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Paula Fernanda de Azevedo
ORCID: https://orcid.org/0000-0003-4327-6800
Universidade Estadual do Norte do Paraná, Brazil
E-mail: paula.fer.azevedo@hotmail.com
Ana Carolina de Almeida
ORCID: https://orcid.org/0000-0003-1048-1496
Universidade Estadual do Norte do Paraná, Brazil
E-mail: ana.almeida40@ufv.br
Rodrigo Domiciano Marques
ORCID: https://orcid.org/0000-0002-5933-9776
Universidade Estadual do Norte do Paraná, Brazil
E-mail: rodrigodmarques592@gmail.com
Christiane Luciana da Costa
ORCID: https://orcid.org/0000-0002-2659-1369
Universidade Estadual do Norte do Paraná, Brazil
E-mail: christiane@uenp.edu.br
Anderson Roberto Benedetti
ORCID: https://orcid.org/0000-0001-6523-3196
Universidade Estadual do Norte do Paraná, Brazil
E-mail: ander_bio@live.com
Luis Eduardo Azevedo Marques Lescano
ORCID: https://orcid.org/0000-0003-0874-9211
Universidade Estadual do Norte do Paraná, Brazil
E-mail: edulescano@yahoo.com.br
Mayra Costa da Cruz Gallo de Carvalho
ORCID: https://orcid.org/0000-0002-1559-3828
Universidade Estadual do Norte do Paraná, Brazil
E-mail: mayra@uenp.edu.br
Leopoldo Sussumu Matsumoto
ORCID: https://orcid.org/0000-0001-5102-545X
Universidade Estadual do Norte do Paraná, Brazil
E-mail: leopoldo@uenp.edu.br

Abstract
Cassava root rot causes significant production losses. Difficulties of management, along with the lack of chemical fungicides officially registered by the Ministry of Agriculture, Livestock and Supply (MAPA), require alternative control methods. This study investigated the *in vitro* antagonistic activity of *Trichoderma harzianum* as well as a biological fertilizer MICROGEO® on *Fusarium solani*. The phytophatogenic strains of *F. solani*, called F1 and F2 were isolated from rotted cassava tubers and *T. harzianum*, strain ESALQ 1306, from a biological fungicide. Continuous liquid composting of bovine ruminal content, water and MICROGEO® produced the biological fertilizer. Dual culture method was used at the bioassay with *T. harzianum*. Sterilized (St) and unsterilized (USt) biological fertilizer were tested in different concentrations (% v/v) diluted in the culture media. Colony diameters were measured daily in order to establish the mycelial growth velocity index, inhibition percentage, aside from the sporulation rate and spore germination percentage. The mycelial growth of *F. solani* isolates was interrupted after hyphae encounter with *T. harzianum*, due to the occurrence of mycoparasitism, but without influence on the sporulation rate. Sterilized biological fertilizer induced no biocontrol, whereas the unsterilized product (concentration 2.5%) inhibited approximately 64% and 85% of the mycelial growth of isolates F1 and F2, respectively. Moreover, spore germination declined with increasing concentration. In conclusion, *T. harzianum* and the unsterilized biofertilizer showed *in vitro* antagonistic activity on *F. solani*.
Keywords: Biological control; Biological fertilizer; Mycoparasitism; Mycelium growth; *Fusarium* root rot.

Resumo
A podridão radicular da mandioca ocasiona perdas relevantes em sua produção. O difícil manejo, aliado à indisponibilidade de fungicidas químicos registrados ao Ministério da Agricultura, Pecuária e Abastecimento (MAPA)

demandam alternativas de controle. O objetivo deste trabalho foi verificar a atividade antagonista *in vitro* de *Trichoderma harzianum* e adubo biológico MICROGEO® sobre *Fusarium solani*. *F. solani* (F1 e F2) foram isolados de raízes de mandioca e *T. harzianum*, linhagem ESALQ 1306, de um fungicida biológico. O adubo biológico foi produzido por compostagem líquida contínua de MICROGEO® com conteúdo ruminal bovino e água. Para o bioensaio com *T. harzianum* utilizou-se o método de cultura pareada. O adubo biológico foi testado esterilizado (E) e não esterilizado (NE) em diferentes concentrações (% v/v) diluídas nos meios de cultura. Avaliou-se o crescimento micelial pela medição do diâmetro das colônias, obtendo-se o índice de velocidade de crescimento micelial (IVCM), a porcentagem de inibição, a taxa de esporulação e porcentagem de germinação dos esporos. O crescimento micelial de *F. solani* em cultivo pareado com *T. harzianum* foi interrompido após o encontro de suas hifas pela ocorrência de micoparasitismo, porém sem interferência na esporulação. O adubo biológico esterilizado não promoveu biocontrole, porém seu efeito não esterilizado à 2,5% inibiu aproximadamente 64% e 85% o crescimento dos fungos F1 e F2, respectivamente. Além disto, observou-se declínio na germinação dos esporos conforme aumento da concentração do produto. Conclui-se que o fungo *T. harzianum* e o adubo biológico não esterilizado demonstraram atividade antagonista *in vitro* sobre *F. solani*.
Palavras-chave: Biocontrole; Biofertilizante; Micoparasitismo; Crescimento micelial; Fusariose.

