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Vermicompost and trichoderma in the development of cherry group tomato seedlings

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ABSTRACT

Trichoderma is used as crop growth promoters. However, they may have harmful effects at higher concentrations. The objective of this work was to evaluate the vermicompost effect formulated with different concentrations of sandy soil, bioenriched with higher than recommended doses of trichoderma, on the germination, initial development and chemical composition of the cherry tomato Creole shoot. The treatments (T) used were substrate composed of vermicompost and sandy soil at 25 kg kg-1 (low), 50 kg kg-1 (medium) and 75 kg kg-1 (high), respectively. The Ecotrich bioproduct was added to the substrate at the following concentrations T1) 0.0; T2) 108; T3) 109; T4) 1010 cfu L-1. Afterwards, three cherry tomato seeds were sown per cell in trays containing the mixture, totaling 90 seeds per treatment. At 30 DAS, height, stem diameter, chlorophyll a, b and total, fresh and dry root and shoot phytomass, root volume and area, leaf area and leaf chemical analysis were evaluated. Bioproduct concentrations, compared to control without trichoderma, did not provide superior results for shoots and roots at 30 DAS. However, plants cultivated with higher concentration of vermicompost were less negatively affected at higher doses of the bioproduct.

Keywords: Solanum lycopersicum; Trichoderma harzianum; Organic substrate

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1 INTRODUCTION

Bioinoculation of vermicompost with trichoderma has been proposed as a substrate enrichment alternative by several researchers. From this association there is a significant increase in mustard seed production compared to plants grown in medium consisting of the recommended dose of nitrogen, phosphorus and potassium (HAQUE et al., 2010). This interaction also allowed to verify superior responses for the parameters height, dry biomass and root length of tomato plants when compared to uninoculated control and treatments with bacterias *Pseudomonas fluorences* and *Bacillus subtilis*, which proves the superior efficiency of trichoderma and good interation with vermicompost in relation those bioagents (BASCO et al., 2017).

In fact, the effects of trichoderma on plants are influenced by the characteristics of the medium where it will be applied (Hajieghrari, 2010; Marín-Guirao, 2016), and the presence or absence of organic material in the environment may be fundamental for the beneficial interaction between plant and microorganism. While the presence of organic matter in association with trichoderma proved to be more efficient for soil microbiome maintenance and tomato seedling growth in relation to chemical fertilizer treatments (Pang et al., 2017), nutrient scarcity in substrates with low organic content can cause interspecific competition with advantage to trichoderma because it has better nutrient absorption efficiency than plants (Li et al., 2015). Contradictorily, Benítez et al. (2004) state that trichoderma provides better results in plant species grown in poor soils.

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Antibiotics and fungal synthesized plant growth regulators that are reported to be responsible for increasing plant nutrient uptake (Machado et al., 2012; Singh et al., 2016; Wiethan, 2018), plant organ size and crop yield (Vinale et al., 2014) may also cause phytosanitary or physiological damage to plants as a result of phytotoxicity and pathogenicity (Frankenberger & Muhammad, 1995). For example, Hassan et al. (2013) found that trichoderma overdose causes inhibition of *Striga hermonthica* seed germination. The authors relate the negative effect to the fact that trichoderma synthesizes secondary metabolites that may have a phytotoxic effect on certain plant species and at certain concentrations that may become antagonistic. Neumann & Laing (2007) also found that a high dose of trichoderma causes lettuce phytotoxicity due to the ability of the fungus to inhibit ammonium-nitrate cation-converting bacteria in the environment, which caused a reduction in the length of vegetable root meristems (Liu et al., 2013).

On the other hand, vermicomposts are indicated as highly capable of reducing the concentration of various substances in the culture medium (Pettit, 2004; García et al., 2014; Pereira et al., 2014). This is because humic vermicompost fractions are able to adsorb and then stabilize and inactivate cations, toxic metals and herbicides (Pettit, 2004; García et al., 2014). And despite the adsorbent ability of vermicompost, research on the intrinsic relationships between vermicompost and trichoderma and, as stated by Pereira et al. (2014), its application in the retention of organic compounds, such as those synthesized by bioagent, is still little explored.

In view of the above, the objective was to evaluate the vermicompost effect formulated with different concentrations of sandy soil, enriched with higher than recommended doses of trichoderma, on the germination, initial growth and chemical composition of the cherry tomato creole shoot.

