Revista Ciência Agronômica, v. 56, e202493672, 2025
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Biocontrol of *Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, *Fusarium* spp., and the agronomic performance of soybean by *Trichoderma* spp.[1]

Lincon Rafael da Silva[2], Ana Beatriz Zacaroni[3], Ana Luiza Bezerra Cardoso[4], Angelo Aparecido Barbosa Sussel[5], Alexei de Campos Dianese[5], André Ferreira Pereira[6], Sueli Corrêa Marques de Mello[3*]

ABSTRACT - Soybean (*Glycine max* L.) is a globally important legume that is susceptible to a variety of pathogens throughout its development stages. One effective option for controlling these diseases is the use of biological products derived from fungi of the genus *Trichoderma*. This study aimed to select *Trichoderma* strains, maintained in culture collections, as control agents for the pathogens *Sclerotinia sclerotiorum*, *Sclerotium rolfsii,* and *Fusarium* spp. Additionally, it sought to identify strains among those selected that can enhance the agronomic characteristics of soybean plants in the field. Experiments were carried out *in vitro*, in a greenhouse, and in an experimental field. The *T. koningiopsis* CEN209 strain showed performance *in vitro* and in the greenhouse against *S. sclerotiorum*, *S. rolfsii* and *Fusarium* spp., while the *T. afroharzianum* CEN230 and *T. rifaii* CEN288 strains obtained better performances against *S. sclerotiorum* and *S. rolfsii*. The strains *T. azevedoi* CEN1242 and *T. koningiopsis* CEN1513 significantly increased the final soybean yield under field conditions without the occurrence of soil-borne diseases.

Key words: White mold. *Glycine max.* Biological Control. Field trials.

DOI: 10.5935/1806-6690.20250054
Editor-in-Chief: Agricultural Engineer. Manoel Barbosa Filho - manoel.filho@ufc.br
*Author for correspondence
Received for publication 02/07/2024; approved on 27/11/2024
[1]This study is part of a research project carried by Embrapa Genetic Resources and Biotechnology, funded by Bionat Biological Solutions and National Council for Scientific and Technological Development (CNPq)
[2]Laboratory of Phytopathology, State University of Goias (UEG), Quirinopolis-GO, Brazil, lincon@ueg.br (ORCID ID 0000-0003-3148-8232)
[3]Laboratory of Phytopathology, Embrapa Genetic Resources and Biotechnology, Brasilia-FD, Brazil, anabeatriz.zacaroni@gmail.com (ORCID ID 0009-0003-0996-7031), sueli.mello@embrapa.br (ORCID ID 0000-0002-7626-3857)
[4]Postgraduate Program in Phytopathology, University of Brasilia (UnB), Brasilia-FD, Brazil, analuizabezerra.c@hotmail.com (ORCID ID 0000-0002-1955-7840)
[5]Laboratory of Phytopathology, Embrapa Cerrados, Brasilia-FD, Brazil, angelo.sussel@embrapa.br (ORCID ID 0000-0002-6959-9483), alexei.dianese@embrapa.br (ORCID ID 0000-0002-0968-8206), andre.ferreira@embrapa.br (0000-0002-5572-4466)
[6]Laboratory of Soybean Genetics and Breeding, Embrapa Cerrados, Brasilia-FD, Brazil, andre.ferreira@embrapa.br (ORCID ID 0000-0002-5572-4466)

INTRODUCTION

Soybean (*Glycine max* L.) is a legume of global importance, with seeds containing approximately 20% oil and 40% protein (Clemente; Cahoon, 2009). The seeds are crushed to extract vegetable oil and produce biodiesel. The resulting meal from processed seeds is a primary source of protein used in the formulation of balanced foods for human and animal nutrition (Dall´Agnol, 2016; Toloi *et al*., 2021). In half a century of cultivation, the area planted with soybeans in Brazil has exceeded 44 million hectares, and its production in the 2023/2024 season exceeded 147 million tons. According to a report released by the National Supply Company of Brazil, the harvest in 2025 is expected to reach 167 million tons (CONAB, 2025).