Resumen
La pudrición de raíces de yuca provoca pérdidas en su producción. El difícil manejo, la indisponibilidad de fungicidas químicos registrados en el Ministerio de Agricultura, Ganadería y Abastecimiento (MAPA), demandan alternativas de control. El objetivo de este trabajo fuer verificar la actividad antagonista *in vitro* de *Trichoderma harzianum* y fertilizante biológico MICROGEO® sobre *Fusarium solani*. *F. solani* (F1 y F2) se obtuvieron de raíces de yuca y *T. harzianum*, cepa ESALQ 1306, de un fungicida biológico. El fertilizante biológico se produjo por compostaje líquido de MICROGEO® con contenido de rumen bovino y agua. Para el bioensayo con *T. harzianum* se utilizó el método de cultivo pareado. El fertilizante biológico se probó esterilizado (E) y no esterilizado (NE) en diferentes concentraciones diluidas en medio de cultivo. El crecimiento micelial se evaluó midiendo el diámetro de las colonias, obteniendo el índice de crecimiento micelial (IVCM), el porcentaje de inhibición, la tasa de esporulación y el porcentaje de germinación de esporas. El crecimiento micelial de *F. solani* se interrumpió después del encuentro de sus hifas con *T. harzianum* por micoparasitismo, pero sin interferencia con la esporulación. El fertilizante biológico esterilizado no promovió el biocontrol, pero su efecto no esterilizado al 2.5% inhibió aproximadamente 64% y 85% del crecimiento de los hongos F1 y F2, respectivamente. Hubo una disminución en la germinación de las esporas con el aumento en la concentración del producto. Se concluye que el hongo *T. harzianum* y el fertilizante biológico no esterilizado demostraron actividad antagonista *in vitro* sobre *F. solani*.
Palavras clave: Biocontrol; Biofertilizante; Micoparasitismo; Crecimiento micelial; Fusariosis.

1. Introduction

The capacity of adaptation to different cultivation conditions and the versatility of uses make cassava (*Manihot esculenta* Crantz) both socially and economically a highly relevant crop. The whole plant can be exploited, although the major interest is focused on the tuberous, starch-rich roots, which are worldwide an energy source in the diet of populations (Boas et al., 2016). From the economic point of view, it is the crop with the world's sixth highest production and Brazil is the fourth largest producer of cassava root (Conab, 2019).

Diseases such as cassava root rot, caused by fungi of the genus *Fusarium*, affect the plant health and are responsible for significant yield losses, hampering sales (Boas et al., 2016). Aside from affecting the quality of the harvested roots, some species of the genus are able to produce mycotoxins, which can be harmful to plants, animals and humans. These plant pathogens also colonize alternative host plants and survive in the soil for long periods under adverse conditions, foiling an effective plant health management (Kim & Vujanovic, 2016).

Although not very sustainable, chemical control by fungicide applications is one of the keys and most constantly applied measures of plant disease control (Kim & Vujanovic, 2016). However, the lack of chemical fungicides registered by the Ministry of Agriculture, Livestock and Food Supply (MAPA) to control this cassava disease requires alternative control methods (Mapa, 2021).

Biological control is an alternative for the use of agrochemicals in the management of agricultural diseases. It involves the use of living organisms, called biocontrol agents, and/or their metabolites against undesirable pathogens, in order

to combat them or reduce their development and harmful effects on plants (Hridya et al., 2013). With regard to the interaction between fungi and/or bacteria with plant pathogenic fungi, e.g., *Fusarium* spp., biocontrol can occur through antibiosis (secretion of inhibitory extracellular metabolites), competition for nutrients and sites of infection and mycoparasitism or hyperparasitism. Other types of interactions that benefits the plant development at the expense of the pathogen are related to the induction of host resistance as well as growth promotion (Silva et al., 2016).

Species of the fungus *Trichoderma* are promising for the biocontrol of plant pathogens. These fast-growing soil microorganisms are suited for development under diverse conditions and have parasite and antagonistic behavior to many soil-borne plant pathogenic fungi, e.g., *Phytophtora* sp., *Fusarium* spp., *Sclerotinia sclerotiorum* and *Rizhoctonia solani*, on different crops. In addition, they have shown to be avirulent plant symbionts and may induce plant resistance and growth (Qualhato et al, 2013).

Another promising alternative for the suppression of a disease is the manipulation physical, chemical and microbiological characteristics of the soil by means of management practices that enrich biodiversity and soil microbial activity, creating an environment deleterious to the plant pathogen (Barros et al., 2014). In this context, biological fertilizers serve not only as complement of mineral nutrition, but also to restore the microbial soil biodiversity (Bhardwaj et al., 2014; Zanuncio et al., 2020).

Biological fertilizers, also called biofertilizers, contain macro and micronutrients required for plant nutrition, as well as living beneficial microorganisms that can confer plant-hormonal and suppressive effects against different plant pathogens (Barros et al., 2020) in different crops, as described by Hridya et al. (2013) for *Phytophthora palmivora* on cassava and by Shen et al. (2013) for *Fusarium oxysporum* f. sp. *cubense* on banana trees. The fertilizers are applied on crop leaves or directly to the soil (Medeiros & Lopes, 2006), and their effect in disease suppression is related to their microbiological diversity, the soil microbiota and factors such as soil type, climatic conditions and management (Akter et al., 2013).

The purpose of this study was to determine the *in vitro* antagonistic activity of *T. harzianum* and biological fertilizer MICROGEO® on *F. solani*, causal agent of cassava root rot.