2 MATERIALS AND METHODS

The work was carried out in an agricultural greenhouse and in the Tissue Analysis Laboratory of the Soil Department, Plant-Microorganism Interaction and Plant Physiology Laboratories of the Department of Biology of the Federal University of Santa Maria. The manure-based vermicompost was supplied by the Department of Soils, UFSM. As source of the biological agent, the commercial bioproduct Ecotrich WP formulated in powder form based on the fungus Trichoderma harzianum IBLF006 was used, at the concentration 10^{10} cfu g^{-1} of commercial product registered in MAPA under no. 04213 as contact microbiological fungicide, indicated for leaf application for the control of *Sclerotinia sclerotiorum*. In an efficiency test, the company applied 150 g ha^{-1} to lettuce and 200 to 250 g p c ha^{-1} to soybean.

The experimental units were represented by polyethylene germination trays, each composed of five cells. As substrate was used the association of vermicompost produced from cattle manure with soil classified as dystrophic red typical Argisoil, textural class Franco sandy, composed of 60% sand, 30% silt and 10% clay, in concentrations 25. kg kg^{-1} (low), 50 kg kg^{-1} (average) and 75 kg kg^{-1} (high) both previously sieved in 0.5 cm thick mesh. The substrate was inoculated with trichoderma solution at the substrate concentrations of 10^8, 10^9 and 10^{10} cfu L^{-1}, except for the control, where only distilled water was applied. After the substrate was placed in the trays, one day later, three seeds of creole cherry tomato were sown per cell at 0.5 cm depth, totaling 90 seeds per treatment. The experimental units were kept in agricultural greenhouse under controlled conditions of temperature (28 ° C) and humidity (substrate and environment).

To determine the emergence percentage, the number of seedlings emerged up to 12 days after sowing (DAS) was recorded daily, when thinning was performed, leaving one seedling per cell. For purposes of comparison and differentiation of development, at 30 DAS, the height and diameter of the seedling collection were measured with the aid of a ruler and digital caliper, respectively, and the chlorophyll leaf content was determined using the electronic meter ClorofiLOG.

The seedlings were cut at the height of the neck and weighed to determine fresh root and shoot biomass. Then all plants were analyzed with the aid of the Epson XL 10000 professional scanner, using a 600 dpi definition for root morphology and 400 dpi for leaf measurements. The images obtained in three dimensions were analyzed by the WinRHIZO Pro 2013 program, where the characteristics of leaf and root surface area (cm^2) and root characteristics total length (cm), volume (cm^3) and mean diameter (mm) were quantified. The aerial part of the plants were placed in paper envelopes and taken to a drying oven with forced ventilation at a temperature of 65 ° C until they reached constant weight for weighing and determination of dry weight.

To determine the nutrients Ca, Mg, N and K of the shoot, the dry material was ground with the aid of porcelain crucible and pistil and subjected to analysis of plant tissues, as proposed by Tedesco et al. (1995).

Analysis of variance (ANOVA) and means compared by Scott-Knott test ($p \leq 0.05$) were used. Statistical analyzes were performed with the aid of SISVAR 5.6 (FERREIRA,)

3 DISCUSSION

For emergence, height, stem diameter, root and shoot dry phytomass and total chlorophyll of cherry tomato seedlings produced in increasing concentrations of vermicompost inoculated with increasing concentrations of trichoderma, interactions between the factors dose of trichoderma and concentrations of vermicompost (Table 1). There was a tendency of reduction in these characteristics in seedlings submitted to the highest concentrations of the microorganism (109 and 1010 cfu L^{-1}) and cultivated in substrate with low concentration of vermicompost. In the presence of higher vermicompost content the parameters seedling height, stem diameter and chlorophyll a and total were similar to treatments without trichoderma.

The effect of trichoderma on seedling emergence and plant growth seems to be dependent on its concentration in the culture medium. Answers about the amount of trichoderma in horticultural species were evidenced by Singh et al. (2016), after verifying that each crop presented an optimal concentration range of the bioproduct to promote plant development. The authors reported that lower concentrations of 10^3, 10^4 and 10^6 spores mL^{-1} stimulate seed germination and tomato, eggplant, bell pepper, okra, gourd and guar growth, while higher concentrations of 10^8 spores mL^{-1} inhibited seed spores. root length of tomato seedlings and from 10^6 mL^{-1} eggplant spores, concentrations that also reduce the further development of these species in a partially controlled environment.