The expansion of cultivation and monoculture has intensified the incidence of several pathogens in all phases of its development, making diseases a limiting factor for its productivity. (Han *et al*., 2021; Roth *et al*., 2020). Soybean (*Glycine max* L.) is a legume of global importance and can be affected by a variety of pathogens at all stages of its development.

Among these pathogens are the soil fungi *Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, and *Fusarium* spp. (Han *et al*., 2021; Mahartha; Suprapta, 2018; Westrick *et al*., 2019). These soil fungi can form survival structures such as sclerotia and chlamydospores, which enable them to survive for long periods under adverse environmental conditions (Aoki *et al*., 2003; Smolińska; Kowalska, 2018).

One of the options for controlling these diseases is the use of biological products based on fungi of the genus *Trichoderma*. These fungi can colonize sclerotia, spores, and chlamydospores structures that are generally difficult to reach with synthetic fungicides (Macena *et al*., 2020; Meena *et al*., 2017). Additionally, *Trichoderma* species compete with pathogens for space and nutrients, produce secondary compounds toxic to other fungi, and can directly contribute to biological control. They also promote plant growth (Carvalho *et al*., 2015; Khan *et al*., 2020; Marques; Martins; Mello, 2018; Silva *et al*., 2021) and induce plant resistance to diseases. Due to these characteristics, *Trichoderma* has become an active ingredient used in the development of biofungicides and plant growth promoters (Widmer, 2019). In recent years, Brazil has emerged as the largest producer and user of biocontrol agents in the world. Currently, there are 142 registered bioproducts in the country (Bettiol; Medeiros, 2023), and the genus *Trichoderma* stands out among the fungal biological control agents.

The indiscriminate use of synthetic fungicides for controlling fungal diseases can lead to the emergence of resistant pathogen strains and contaminate soil and food. Therefore, ecologically sound biocontrol practices are desirable, in order to reduce dependence on these products (Srivastava *et al*., 2016). In this context, the selection of *Trichoderma* strains to develop new active ingredients has been the focus of several research groups worldwide (O'Brien, 2017; Silva *et al*., 2020; Wonglom *et al*., 2019). Numerous studies in the literature indicate that the behavior of *Trichoderma* strains can be specific regarding their antagonistic action against pathogens and plant growth promotion (Alfiky; Weisskopf, 2021; Rivera-Méndez *et al*., 2021; Silva *et al*., 2022a). Thus, developing biofungicides requires preliminary studies to evaluate and select strains with good competitive potential, antagonistic properties, and the ability to produce secondary metabolites with toxic effects on the target pathogen.

Given the above, this study aimed to select *Trichoderma* strains from a culture collection to act as control agents against diseases caused by the pathogens *Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, and *Fusarium* spp. Additionally, it sought to identify strains among those selected that could increase the productivity.

MATERIAL AND METHODS

Origin and maintenance of strains

The *Trichoderma* strains (Table 1) and the pathogens *S. sclerotiorum* (CEN1147), *S. rolfsii* (CEN216), *Fusarium crassistipitatum* (CEN1636), and *Fusarium* sp. (CEN1631) used in this study belong to the Collection of Biological Control Agents of Phytopathogens and Weeds at Embrapa Genetic Resources and Biotechnology (Brasilia, Federal District, Brazil). The cultures, maintained in liquid nitrogen (N_2), were reactivated on Potato Dextrose Agar (PDA) Merck® medium and stored at 6 °C in screw-capped test tubes containing 20 mL of PDA. For each experiment, samples taken from these test tubes were cultured for five days in Petri dishes (90 x 15 mm) containing 20 mL of the same medium.