2. Materials and Methods

2.1 Isolation and identification of *Fusarium solani* and *Trichoderma harzianum*

For the isolation of *Fusarium*, samples of cassava roots with rot symptoms were obtained from properties in the region of Paranavaí-PR. Fragments were cultured in Petri dishes containing Potato-Dextrose-Agar (PDA). Monosporic isolates were obtained to ensure pure cultures. The isolate morphology was differentiated by macroscopic (pigmentation) and microscopic (hyphae and conidia morphology under an optical microscope) observations of the colony. *Fusarium* isolates were submitted to molecular identification for *F. solani* confirmation. Genomic DNA was extracted according to the protocol proposed by Gontia-Mishra et al. (2014). The ITS region of the *F. solani* genome was amplified by Polymerase Chain Reaction (PCR) using the primers ITS-F (5´-CCAGAGGACCCCCTAACTCT-3') and ITS-R (5'-CTCTCCAGTTGCGAGGTGTT-3') (Arif et al., 2012).

The *T. harzianum* fungus strain ESALQ 1306 was isolated by serial dilution of a biological fungicide in PDA culture medium.

2.2 Antagonist action of *Trichoderma harzianum* on *Fusarium solani*

The antagonistic action of *T. harzianum* on *F. solani* was evaluated *in vitro*, by the modified dual culture method proposed by Dennis & Webster (1971). Each Petri dish (150 mm diameter), containing PDA culture medium, received two

agar disks (8 mm diameter) with fungal mycelium, one *F. solani* and one *T. harzianum* colony disc, on opposite sides and 1.5 cm away from the edges of the plate. *F. solani* mycelial discs were taken from 10-days-old colonies and transferred to the Petri dish 96 h before *T. harzianum*, according to the methodology adapted from Carvalho et al. (2008). As controls, the fungi were grown separately, from a mycelium disc placed in the center of the plate. The Petri dishes were incubated in a BOD incubator at 25°C, with a photoperiod of 12 h for 9 days. Mycelial growth (mm) of the fungi was determined by measuring the colony diameters daily, to calculate the Mycelial Growth Velocity Index (MGVI), in mm day^{-1}, as proposed by Oliveira (1991).

2.3 Sporulation analysis of *Fusarium solani*

For the sporulation analysis of *F. solani* isolates, a methodology was adapted from Guzmán-de-Peña & Ruiz-Herrera (1997), counting the spores of three mycelial discs (8 mm diameter) collected from the central, intermediate and peripheral region of the colonies on the 10th day of development. Samples from each colony were transferred to test tubes containing 10 mL of 0.05% Tween 80 solution and vortexed for 2 min. The counts were performed in a Neubauer chamber under light microscopy. Sporulation was expressed in number of spores cm^{-2} of colony.

2.4 Macroscopic and microscopic morphology of *Fusarium solani*

The macroscopic morphology was evaluated as proposed by Pitt & Hocking (1997) and the microscopy was analyzed by means of dual microcultivation of the fungi on microscope slides and later observation under optical microscope.

2.5 Determination of the microbial community of biological fertilizer

Continuous liquid composting of bovine ruminal content, water and the component MICROGEO® produced the biological fertilizer used. The functional groups of microorganisms (population of total fungi, heterotrophic bacteria, cellulolytic, proteolytic, amilolytic, actinomycetes, free-living nitrogen-fixing and *Pseudomonas fluorescens*) in the broth were determined by serial dilution and inoculation on selective culture media.

2.6 Effect of the biological fertilizer on mycelial growth of *Fusarium solani*

The tests with the biological fertilizer were carried out *in vitro*, with the unsterilized (USt) and sterilized (St) product. For sterilization, a fertilizer sample was submitted to: pH correction to 6.8-7.2; filtering through common filter paper; centrifugation at 4,000 rpm for 20 min; centrifugation of the supernatant at 10,000 rpm for 10 min and sterilization by vacuum pump filtration (0.22 μm Millipore membrane).

The sterilized biological fertilizer (St) was tested in TSA (tryptic-soy-agar) medium at concentrations (%v/v): St1 (control), St2 (2.5%), St3 (5.0%), St4 (10.0%), St5 (20.0%) and St6 (40.0%). The unsterilized biological fertilizer (USt) was verified in TSA medium at concentrations: USt1 (control), USt2 (0.3125%), USt3 (0.625%), USt4 (1.25%), USt5 (2.5%), USt6 (5.0%), USt7 (10.0%), USt8 (20.0%) and USt9 (40.0%). The product was diluted in the culture media after autoclaving but before solidification.

F. solani mycelium discs (8 mm diameter) were transferred to the center of the Petri dishes (90 mm diameter) containing the culture medium plus the biological fertilizer. Incubation conditions were 25 °C with a photoperiod of 12 h for 7 days. The mycelial diameter of the *F. solani* isolates was measured daily in the two perpendicular directions of the Petri dish to obtain the mean of the diameters (in mm) of each colony to calculate the Mycelial Growth Velocity Index (MGVI), according to Oliveira (1991). For the evaluation of mycelial growth inhibition, the values of colony diameters on the last day of incubation were used and the formula proposed by Silva et. al (2014).