In evaluating secondary metabolites, Contreras-Cornejo et al. (2009) have suggested that trichoderma may induce plant growth through an auxin-dependent mechanism by finding that fungal biosynthesized indoleacetic acid increases root length in Arapdopsis in certain amounts. On the other hand, exogenous auxins, such as those secreted by trichoderma, may affect the development of highly sensitive tissues, such as those of the root system (Tanimoto, 2005) and young stems (Thimann, 1937). Contradictorily, in the present work, the higher concentration of trichoderma, regardless of the vermicompost content, provided a larger root area in the seedlings, which, according to Björkman (2004), can be explained by the fact that the tomato roots do not have sensitivity to the substances under the conditions established in this work.

Similar results to the present study were found by Wiethan et al. (2018), who observed that all trichoderma concentrations tested in lettuce seedlings grown in the same concentration of vermicompost had a reducing effect on the root and shoot development when compared to the treatment without trichoderma. Higher than recommended doses of 8 and 16 10^{11} conidia kg^{-1} reduced germination, concentration of these nutrients in the leaves and inhibited the development of vegetables compared to other treatments.

The concentration of vermicompost in the growing environment can determine the potential for reducing substances that may be harmful to plant growth (Frankenberger and Muhammad, 1995). Similarly to the present work, data obtained by Marín-Guirao et al. (2016) investigating the effect of trichoderma on the growth of seedlings grown on substrates with different organic material contents, showed pathogenicity in tomato and spinach when the maximum trichoderma concentration tested (10^8 conidia mL^{-1}) was associated with the substrate without organic matter, compared to vegetables grown on substrate rich in organic matter and with lower doses of the biostimulant (10^7 and 10^6 conidia mL^{-1}).

The relationship between the rate of synthesis and retention or biodegradation of trichoderma secondary metabolites determines the effect of bioagent on plant growth (Vinale et al., 2014). However, this process will be dependent on the physical and chemical properties of the compounds to be adsorbed (Pereira et al., 2014). Several works demonstrate the affinity between the two types of substances. Canellas et al. (2002) found exchangeable auxin groups in the macrostructure of vermicompost humic substances after analyzing the vermicompost structure.

Humic substances also influence the enzymes involved in plant growth regulation (Pettit, 2004). Humic acid fractions prevent the destruction of the indolacetic acid oxidase (IAA-oxidase) enzyme system responsible for the degradation of indolacetic acid through a mechanism involving the action of phenolic groups of humic acids and thus phytohormonium continues to stimulate the processes of plant growth (Mato et al., 1971).

On the other hand, these humic substances also bind to indolacetic acid (Muscolo 1999). The adsorption of substances responsible for plant growth by vermicompost is reported by Arancon et al. (2006) and later by Tejada and Benítez (2015), who determine that the binding sites present in vermicompost humic fractions are capable of retain plant hormones and make them gradually available to plants. Auxins, kinetins and gibberellins have also been reported by Kiyasudeen et al. (2016) as phytohormones capable of being retained in vermicompost humatos and fulvates and released so that the biostimulant action persists for a longer period, without negative effects during plant growth.

4 CONCLUSIONS

Seed germination, initial growth and shoot chemical composition of cherry tomato seedlings grown in the presence of higher vermicompost content are less prone to damage caused by bioactive compounds secreted by trichoderma when in high concentrations, as used in this work. probably due to the ability of vermicompost humic substances to reduce the concentration available to plants of bioagent synthesized substances that may have a detrimental effect on plant development.

Table 1 - Emergence, plant height, stem diameter, root area, root dry mass, shoot dry mass, chlorophyll A and total chlorophyll as a function of treatments. Santa Maria – UFSM, 2018

