T. koningiopsis*, whose mean value was 9.94. This last species in turn, did not differ significantly from the other three, *Trichoderma* sp., *T. asperelloides* and *T. asperellum*, achieving averages of 7.26, 7.88 and 8.60 g, respectively of fresh root mass.

Table 1. Fresh root mass (F.R.M) of different lettuce cultivars 30 days after inoculation (DAI) with *Trichoderma* spp.

Strains of *Trichoderma*	F.R.M per cultivar (g)		
	BRS Leila	BRS Lélia	BRS Mediterrânea
Trichoderma sp.	6.80±1.66b	9.15±2.71a	7.26±2.27bc
T. virens	11.97±3.38a	7.43±3.13a	13.28±2.01a
T. asperellum	7.90±1.19ab	9.92±1.77a	8.60±1.33b
T. asperelloides	8.18±2.37ab	8.85±2.03a	7.88±2.99b
T. koningiopsis	8.30±2.23ab	8.53±2.22a	9.94±1.22ab
Control	6.72±2.38b	6.96±3.05a	3.30±1.79c

Means followed by the same letter in the same column do not differ significantly at the 5% level by the Tukey test.

Considering the variable fresh mass of aerial parts of lettuce plants, there was no significant difference among *Trichoderma* strains when tested with the cultivars, except with

'BRS Mediterrânea'. For this cultivar, better performance was observed (20.14 g) when treated with *T. virens*, meaning an increase of 80% compared to the control treatment, also differing from *Trichoderma* sp. The latter did not differ from *T. asperellum, T. asperelloides* and *T. koningiopsis* treatments, nor from the control (Table 2).

Table 2. Fresh mass of aerial part (F.M.A.P) of different lettuce cultivars 30 days after inoculation (DAI) with *Trichoderma* spp.

Strains of *Trichoderma*	F.M.A.P per cultivar (g)		
	BRS Leila	BRS Lélia	BRS Mediterrânea
Trichoderma sp.	11.62±3.46a	11.57±4.80a	11.66±3.33bc
T. virens	13.70±2.02a	9.83±7.63a	20.14±4.66a
T. asperellum	10.78±1.33a	15.17±5.79a	16.30±4.35ab
T. asperelloides	10.90±3.73a	12.58±3.05a	15.14±5.31ab
T. koningiopsis	12.40±1.90a	13.20±2.89a	16.54±3.69ab
Control	7.48±2.52a	9.60±4.54a	4.34±2.26c

Means followed by the same letter in the same column do not differ significantly at the 5% level by the Tukey test.

Regarding the variable height of plants, there was no statistical difference among the treatments with *Trichoderma*, for the three cultivars tested. The cultivar BRS Mediterrânea was the only one for which there was a significant difference in treatments with *Trichoderma* spp. in relation to the control treatment. Nonetheless, the height averages ranged from 14.04 to 16.00 cm, meaning an increase of 36 to 45%, compared to the treatment without *Trichoderma* (Table 3).

Table 3. Plant height (P.H) of different lettuce cultivars at 30 days after inoculation (DAI) with *Trichoderma* spp.

Strains of *Trichoderma*	P.H per cultivar (cm)		
	BRS Leila	BRS Lélia	BRS Mediterrânea
Trichoderma sp.	13.00±0.00a	11.78±2.02a	14.04±1.44a
T. virens	14.00±0.82a	10.28±3.25a	16.00±1.15a
T. asperellum	11.50±1.29a	11.43±1.65a	15.30±1.10a
T. asperelloides	13.00±1.41a	12.75±1.72a	15.30±1.60a
T. koningiopsis	14.25±0.96a	11.83±2.77a	15.06±0.44a
Control	12.10±0.22a	11.00±1.00a	8.90±1.02b

Means followed by the same letter in the same column do not differ significantly at the 5% level by the Tukey test.

With respect to the length of the roots, considering the cultivar BRS Leila, all treatments with *Trichoderma* differed from the control, however not differing from each other. For the cultivar BRS Lélia, *T. asperellum* showed to be statistically different from *Trichoderma* sp., the latter showing a lower mean value (15.25 g). For the cultivar BRS Mediterrânea, the best result was obtained with *T. asperelloides*, which differed statistically from the others, with an average value of 22.06 cm. This result showed 40% more root length compared to the control. *T. asperellum* also showed a 23% increase in root length (17.02 cm) and thus also differed significantly from the control (Table 4).

Table 4. Root length (R.L) of different lettuce cultivars at 30 days after inoculation (DAI) with *Trichoderma* spp.

Strains of *Trichoderma*	R.L per cultivar (cm)		
	BRS Leila	BRS Lélia	BRS Mediterrânea
Trichoderma sp.	17.75±2.75a	15.25±0.76b	16.24±2.15bc
T. virens	15.75±2.22ab	18.03±2.00ab	16.58±1.43bc
T. asperellum	16.50±1.29ab	19.50±3.27a	17.02±1.15b

T. asperelloides	15.75±2.22ab	17.33±0.52ab	22.06±2.12a
T. koningiopsis	15.75±1.50ab	17.00±1.26ab	15.46±1.34bc
Control	13.50±1.94b	16.90±3.13ab	13.10±3.25c

Means followed by the same letter in the same column do not differ significantly at the 5% level by the Tukey test.

Plant growth promotion by fungi of the *Trichoderma* genus can be highly variable due to several factors including type of crop, growing conditions, inoculum rate and type of formulation. Some of these products are formulations with mixtures of strains of the same species and, in other cases, with mixtures of different species. This attests that there is a growing search for more efficient products, both in terms of controlling plant diseases and in terms of promoting plant growth by these fungi.