Dual culture experiment

To evaluate the antagonism of *Trichoderma* spp. against each of the pathogens, the dual culture technique in Petri dishes was employed, as described by Dennis and Webster (1971). Initially, screening was conducted with 60 strains. Agar discs (5 mm Ø) containing mycelium were taken from three-day-old colonies of both the pathogen and the antagonist and simultaneously placed on the surface of

solidified PDA (20 mL) at opposite ends, 5 mm from the edges of the Petri dishes. This setup was maintained at a temperature of 24 ± 1 °C with a photoperiod of 12 hours. The control treatment consisted of plates containing only the pathogen, without the antagonist. After complete colonization of the PDA medium in the control plates, the diameter of the pathogen colonies was measured using a digital caliper (Digimess©). The average values of mycelial growth inhibition were calculated using the formula: I = (C – T) /C x 100, where I represents the percentage of inhibition, C represents the diameter values of the control treatment culture, and T represents the diameter values of the treatments with the antagonist, measured in millimeters. The experiments were conducted in a completely randomized design with three replicates per treatment and were repeated twice.

Greenhouse tests with *Sclerotinia sclerotiorum* and *Sclerotium rolfsii*

The same 60 *Trichoderma* strains were evaluated for their ability to suppress *S. sclerotiorum* and *S. rolfsii* in a greenhouse. For this purpose, parboiled rice grains were placed in Erlenmeyer flasks, moistened with distilled water at a rate of 60% (w/v), and then sterilized at 120 °C for 20 minutes. Subsequently, each flask received 10 mycelium discs (5 mm Ø) colonized by *Trichoderma* spp., or by the pathogens *S. sclerotiorum* or *S. rolfsii.* The cultures were maintained at a temperature of 24 ± 1 °C with a photoperiod of 12 hours. The rice was stirred daily to promote gas exchange, break the mycelium, and increase the surface contact between the fungus and the substrate. To prepare the *Trichoderma* suspension, after seven days of cultivation, 100 mL of sterilized water were added to each flask. The concentration of conidia/mL of water was determined using a Neubauer chamber, and this suspension was adjusted to 1 x 10^8 colony-forming units (CFU)/mL. For the preparation of pathogen inoculum, at seven days of cultivation, the colonized rice was dried for three days and then ground in a blender. Finally, disposable cups with 50 mL of pre-moistened vermiculite were added with 100 mL of dry vermiculite containing 0.5 g of pathogen structures. Twenty-four hours later, each cup received 10 mL of *Trichoderma* inoculum suspension, and after another 24 hours, they were seeded with four soybean seeds. The control treatments consisted of vermiculite + ground rice uncolonized (C1); vermiculite alone (C2); and vermiculite + ground rice colonized with *S. sclerotiorum* or *S. rolfsii* (C3). Three readings of the number of seedlings were taken, obtaining the emergence speed index (ESI). The percentages of live seedlings were calculated from readings taken at the last evaluation, 12 days after sowing. The experiments were conducted in a completely randomized design with three replicates for each *Trichoderma* strains and were repeated twice.

Greenhouse tests with *Fusarium* spp.

Sixteen *Trichoderma* strains selected from the experiments described in dual culture experiment and greenhouse were tested for their suppression of *F. crassistipitatum* (CEN1636) and *Fusarium* sp. (CEN1631), which are pathogens affecting soybean cultivation. The *Fusarium* spp. strains were obtained from soybean plants showing symptoms of fusarium wilt, collected from an area with a history of the disease. The experiment was conducted and evaluated as described in greenhouse tests.

Agronomic performance of soybean plants under field conditions

The agronomic performance of soybean was evaluated in the field over two consecutive harvest seasons (2019/2020 and 2020/2021) using 10 *Trichoderma* strains. The strains used were *T. afroharzianum* (CEN234, CEN287, and CEN1546), *T. asperelloides* (CEN162, CEN1532, CEN1533, and CEN1277), *T. azevedoi* (CEN1242), *T. koningiopsis* (CEN1513), and *T. lentiforme* (CEN1294). Antagonist suspensions were prepared as described in greenhouse and used at a ratio of 3 mL per 330 g of soybean seeds (M7110 IPRO-Monsoy®), contained in plastic bags. Seeds and *Trichoderma* suspensions were mixed by manual agitation and left to dry overnight on a bench. Subsequently, the seeds were placed in paper envelopes, randomized, and taken to the experimental field. In the first harvest season, sowing took place on December 6, 2019, and in the second season 2020/2021, on December 4, 2020. The plots consisted of four rows of five meters in length planted with soybeans spaced 40 centimeters apart, with a density of 16 plants per linear meter. To compose the controls (plots without *Trichoderma*), soybean seeds were treated with 3 mL of sterilized water.