Treatments	Trichoderma concentrations (ufc L^{-1}) 0	10^8	10^9	10^{10}
Vermicompost concentrations ($g.g^{-1}$)	**Number of seedlings emerged** (days)			
25	70,00 Aa[1]	66,66 Aa	35,00 Bb	43,33 Bb
50	61,67 Aa	63,33 Aa	55,00 Ab	58,33 Ab
75	58,33 Aa	73,33 Aa	71,67 Aa	80,00 Aa
CV (%) = 26,57%				
	Plant height (cm)			
25	9,560 Aa	8,596 Aa	7,075 Bb	6,213 Bb
50	9,603 Aa	10,030 Aa	8,392 Ab	10,673 Aa
75	10,627 Aa	11,076 Aa	11,205 Aa	12,057 Aa
CV (%) = 17,40				
	Waistband diameter (cm)			
25	2,114 Ab	2,024 Ab	2,538 Ab	2,354 Ab
50	2,293 Bb	3,328 Aa	2,911 Ab	2,606 Bb
75	3,467 Aa	3,406 Aa	3,329 Aa	3,443 Aa
CV (%) = 12,89				
	Root area (cm^2)			
25	31,69 Ab	34,92 Ab	16,03 Ac	42,14 Aa
50	36,98 Ab	44,34 Ba	35,49 Bb	43,34 Aa
75	38,80 Ab	46,10 Ba	41,75 Cb	49,38 Aa
CV (%) = 7,43				
	Chlorophyll a (ICF)			
25	277 Aa	187 Bb	212 Bb	242 Aa
50	247 Aa	255 Aa	261 Aa	243 Aa
75	249 Aa	267 Aa	263 Aa	264 Aa
CV= 12,5				
	Total V (ICF)			
25	351 Aa	238 Bc	265 Bc	300 Ab
50	314 Aa	317 Aa	321 Aa	321 Aa
75	314 Aa	338 Aa	330 Aa	306 Aa
CV = 12,25				

		Root dry mass (g)		
25	0,023 Ab	0,032 Aa	0,021 Bb	0,022 Bb
50	0,028 Aa	0,026 Aa	0,026 Ba	0,025 Ba
75	0,026 Ab	0,024 Ab	0,035 Aa	0,036 Aa
CV (%) = 12,00				
		Shoot dry mass (g)		
25	0,32 Aa	0,129 Ba	0,104 Ba	0,097 Ba
50	0,14 Ab	0,159 Aa	0,161 Aa	0,176 Aa
75	0,16 Ab	0,198 Aa	0,156 Aa	0,171 Aa
CV (%) = 52,63				

Averages followed by the same lowercase letter in the column and lowercase in the row do not differ by the Scott-Sknott test at the 5% probability level.

Table 2 - Root Volume (VR), Shoot fresh mass (SFM), Leaf Area (LA), nitrogen(N), potassium (k), calcium (Ca) e magnesium (Mg) depending on the treatments. Santa Maria UFSM– RS, 2018

Vermicompost concentration (Kg Kg^{-1})	**RV (cm^3)**	**SFM (g)**	**LA (cm^2)**	**N (g.Kg^{-1})**	**K (g.Kg^{-1})**	**Ca (g.Kg^{-1})**	**Mg (g.Kg^{-1})**
25	0,5 a	1,3 c	6,7 b	30,3 b	33,77 b	4,20 a	30,33 a
50	0,5 a	2,0 b	6,9 b	38, 6 b	40,94 a	3,93 a	31,98 a
75	0,5 a	2,7 a	13,9 a	43,4 a	42,75 a	3,20 a	31,85 a
CV (%)	33,3	22,8	103,3	32,6	16,04	33,15	31,56

Averages followed by the same lowercase letter in the column and uppercase in the row do not differ by the Scott-Sknott test at the 5% probability level.

Table 3 - Mean values of root volume (RV), fresh shoot mass (FSM), leaf area (LA), nitrogen (N), potassium (K), calcium (Ca) and magnesium (Mg). Santa Maria UFSM RS, 2018

Dose de trichoderma (UFC L^{-1})	**RV (cm^3)**	**FSM (g)**	**LA (cm^2)**	**N (g.Kg^{-1})**	**K (g.Kg^{-1})**	**Ca (g.Kg^{-1})**	**Mg (g.Kg^{-1})**
0	0,4177 a	2,2 a	9,0 a	43,22 a	39,52 a	3,48 a	31,56 a
10^8	0,5670 a	2,0 a	10,2 a	38,20 a	36,62 a	4,08 a	31,78 a
10^9	0,4999 a	1,9 a	6,39 a	38,80 a	43,98 a	3, 88 a	31,18 a
10^{10}	0,6219 a	2,0 a	10,91 a	29,50 a	36,51 a	3,68 a	31,05 a
CV(%)	33,25	22,7	103,3	32,60	16,04	33,15	31,56

1 Averages followed by the same lowercase letter in the column do not differ by the Scott-Sknott test at the 5% probability level.

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