2.7 Effect of biological fertilizer on spore germination of *Fusarium solani*

The germination of *F. solani* spores (1×10^5 conidia mL^{-1} suspension) occurred on microscopic slides with TSA culture medium plus unsterilized biological fertilizer (USt) in the treatments USt1 (control), USt2 (0.3125%), USt3 (0.625%), USt4 (1.25%), USt5 (2.5%), USt6 (5.0 %), USt7 (10.0 %), USt8 (20.0%), and USt9 (40.0%). Germination was assessed by counting of germinated and non-germinated spores in four randomly chosen fields of vision, evaluating a minimum of 100 spores per field, to determine the germination percentage.

2.8 Statistical analysis of the results

The bioassays were conducted in a completely randomized design with four replications. The results were subjected to analysis of variance. The means obtained from the bioassay with *T. harzianum* were compared by the t-test ($p < 0.05$) and regression analysis and Tukey's test ($p < 0.05$) were applied to the means of the bioassay with biological fertilizer.

3. Results and Discussion

3.1 Morphological and molecular identification of the plant pathogens

Monosporic fungus cultures allowed the identification of two *Fusarium* isolates, called F1 and F2, differentiated by morphological characteristics of the colonies in macroscopic (F1 with white-pigmented mycelium and F2 with pink-pigmented mycelium) and microscopic (F1 with sickle-shaped and F2 with oval conidia) characteristics. However, the identification of *Fusarium* species based only on the morphological characteristics of the fungus requires the skills of specialized taxonomists. Thus, the molecular identification of the *Fusarium* isolates F1 and F2 confirmed the *F. solani* species, according to the observation of 354 bp fragments obtained from the amplification of the ITS region of the species.

3.2 Antagonist action of *Trichoderma harzianum* on *Fusarium solani*

The dual culture assay of *T. harzianum* with *F. solani* isolates demonstrated an interruption in the mycelial growth of plant pathogens at the moment their hyphae meet with those of *T. harzianum,* on the 7th days after subculturing (DAS) (Table 1). The biocontrol however was only perceptible on the 8th DAS of the plant pathogens, due to the statistical difference in the colony diameters, compared to the controls. Before that, there was no inhibitory action of *T. harzianum*, since the MGVI (mm day^{-1}) of isolates F1 and F2 did not differ from the controls (Table 1).

One of the characteristics of *Trichoderma* sp. is the faster development, which is relevant for its performance as a biocontrol agent (Qualhato et al., 2013). The mean mycelial growth of *T. harzianum* was 40.77 mm day^{-1}, which is approximately four times the MGVI of isolate F1 (10.87 mm day^{-1}) and three times the MGVI of isolate F2 (12.91 mm day^{-1}) of *F. solani*. Although *T. harzianum* was transferred to the Petri dish 96 h after *F. solani*, it rapidly reached and surpassed the mycelial growth of the plant pathogens, aside from continuing to grow and sporulate on their colonies. Similar results were reported by Pereira (2009), in an analysis of the antagonism of ten *Trichoderma* isolates against *Fusarium* sp. isolates, causing vascular wilt of tomato. In their study, all *Trichoderma* sp. isolates grew faster than the tested plant pathogens.

Table 1. Mean diameter of colonies of F1 and F2 isolates of *Fusarium solani* in dual culture with *Trichoderma harzianum*.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9***	**MGVI** mm day^{-1}**
	Colony diameter (mm)									
	F1									
C[1]	4.00 a	15.50 a	26.80 a	38.90 a	52.60 a	64.97 a	76.10 a	88.57 a	100.63 a	10.87 a
DC[2]	4.00 a	16.00 a	28.80 a	39.60 a	52.80 a	65.60 a	74.80 a	74.80 b	74.80 b	10.69 a
CV[3] (%)	1.25	3.89	5.41	2.69	2.17	1.72	3.28	3.41	3.38	3.28
	F2									
C[1]	6.10 a	20.70 a	34.90 a	49.00 a	63.70 b	77.10 b	89.30 a	101.23 a	114.57 a	12.76 a
DC[2]	6.40 a	20.80 a	36.80 a	50.40 a	66.40 a	79.20 a	90.40 a	91.60 b	91.60 b	12.91 a
CV[3] (%)	11.17	4.70	4.41	2.95	1.72	1.78	1.47	2.48	2.33	1.47

[1] Control, [2] Dual culture, [3] Coefficient of Variation, * Days After Subculturing (DAS), ** Mycelial Growth Velocity Index calculated from the diameters of the *F. solani* colonies on the 7th DAS. Means followed by the same letter in the column do not differ from each other by the t test at 5% probability.
Source: Authors.

Mycoparasitic action of *T. harzianum* on the *F. solani* isolates F1 and F2 was observed after direct contact of their hyphae. A microscopic analysis of the mycelium in the encounter region showed coiling of the *T. harzianum* hyphae on host hypha. The consequences of this event may have induced a mechanical and enzymatic degradation (chitinase production) of the cell wall of the host fungi, as well as absorption of their nutrients, causing their death (Kim & Vujanovic, 2016).

The macroscopic analysis of the colonies showed changes in the mycelium appearance and color of *F. solani* isolates, according to the overlapping and sporulation of *T. harzianum* on plant pathogenic hyphae. Similarly, Siameto et al. (2010) observed antagonism of *T. harzianum* isolates against against the fungi *Rhizoctonia solani*, *Pythium* spp. and *Fusarium* spp. These authors attributed the reduction in plant pathogen growth to the competition for nutrients and space, as well as the coiling and penetration of *T. harzianum* hyphae around and into hyphae of the host fungi, causing their disintegration, confirming a strong hyperparasitism.