Fifteen days after sowing, a spray application was conducted using a *Trichoderma* spore suspension produced as described earlier. A volume of 250 milliliters of suspension per plot was applied along the planting line using a manual sprayer with directed spray. The following agronomic parameters were recorded: plant height at 60 days after planting (DAP); number of nodules, internodes, and pods at 70 DAP; and dry weight of grains from each plot at 120 DAP. Based on the weight of grains produced per treatment, yield was estimated in kilograms and sacks per hectare, and then the increase/decrease in terms of sacks per hectare compared to the control was calculated.

Statistical analysis

The data obtained were subjected to analysis of variance, and the means were compared using the

Scott-Knott test at a 5% probability level, with the assistance of the Sisvar 5.7 software (Ferreira, 2011). The data obtained in the greenhouse were transformed by the square root of x + 0.5.

RESULTS AND DISCUSSION

Dual culture experiment

All *Trichoderma* strains tested exhibited inhibitory activity against the mycelial growth of the pathogens (Table 1). The best results against *S. sclerotiorum* were obtained with strains CEN1513, CEN1562, CEN1399, CEN288, CEN281, CEN287, CEN234, CEN1283, CEN235, CEN209, CEN230, CEN155, CEN203, CEN1417, CEN1514, CEN208, CEN1546, CEN1336, and CEN197, whose average inhibition values ranged from 69.17% (CEN1513) to 53.06% (CEN197), classified as "a" and "b". Among these 19 strains, 12 strains showed similar results against *F. crassistipitatum* (exceptions were strains CEN1562, CEN1399, CEN1283, CEN155, CEN1514, CEN208, and CEN197). It is also noteworthy here that five other strains (CEN254, CEN223, CEN144, CEN1242, CEN1294) exhibited average inhibition values against *F. crassistipitatum* ranging from 65.88 (CEN1294) to 53.43 (CEN144), thus classified as "a" and "b" in the mean comparison test used, totaling 17 pre-selected strains against this pathogen. In contrast, concerning the pathogen *S. rolfsii*, average inhibition values of growth were much lower compared to those obtained with *S. sclerotiorum* and *F. crassistipitatum*. However, most strains fell within the range of 40.43% (CEN1513) to 30.60% (CEN225). Therefore, strains classified with the letter "a" in the Scott-Knott test and considering their performance against the other two pathogens targeted in this study were considered for subsequent tests, as shown in Table 1.

In the end, 10 strains were chosen from those that showed the best performance against all three pathogens simultaneously. They are CEN230, CEN234, CEN235, CEN281, CEN287, and CEN1546, belonging to the species *T. afroharzianum*; CEN1513 and CEN209, from the species *T. rifaii*; and CEN288 and CEN1336, belonging to the species *T. lentiforme* (Table 1).

Table 1 - Antagonistic action of *Trichoderma* spp. on *Sclerotinia sclerotiorum* (CEN1147), *Sclerotium rolfsii* (CEN216), and *Fusarium crassistipitatum* (CEN1636), expressed as average values of mycelial growth inhibition of the pathogen in dual culture

