Original Research Article

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

ABSTRACT

Fungi of the *Trichoderma* genus are present in practically all types 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, by an indirect biological control mechanism. Because of these, many products containing *Trichoderma* strains are used to improve seed health, providing better development of roots and aerial parts of plants. In view of this fact, research work was carried out in a greenhouse 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 was 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). Control treatments 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% in fresh root mass, 45% in fresh mass of aerial part, with *T. virens*; 15% in plant height with *T. koningiopsis*, and 23.94% in root length, with *Trichoderma* sp. For 'BRS Lélia' the highest values of fresh root mass, fresh mass of aerial part and root length were 30%, 36.71%, and 13.33% with *T. asperellum*. *Trichoderma asperelloides* provided 13.72% increase in height compared to the control. 'BRS Mediterrânea' showed increments of 75% of fresh root mass, 78.45% of fresh mass of aerial part, and 44.37% of height with *T. virens.* With *T. asperelloides*, 40.61% was observed in root length. The strain *T. virens* performed better in all the analyzed variables, except for root length.

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

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*. This important genus contains more than 300 accepted species. Besides, these fungi are considered cosmopolitan, inhabiting different ecological niches, and they are able to colonize practically all types of soil [5,6]. Several *Trichoderma* species perform antagonism against phytopathogenic agents, especially bacteria and fungi. Their 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 seedling health in lettuce by various paths. They promote the development of roots and aerial parts and make plants more resistant to invasion by phytopathogens [8].

Numerous research papers report positive effects of *Trichoderma* application as a plant growth promoter in different cultivated plant species [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 different mechanisms at the same time. Among these mechanisms are the synthesis of growth-stimulating substances, such as phytohormones and the ability to solubilize nutrients present in soil and make them available to be absorbed by the roots of plants, while reducing costs of 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 this context, a study was carried out in the greenhouse 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 addition to a strain unidentified at the species level, in three new crisp lettuce cultivars.

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 types of lettuce ('BRS Leila', 'BRS Lélia' and 'BRS Mediterrânea'). The strains used belong to the Collection of Biological Control Agents at Embrapa Genetic Resources and Biotechnology, Brasilia, Federal District, Brazil. They 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 colonized medium 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.

2.2 *In vivo* growth promotion tests

Suspensions of *Trichoderma* spp. were evaluated 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 2% sodium hypochlorite solution for 1 minute, then were rinsed twice in 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. Irrigation was daily and no fertilizer was used. In order to keep one plant per pot, thinning was 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 on a precision balance 30 days after inoculation of *Trichoderma* spp. [11].

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 a 3 x 5 factorial randomly distributed. The values of root and fresh mass of aerial part, plant height and root length were subjected to analysis of variance (ANOVA) and the means were compared by the Tukey test ($P \leq 0.05$), using the R *software* [13].

3. RESULTS AND DISCUSSION

Thirty days after inoculation, positive differences in root fresh mass, fresh mass of aerial part, plant height and root length varied 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 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, for which the 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) for 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 amount *Trichoderma* strains when tested with the cultivars, except with 'BRS Mediterrânea'. For this cultivar, a 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 amount 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, although they did not differ from each other. For the cultivar BRS Lélia, *T. asperellum* showed to be statistically different from *Trichoderma* sp., the latter presenting 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.

The effect of *Trichoderma* spp. on plant development can be observed in seed germination, rooting, sprouting of cuttings, growth of branches, increase in leaf area, delay in senescence, accumulation of organic matter (fresh and dry mass) and increase in crop yield [12, 14]. These effects are highly variable, due to a number of factors, including crop type, growing conditions, inoculum rate, and formulation type. Ozdemir et al. [15] showed that the application of *T. harzianum* in lettuce plants promotes higher chlorophyll content in the leaves, a photosynthetic pigment that is directly related to the production of plant biomass. This attests to the growing interest in the study of *Trichoderma* spp. both in the control of plant diseases and in the promotion of plant growth by these fungi.

In this present study, there was a higher fresh root mass in two of the 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 [16,17,18,19,20). Yedidia et al. [16] suggest that this effect of greater root growth in plants inoculated with *Trichoderma* is due to the colonization of the fungus in the rhizosphere, providing a positive effect on the mycorrhizal interaction with plants. More highly developed roots allow plants to explore a greater volume of soil and consequently absorb a higher amount of available nutrients. Among all the cultivars evaluated, only BRS Mediterrânea showed a significant increase in fresh mass of the aerial part compared to the control. This positive effect of *Trichoderma* species is very important for increasing lettuce yield, as the final commercialized product is its leaves. The better the appearance of the harvested product, the greater the profits for producers. Pereira et al. [14] obtained an increase in fresh mass with *T. harzianum* and *T. asperellum* strains, where the gain of fresh mass of the aerial part was approximately 40% higher than the control. Steffen et al. [21] 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.

The best plant height performance obtained with the BRS Mediterrânea cultivar is close to the values verified with the Regina cultivar in the study conducted by Silva et al. [22], in 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 positive 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 a result of sustainable production [7,23,24,25].

The data obtained here regarding the promotion of root length in lettuce plants are in accordance with Silva et al. [22]. These authors suggested that *Trichoderma* spp. act as root growth promoters as well. According to Altomare et al. [26], there is a balance of nutrients in the soil that is influenced by the microflora, directly affecting nutrient 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 the plant`s healthy development.

Several studies have been conducted with species of *Trichoderma* to verify the relationship of its inoculation with increased vegetative growth and productivity. It was shown that growth promotion by *Trichoderma* species was dependent on their ability to colonize plant roots [27]. When *T. brevicrassum* TC967 colonized the surface of the cucumber roots, cucumber growth was promoted [28]. Ousley et al. [29] 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.

The potential of *Trichoderma* spp. in plant growth promotion, as in the case studied in this work, may be related to the increase in the synthesis of plant hormones such as auxins and ethylene [30]. 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 [31].

Many products containing *Trichoderma* have been commercially available since the rise of biological control in modern agriculture. Their availability provided an opportunity for farmers become familiar with the benefits of applying these fungi on their crops. Plant growth promotion microorganisms for agriculture, biotechnology and nanotechnology exploit actinomycetes, bacteria, fungi and cyanobacteria, and their usage makes it possible to produce vegetables without chemical fertilizers and phytosanitary products. Combining all their natural multidimensional attributions in microbiology, based on the ability of *Trichoderma* inoculation to increase plant growth and stimulate plant defense mechanisms, researchers are continuing to explore these species` effectiveness in controlling soil-transmitted fungal and bacterial diseases.

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

4. CONCLUSION

1. *Trichoderma virens* was the species that most contributed to an increment in 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 used 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. The research was not funded by the producing company; instead, it was funded by personal efforts of the authors.

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