The antagonism of *Trichoderma* sp. mainly involves the mechanisms of antibiosis (release of inhibitory extracellular compounds such as gliotoxins), parasitism (direct contact with production and release of enzymes that disintegrate the cell wall of the parasited fungus, e.g., chitinases) and competition for nutrients and space (Kim & Vujanovic, 2016). Despite the interruption in the mycelial growth of *F. solani* isolates F1 and F2 by the action of *T. harzianum* after mycelium contact, there was no statistical difference (Tukey test $p < 0.05$) in their sporulation rate (mean spore number/cm² of colony) compared to their control colonies. Isolate F1 sporulation rate was 1.2547×10^7 spores/cm² (control F1: 1.2049×10^7 spores/cm²) and isolate F2 sporulation rate was 3.4066×10^7 spores/cm² (control F2: 3.1566×10^7 spores/cm²). The results suggest that *T. harzianum* was not able to produce sporulation-inhibiting extracellular compounds, what differs from Santos (2008), who reported inhibition of sporulation of *Fusarium oxysporum* by metabolite production due to antibiosis.

The *in vitro* results demonstrated that *T. harzianum* is a fungus that could potentially be used for the biological control of *Fusarium* root rot. Apart from enriching the soil microbiota, they are antagonistic to *Fusarium solani* and other soil fungi due to their biocontrol mechanisms (Qualhato et al., 2013).

3.3 Microbial composition of biological fertilizer

The microbial population obtained from the biological fertilizer showed a considerable diversity of microorganisms: total fungi (3.97 LogUFC mL^{-1}), heterotrophic bacteria (7.01 LogUFC mL^{-1}), actinomycetes (4.80 LogUFC mL^{-1}),

Pseudomonas fluorescens (3.50 LogUFC mL^{-1}), microorganisms participating in the biogeochemical cycles of carbon (cellulolytic: 4.88; proteolytic: 5.07 and amylolytic: 5.21 LogUFC mL^{-1}), nitrogen (free-living, nitrogen-fixing bacteria – flNFB: 6.14 LogUFC mL^{-1}). The presence of actinomycetes and *Pseudomonas fluorescens* is fundamental, since these microorganisms are considered strong biocontrol agents (Hridya et al., 2013). Only part of the microbial population in selective media is countable, i.e., the real is greater than the detected population (Tratch & Bettiol, 1997).

The microbial composition contained in the biological fertilizer is relevant for its performance in terms of disease suppression. Generally, phytosanitary problems associated with soil fungi such as *Fusarium* spp. are related to a decrease in the soil microbial diversity (Palmieri et al., 2017). Thus, the restructuring of the microbiota by the application of a biological fertilizer full of microorganisms, apart from accelerating the degradation of soil biological matter and providing plant-accessible nutrients, invigorates the control of diseases caused by soil plant pathogens, by creating a competitive and pathogen-hostile environment (Bhardwaj et al., 2014). The suppressiveness to cassava root rot caused by *F. solani* can be improved by an increase of its biodiversity by using adequate cultivation and management system in the area, and the disease severity was highest in soils used for monoculture crops in the past (Barros et al., 2014). Analyzing the mechanisms of control, Bonilla et al. (2012) stated that the suppression of diseases should not be attributed to the entire microbial population of the biofertilizer, but to the biocontrol activity and mechanisms used by specific groups of microorganisms.

3.4 Effect of the biological fertilizer on *Fusarium solani* development

The sterilized biological fertilizer had no significant effect on the MGVI of *F. solani* isolate F1 when cultured in TSA medium (Figure 1a). However, the MGVI of isolate F2 decreased slightly (Figure 1b), with a mean inhibition percentage of only 5.74% at the highest concentration.

For the unsterilized biological fertilizer (USt), the effect on fungal mycelial growth of both *F. solani* isolates was similar. There was a marked decrease in MGVI until a product concentration of 2.5% (USt5) (Figures 2a and 2b), which did not differ from the higher concentrations by the Tukey test (p <0.05).

The unsterilized biological fertilizer at a concentration of 2.5% (USt5) promoted inhibitions of approximately 64% in mycelial growth of isolate F1 (Figure 3a) and 85% inhibition of isolate F2 (Figure 3b). As observed for the MGVI of the fungi (Figures 2a and 2b), at concentrations above 2.5% of the unsterilized product, its effect on the inhibition percentage did not differ statistically by the Tukey test (p <0.05).

Similar results were reported by Tratch and Bettiol (1997) regarding the effect of a biofertilizer produced by anaerobic fermentation of cattle manure enriched with biological compounds on the mycelial growth of *F. oxysporum* f. sp. *phaseoli*. In this study, they observed that the biocontrol of the plant pathogen was effective at a product concentration of 10% and higher, not differing at the highest concentrations tested.

For foliar applications, biofertilizers are generally sprayed at concentrations ranging from 0.1 to 5.0% of the product diluted in water. Due to their nutritional and microbial composition, the application of very high concentrations may interfere at the plant development, causing physiological stress, apart from favoring vegetative plant growth and delaying the reproductive stage (Medeiros & Lopes, 2006). The technical recommendations of the manufacturer of this biological fertilizer suggest product doses of 3 to 5% diluted in water.