ID	*Trichoderma* species	Inhibition of mycelial growth (%)		
		S. sclerotiorum	*S. rolfsii*	*F. crassistipitatum*
CEN1513	*T. koningiopsis*	65.31 a*	40.43 a	69.17 a
CEN1562	*T. longibrachiatum*	63.95 a	30.06 b	27.77 f
CEN1399	*T. longibrachiatum*	61.90 a	27.87 b	6.42 g
CEN288	*T. rifaii*	61.22 a	37.70 a	55.87 b
CEN281	*T. afroharzianum*	59.18 a	34.42 a	62.63 a
CEN287	*T. afroharzianum*	59.18 a	32.24 a	57.63 b
CEN234	*T. afroharzianum*	58.50 a	32.79 a	55.01 b
CEN1283	*Trichoderma* sp.	57.82 b	25.14 c	50.51 c
CEN235	*T. afroharzianum*	55.10 b	34.97 a	63.54 a
CEN209	*T. koningiopsis*	55.10 b	39.89 a	56.88 b
CEN230	*T. afroharzianum*	55.10 b	35.52 a	55.43 b
CEN155	*T. afroharzianum*	55.10 b	24.04 c	44.68 c
CEN203	*T. koningiopsis*	54.42 b	30.60 b	54.80 b
CEN1417	*T. afroharzianum*	54.42 b	31.15 b	53.29 b
CEN1514	*T. asperelloides*	54.42 b	32.24 a	47.54 c
CEN208	*Trichoderma* sp.	54.42 b	28.42 b	40.70 d
CEN1546	*T. afroharzianum*	53.74 b	32.79 a	63.79 a
CEN1336	*T. lentiliforme*	53.74 b	33.33 a	53.16 b
CEN197	*T. afroharzianum*	53.06 b	34.43 a	45.72 c
CEN254	*T. afroharzianum*	51.70 c	32.24 a	64.53 a

Continuation Table 1

CEN1282	*T. azevedoi*	51.70 c	36.07 a	41.36 c
CEN228	*T. peberdyi*	51.70 c	27.32 b	25.88 f
CEN223	*T. lentiforme*	51.02 c	27.32 b	55.97 b
CEN1559	*T. asperelloides*	51.02 c	31.70 b	48.41 c
CEN144	*T. atroviride*	50.34 c	34.43 a	53.43 b
CEN1293	*T. azevedoi*	49.66 c	33.88 a	40.12 d
CEN211	*T. peberdyi*	49.66 c	33.88 a	29.44 f
CEN168	*T. azevedoi*	48.98 c	29.51 b	39.86 d
CEN221	*Trichoderma* sp.	48.98 c	32.79 a	34.81 e
CEN141	*T. afarasin*	48.98 c	20.22 c	34.14 e
CEN232	*T. peberdyi*	48.98 c	27.87 b	26.92 f
CEN162	*T. asperelloides*	48.30 c	31.70 b	44.23 c
CEN1241	*T. azevedoi*	48.30 c	30.06 b	39.08 d
CEN256	*T. peberdyi*	48.30 c	30.60 b	27.99 f
CEN158	*T. afroharzianum*	47.62 c	27.87 b	51.02 c
CEN1533	*T. asperelloides*	47.62 c	32.24 a	47.32 c
CEN198	*T. peberdyi*	46.94 c	23.50 c	27.21 f
CEN1242	*T. azevedoi*	46.26 c	28.42 b	59.95 b
CEN1542	*T. asperelloides*	46.26 c	34.43 a	41.88 c
CEN202	*T. rifaii*	45.58 c	24.59 c	39.59 d
CEN226	*T. peberdyi*	45.58 c	34.97 a	23.08 f
CEN225	*T. peberdyi*	42.86 d	30.60 b	27.51 f
CEN1277	*T. asperelloides*	42.86 d	31.15 b	27.50 f
CEN1532	*T. asperelloides*	42.18 d	28.96 b	45.84 c
CEN1561	*T. austroindianum*	42.18 d	19.13 c	44.64 c
CEN1555	*T. rifaii*	40.14 d	14.75 d	46.60 c
CEN290	*T. asperellum*	40.14 d	31.15 b	43.93 c
CEN201	*T. lentiforme*	39.46 d	28.96 b	39.21 d
CEN1294	*T. erinaceum*	38.78 d	33.88 a	65.88 a
CEN1558	*T. erinaceum*	38.78 d	14.75 d	48.54 c
CEN1245	*T. brevicompactum*	29.93 f	22.95 c	23.15 f
CEN1274	*T. brevicompactum*	29.25 f	9.29 d	21.38 f
CEN1515	*T. hortense*	27.21 f	24.04 c	43.72 c
CEN1550	*T. ghanense*	13.61 g	22.95 c	12.77 f
CEN242	*T. rifaii*	33.33 e	26.23 b	44.43 c
CEN316	*T. rifaii*	34.01 e	29.51 b	42.62 c
CEN239	*T. rifaii*	34.01 e	18.58 c	34.31 e
CEN238	*T. rifaii*	36.73 e	22.40 c	34.06 e
CEN240	*T. rifaii*	33.33 e	26.78 b	32.37 e
CEN1544	*T. brevicompactum*	35.37 e	19.67 c	31.29 e
CV** (%)		10.78	13.10	10.68