In this present study, there was a higher fresh root mass in two of cultivars tested, BRS Leila and BRS Mediterrânea. The fitness of *Trichoderma* spp. in promoting plant root development has been reported with other plant species including vegetables such as cucumber, strawberry, tomato, pepper, cabbage and beet [14,15,16,17,18). Yedidia et al. [14] suggest that this effect of greater growth of roots inoculated with *Trichoderma* is due to the colonization of the fungus in the rhizosphere, providing a positive effect on the mycorrhizal interaction with plants. Roots much more developed allows plants to explore a greater volume of soil and consequently, absorb a higher amount of available nutrients. Within all the cultivars evaluated, only the BRS Mediterrânea showed a significant increase in fresh mass of aerial part compared to the control. This positive effect of *Trichoderma* species is very important for increasing lettuce productivity, as the final commercialized product is leaves. The greater beautiful and healthy are the lettuces for consumers, greater will be the profits to producers. Pereira et al. [19] reached divergent results with lettuce plants using different strains of *T. harzianum* and *T. asperellum*, where the gain of fresh mass of aerial part was 40% superior from the control. Steffen et al. [20] evaluated the potential of two non-commercial strains of *Trichoderma asperelloides* and *T. virens* for their ability to increase cabbage yield under field conditions and verified an increase of 36.65% and 47.97% in leaf fresh mass commercialized in the first harvest, demonstrating the potential of this fungus in other vegetables.

Comment [L10]: I don't see any divergent results comparing the two sentences; may be the only difference is that they found it in each of the cultivars tested; I suppose it has to be better explained

The best plant height performance obtained with BRS Mediterrânea cultivar is close to the values verified with the Regina cultivar in the study conducted by Silva et al. [21], which they reached an increase of 34% in relation to the control without inoculation of *Trichoderma*. The ability of *Trichoderma* to promote plant height gains has been attributed to the production of phytohormones and greater efficiency in the use of nutrients [10]. The use of these fungi in crop production is an interesting biotechnological tool to increase productivity. Recent discoveries reinforce the idea that some biological control agents can have several positives effects on plants, in addition to disease control. This effect includes the stimulation of plant growth, increased yield, greater bioavailability and nutrient absorption, as well as improving the quality of the commercialized products as results of a sustainable production [7,22,23,24].

The data of the promotion of root length on lettuce plants obtained here are in accordance with Silva et al. [21]. These authors suggested that *Trichoderma* spp. act as root growth promoters as well. According to Altomare et al. [25], there is a balance of nutrients in the soil that are influenced by the microflora, directly affecting their absorption by plant roots. These authors postulate that plant growth promoted by *Trichoderma* may result from the ability of this fungus to provide essential nutrients for plant healthy development.

Several studies have been conducted with species of *Trichoderma* to affirm the relation of it inoculation in increase vegetative growth and productivity. It was shown that the growth promotion by *Trichoderma* species was dependent on their ability to colonization on plants roots [26]. When *T. brevicrassum* TC967 colonized the surface of the cucumber roots, cucumber growth was promoted [27]. Ousley et al. [28] observed that some *Trichoderma* strains inhibited lettuce seed germination but also promoted plant growth, which may also depend on the strain, method of preparation and application of the inoculum.

Comment [L11]: its

Comment [L12]: to colonize plant roots

The potential of *Trichoderma* spp. in plant growth promoting as in the case studied on this work, it may be related to the increase in the synthesis of plant hormones such as auxins and ethylene [29]. Auxins are important in plant development and are associated with vital plant functions such as cell division, multiplication and elongation. Ethylene on the other hand, can reorganize the cell wall microfibrils. This reorganization reduces height growth and provides greater radial growth of plant tissues making them more vigorous [30].

Many products containing *Trichoderma* have been commercially available since the ascension of the biological control in modern agriculture. This event provided a good opportunity for farmers to known all the application benefits of these fungi on their crops. The role of plant growth promoting microorganisms for agriculture, biotechnology and nanotechnology that exploits actinomycetes, bacteria, fungi and cyanobacteria as such, mainly include their applications in the possibility of producing vegetables without the use of chemical fertilizer and phytosanitary products. Combining all their natural multidimensional attributions on microbiology, based on the ability of *Trichoderma* inoculation to increase plant growth and stimulate plant defense mechanisms, research is exploring its effectiveness in controlling soil-transmitted fungal and bacterial diseases.

The results achieved in this study demonstrated the importance of the diversity of *Trichoderma* benefits and showed a positive contribution to BRS Leila, BRS Lélia and BRS Mediterrânea cultivars. The lettuce growth promotion under greenhouse conditions after 30 days of inoculation the strains were great. The growth promotion resulting from the strains tested here, needs further investigation for an application in growth promotion on seedlings as well. These results should further contribute to the knowledge of plant nutrition, biological control of plant diseases, through direct and indirect mechanisms of *Trichoderma*.

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Comment [L14]: The resulting growth promotion of the strains tested here needs further investigations for application in growth promotion also in seedlings.

4. CONCLUSION

1. *Trichoderma virens* was the one that most contributed to the increment of the analyzed variables.

2. Among the cultivars tested, BRS Mediterrânea was the one that best responded to the inoculation of *Trichoderma* spp.

3. Among the tested cultivars, BRS Lélia was the least responsive to *Trichoderma* spp. inoculation, but showed increases in all analyzed variables.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the

advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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