Figure 1. Effect of sterilized biological fertilizer (St) on the mycelial growth velocity index (MGVI) of *Fusarium solani* isolates F1 (a) and F2 (b). Treatments: St1 (control), St2 (2.5%), St3 (5.0%), St4 (10.0%), St5 (20.0%), and St6 (40.0%).
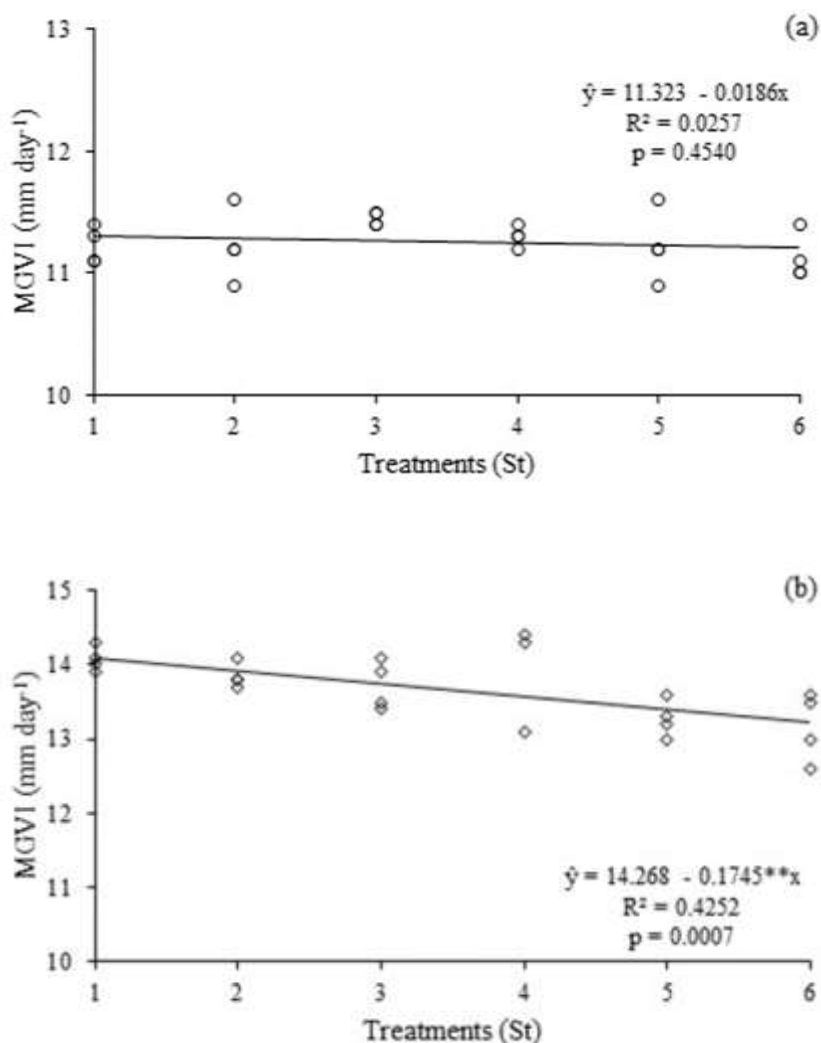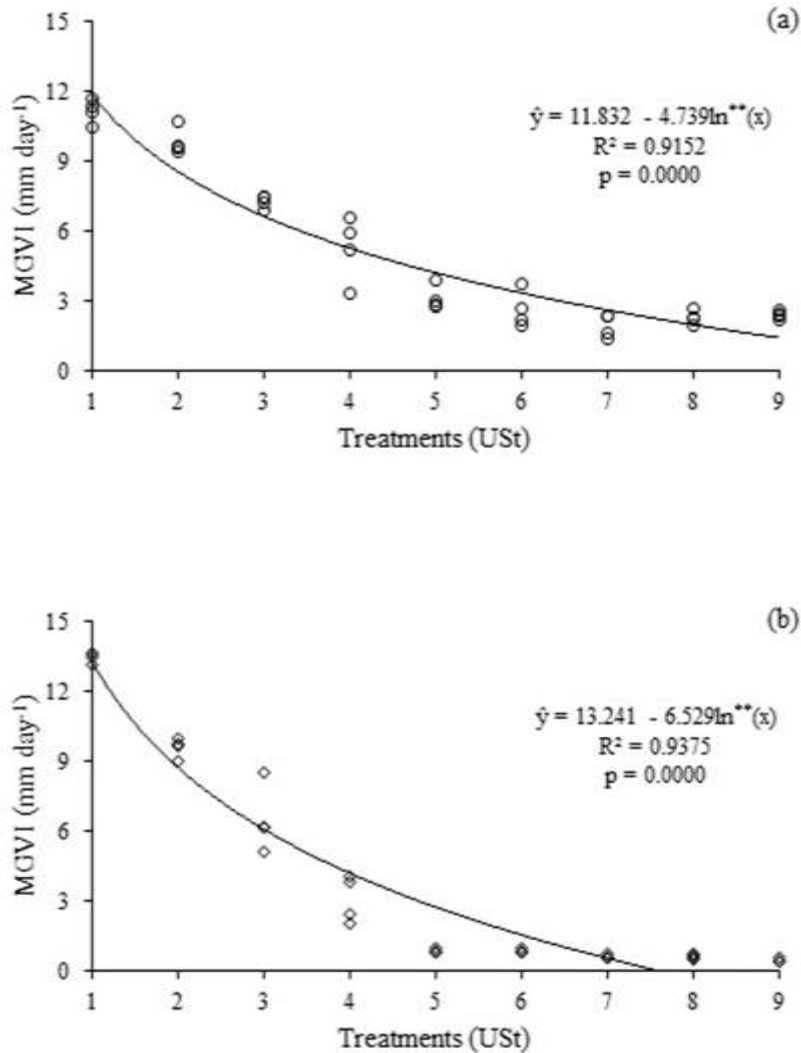

Source: Authors.