*Values followed by the same letter in the column do not differ from each other using the Scott-Knott test at the 0.05% level. **Coefficient of variation

Greenhouse tests with *Sclerotinia sclerotiorum* and *Sclerotium rolfsii*

When the same 60 *Trichoderma* strains were tested against *S. sclerotiorum* and *S. rolfsii* in a greenhouse, 17 of them (CEN1242, CEN1277, CEN1282, CEN1283, CEN1293, CEN1532, CEN1542, CEN1550, CEN1559, CEN197, CEN208, CEN209, CEN221, CEN226, CEN230, CEN288, and CEN316) showed average percentages of live seedlings similar to the controls (C1 and C2), both without pathogen application (Table 2). Among these, 11 strains (CEN1282, CEN1283, CEN1293, CEN1532, CEN1559, CEN197, CEN208, CEN209, CEN230, CEN288, and CEN316) also stood out in terms of the emergence speed index (ESI). Five other strains are included among those that stood out in this regard (CEN141, CNE255, CEN281, CEN1245, and CEN1336), as shown in Table 2.

Table 2 - Average values of percentages of live seedlings and emergence speed index (ESI), transformed by the square root of x + 0.5 in the suppression of *Sclerotium rolfsii* (CEN216) and *Sclerotinia sclerotiorum* (CEN1147) in a greenhouse

Treatments	CEN216		CEN1147	
	Live	ESI	Live	ESI
Trichoderma sp. CEN1283	1.20 a*	0.93 a	1.08 a	0.86 b
T. asperelloides CEN1532	1.15 a	0.98 a	0.95 b	0.84 b
T. rifaii CEN288	1.20 a	0.99 a	1.00 b	0.82 c
T. asperelloides CEN1559	1.13 a	1.00 a	1.00 b	0.81 c
T. koningiopsis CEN203	0.93 b	0.86 b	1.19 a	0.81 c
T. afroharzianum CEN230	1.22 a	0.99 a	0.91 b	0.80 c
T. azevedoi CEN1242	1.10 a	0.85 b	0.91 b	0.80 c
T. azevedoi CEN1282	1.15 a	0.96 a	0.90 b	0.79 c
T. afroharzianum CEN197	1.15 a	0.94 a	0.94 b	0.79 c
T. rifaii CEN316	1.10 a	0.94 a	0.90 b	0.79 c
T. koningiopsis CEN209	1.07 a	0.93 a	1.03 b	0.79 c
T. peberdyi CEN211	0.94 b	0.88 b	0.90 b	0.79 c
T. koningiopsis CEN1513	0.99 b	0.87 b	1.11 a	0.79 c
T. azevedoi CEN1293	1.13 a	0.97 a	0.91 b	0.78 c
Trichoderma sp. CEN208	1.05 a	0.92 a	0.90 b	0.77 c
T. brevicompactum CEN1245	0.99 b	0.91 a	0.91 b	0.77 c
T. afroharzianum CEN254	1.15 a	0.99 a	0.86 c	0.76 c
T. afroharzianum CEN281	1.13 a	0.96 a	0.80 c	0.76 c
T. lentiforme CEN1336	1.07 a	0.93 a	0.81 c	0.76 c
T. afarasin CEN141	1.15 a	0.93 a	0.76 c	0.76 c
T. brevicompactum CEN1274	0.99 b	0.90 b	0.91 b	0.76 c
T. azevedoi CEN1241	0.84 b	0.79 b	0.86 c	0.76 c
T. ghanense CEN1550	1.08 a	0.94 a	1.11 a	0.75 d
T. brevicompactum CEN1544	0.91 b	0.81 b	0.81 c	0.75 d
T. asperelloides CEN1542	1.05 a	0.89 b	0.99 b	0.75 c
T. asperelloides CEN1514	1.00 b	0.88 b	0.90 b	0.75 c
T. peberdyi CEN225	0.95 b	0.82 b	0.94 b	0.75 c
T. afroharzianum CEN1546	1.20 a	1.03 a	0.86 c	0.74 d
T. afroharzianum CEN287	1.20 a	1.01 a	0.87 c	0.74 d
T. asperelloides CEN1277	1.10 a	0.97 a	1.00 b	0.74 d