The behavior of the microbial population present in the biological fertilizer was essential for the *in vitro* biological control of *F. solani*. Since the microbial community is rich and diverse, mechanisms of microbial action can occur simultaneously, e.g., parasitism, competition for nutrients, and production of antimicrobial compounds (Kupper et al., 2009). The results of MGVI (Figure 2) and inhibition percentage of the plant pathogens (Figure 3) indicate that in the presence of plant pathogenic fungi, the microorganisms present in the biological fertilizer were able to produce antimicrobial metabolites by the mechanism of biocontrol, called antibiosis. A relevant fact for the confirmation of this mechanism was the non-inhibition of *F. solani* under the effect of the sterilized product.

The *in vitro* and *in vivo* suppressive effect of biofertilizers on various plant pathogens was described by a number of researchers, in crops such as citrus (Kupper et al., 2009), cucumber (Akter et al., 2013) and banana (Shen et al., 2013). In an experiment of Shen et al. (2013) with *Fusarium* wilt (*Fusarium oxysporum* f. sp. *cubense*) of banana, different biological compounds were tested, as well as a biofertilizer based on pig slurry and amino acids inoculated with an antagonistic *Bacillus amyloliquefaciens* strain. The biofertilizer action reduced the disease incidence to 20%, compared to the other treatments. Aside from the disease suppression by the enrichment and stimulation of the soil microbiota, the crop yield was also increased.

8

Figure 2. Effect of unsterilized biological fertilizer (USt) on the mycelial growth velocity index (MGVI) of F1 (a) and F2 (b) isolates of *Fusarium solani*. Treatments: USt1 (control), USt2 (0.3125%), USt3 (0.625%), USt4 (1.25%), USt5 (2.5%), USt6 (5.0%), USt7 (10.0%), USt8 (20.0%), and USt9 (40.0%).

(a)

$$\hat{y} = 11.832 - 4.739 \ln^{**}(x)$$
$$R^2 = 0.9152$$
$$p = 0.0000$$

(b)

$$\hat{y} = 13.241 - 6.529 \ln^{**}(x)$$
$$R^2 = 0.9375$$
$$p = 0.0000$$

Source: Authors.

The effect of unsterilized biological fertilizer was significant for the percentage of spore germination of *F. solani* F1 and F2. Compared to the control, the germination percentage decreased with increasing product concentration, resulting in a reduction of approximately 34% in the spore germination of isolate F1 and 52% in isolate F2 at the highest test concentration (Figures 4a and 4b).

Figure 3. Inhibition percentage of mycelial growth of *Fusarium solani* isolates F1 (a) and F2 (b) under the effect of different concentrations of unsterilized (USt) biological fertilizer. Treatments: USt1 (control), USt2 (0.3125%), USt3 (0.625%), USt4 (1.25%), USt5 (2.5%), USt6 (5.0%), USt7 (10.0%), USt8 (20.0%), and USt9 (40.0%).
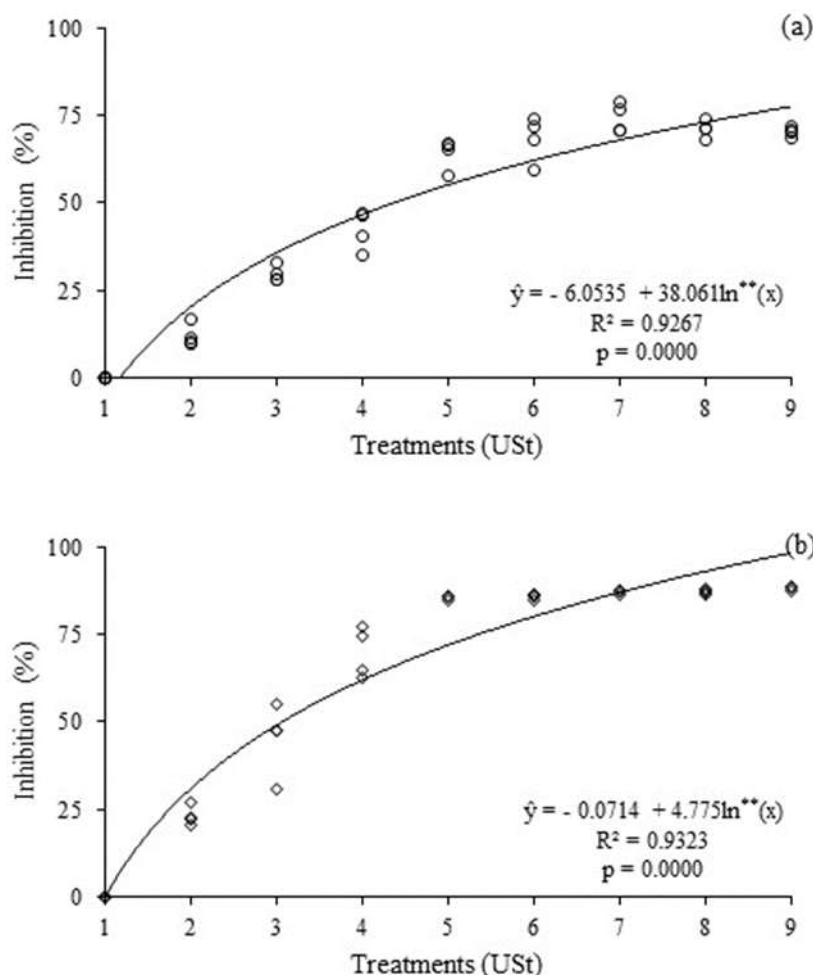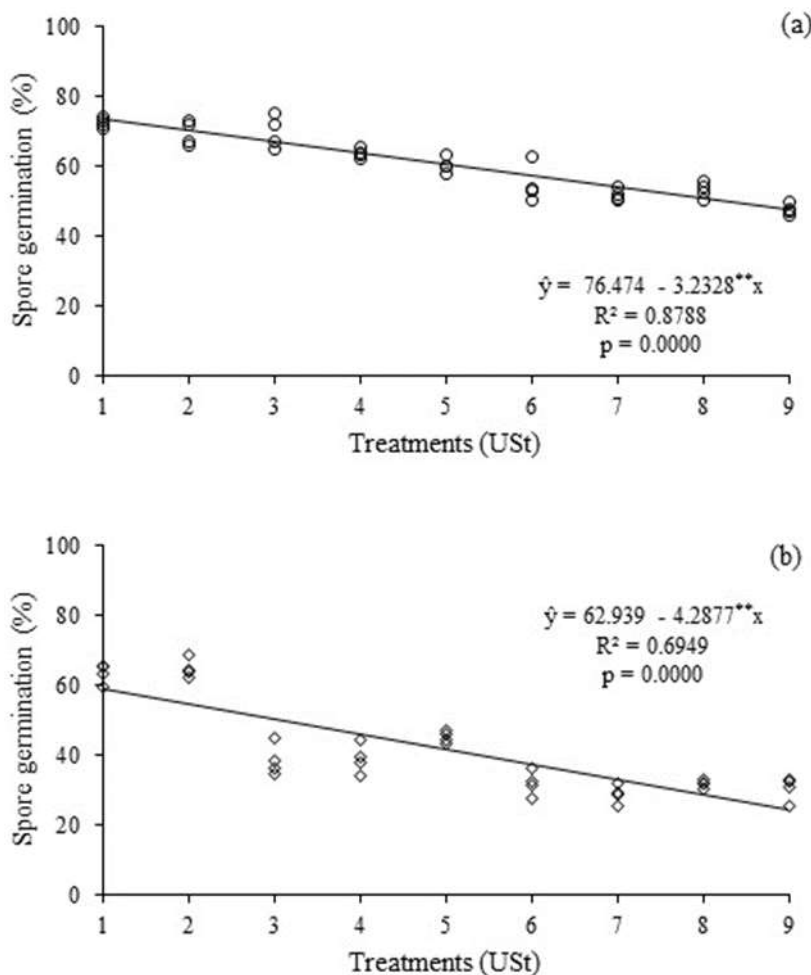

Source: Authors.

The results confirm those of Kupper et al. (2009), who analyzed the effect of different concentrations of two biofertilizers based on cattle manure, one produced under anaerobic (Bio1) and the other under aerobic conditions (Bio2), on the spore germination percentage of *Colletotrichum acutatum*, causal agent of preharvest drop of citrus fruit. The authors observed inhibition rates exceeding 50% of the spore germination in response to the products at concentrations above 10%, particularly for Bio2, with highest inhibition percentages.

The harmful effect of biofertilizers on spore germination of various plant pathogenic fungi was also mentioned by Tratch & Bettiol (1997). For the fungus *Botrytis cinerea*, concentrations above 20% inhibited spore germination completely, while for *Alternaria solani*, a concentration of 10% was sufficient.

In view of the advantages of the use of biofertilizers to maintain the chemical, physical and microbiological characteristics of the soil and its responses in relation to plant pathogen suppression and the development of agricultural crops, their inclusion in sustainable agricultural production systems is becoming increasingly viable (Bhardwaj et al., 2014).

Figure 4. Percentage of spore germination of *Fusarium solani* isolates F1 (a) and F2 (b) under the effect of different concentrations of unsterilized (USt) biological fertilizer. Treatments: USt1 (control), USt2 (0.3125%), USt3 (0.625%), USt4 (1.25%), USt5 (2.5%), USt6 (5.0%), USt7 (10.0%), USt8 (20.0%), and USt9 (40.0%).

Source: Authors.

4. Conclusion

Antagonistic activity of *T. harzianum* on *F. solani* was confirmed *in vitro* and biocontrol occurred by mycoparasitism after the mycelial encounter of the fungi.

Microorganisms presented in the unsterilized biofertilizer MICROGEO®, starting from concentration 2.5% (USt5), caused mycelial growth inhibition in vitro of *F. solani* isolates and induced a reduction in the germination spores.

The proposed management alternatives proved to be relevant for the *in vitro* biological control of *Fusarium solani,* causal agent of the cassava root rot disease. Future research would be important to verify the *in vivo* biological control of *F. solani*.

Acknowledgments

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