Continuation Table 2

T. austroindianum CEN1555	1.03 a	0.86 b	0.76 c	0.74 d
T. asperelloides CEN162	0.97 b	0.85 b	0.94 b	0.74 d
T. afroharzianum CEN234	1.15 a	1.01 a	0.81 c	0.73 d
T. azevedoi CEN168	1.18 a	0.98 a	0.86 c	0.73 d
T. afroharzianum CEN158	1.18 a	0.98 a	0.81 c	0.73 d
T. lentiforme CEN1294	1.15 a	0.98 a	0.76 c	0.73 d
Trichoderma sp. CEN221	1.05 a	0.95 a	0.94 b	0.73 d
T. peberdyi CEN256	0.91 b	0.87 b	0.91 b	0.73 d
T. hortense CEN1515	1.10 a	0.86 b	0.76 c	0.73 d
T. peberdyi CEN198	1.05 a	0.85 b	0.86 c	0.73 d
T. longibrachiatum CEN1562	0.91 b	0.81 b	0.76 c	0.73 d
T. afroharzianum CEN235	1.10 a	0.97 a	0.81 c	0.72 d
T. peberdyi CEN226	1.05 a	0.93 a	0.91 b	0.72 d
T. afroharzianum CEN1417	1.13 a	0.92 a	0.76 c	0.72 d
T. peberdyi CEN228	0.97 b	0.85 b	0.81 c	0.72 d
T. afroharzianum CEN155	1.15 a	1.01 a	0.71 c	0.71 d
T. lentiforme CEN223	1.15 a	1.00 a	0.71 c	0.71 d
T. atroviride CEN144	1.08 a	0.99 a	0.71 c	0.71 d
T. peberdyi CEN232	1.18 a	0.96 a	0.71 c	0.71 d
T. asperelloides CEN1533	1.10 a	0.93 a	0.71 c	0.71 d
T. austroindianum CEN1561	1.10 a	0.93 a	0.71 c	0.71 d
T. rifaii CEN202	1.13 a	0.92 a	0.71 c	0.71 d
T. asperellum CEN201	1.03 a	0.88 b	0.76 c	0.71 d
T. rifaii CEN240	0.97 b	0.85 b	0.71 c	0.71 d
T. rifaii CEN239	0.97 b	0.84 b	0.76 c	0.71 d
T. rifaii CEN290	0.91 b	0.83 b	0.71 c	0.71 d
T. erinaceum CEN1558	0.90 b	0.82 b	0.71 c	0.71 d
T. rifaii CEN238	0.85 b	0.81 b	0.71 c	0.71 d
T. longibrachiatum CEN1399	0.86 b	0.77 b	0.71 c	0.71 d
T. rifaii CEN242	0.80 b	0.75 b	0.71 c	0.71 d
C1	1.22 a	1.08 a	1.22 a	0.99 a
C2	1.22 a	1.08 a	1.22 a	0.97 a
C3	0.71 b	0.71 b	0.71 c	0.71 d
CV** (%)	9.67	7.31	12.76	5.56

C1 = vermiculite + ground rice uncolonized; C2 = vermiculite alone; and C3 + vermiculite + ground rice colonized with *S. sclerotiorum* or *S. rolfsii*. *Values followed by the same letter in the column do not differ from each other using the Scott-Knott test at the 0.05% level. **Coefficient of variation

Among the 60 *Trichoderma* strains, 16 were selected based on the previous results (CEN1277, CEN1283, CEN1294, CEN1532, CEN209, CEN221, CEN230, CEN234, CEN287, CEN288, CEN1546, CEN1513, CEN235, CEN281, CEN1242, CEN1559). These showed better performance in terms of percentage inhibition of mycelial growth *in vitro* and/or a higher number of live seedlings in the greenhouse in the presence of *S. sclerotiorum* CEN1147 and *S. rolfsii* CEN216 (Tables 1 and 2). These strains were then tested against *Fusarium* spp. in the greenhouse tests.

Greenhouse tests with *Fusarium* spp.

The 16 strains selected from dual culture experiment and greenhouse tests with *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* were tested for their ability to suppress *F. crassistipitatum* and *Fusarium* sp. in the greenhouse. There were no significant differences in treatments inoculated with *F. crassistipitatum* (Table 3). However, in treatments inoculated with *Fusarium* sp., strains CEN1277 (*T. asperelloides*), CEN1283 (*Trichoderma* sp.), CEN209 (*T. afroharzianum*), CEN1546 (*T. koningiopsis*), CEN221 (*Trichoderma* sp.), CEN235 (*T. afroharzianum*), CEN281 (*T. afroharzianum*), CEN1294 (*T. lentiforme*), CEN1532 (*T. asperelloides*), and CEN287 (*T. afroharzianum*) showed emergence speed index (ESI) values similar to those obtained with the non-inoculated Control treatments (C1 and C2), while strains CEN1242 (*T. azevedoi)*, CEN230 (*T. afroharzianum*), CEN288 (*T. rifaii*), CEN1513 (*T. koningiopsis*), CEN1559 (*T. asperelloides*), and CEN234 (*T. afroharzianum*) exhibited average ESI values similar to those of the Control (C3) inoculated with the pathogen (Table 3).

Agronomic performance of soybean plants under field conditions

Ten *Trichoderma* spp. strains were chosen from the 60 strains for evaluation of soybean agronomic performance under field conditions. The selection of these strains considered their performance, albeit variable, against the pathogens *S. sclerotiorum*, *S. rolfsii*, and *Fusarium* spp., as observed in the *in vitro* and greenhouse tests, shown in Tables 1, 2, and 3.

The plant height, measured at 60 DAP, ranged from 80 to 88 cm, and no statistical difference was detected between the treatments. As for the number of nodules counted at 70 DAP, the data showed little consistency, as many roots were damaged during harvesting and/or handling (data not shown).

Table 3 - Average values of percentages of live seedlings and emergence speed index (ESI), transformed by the square root of x+0.5 for the suppression of *F. crassistipitatum* (CEN1636) and *Fusarium* sp. (CEN1631) in soybeans

Treatments	CEN1636		CEN1631	
	live	ESI	live	ESI
T. asperelloides CEN1277	1.19 a*	0.88 a	1.22 a	0.90 a
Trichoderma sp. CEN1283	1.22 a	0.89 a	1.22 a	0.89 a
T. koningiopsis CEN209	1.22 a	0.87 a	1.22 a	0.89 a
T. afroharzianum CEN1546	1.22 a	0.90 a	1.22 a	0.89 a
Trichoderma sp. CEN221	1.19 a	0.85 a	1.19 a	0.88 a
T. afroharzianum CEN235	1.22 a	0.89 a	1.22 a	0.88 a
T. afroharzianum CEN281	1.19 a	0.88 a	1.19 a	0.87 a
T. lentiforme CEN1294	1.22 a	0.88 a	1.22 a	0.86 a
T. asperelloides CEN1532	1.15 a	0.88 a	1.15 a	0.86 a
T. afroharzianum CEN287	1.22 a	0.87 a	1.15 a	0.86 a
T. azevedoi CEN1242	1.15 a	0.81 a	1.19 a	0.85 b
T. afroharzianum CEN230	1.22 a	0.87 a	1.19 a	0.84 b
T. rifaii CEN288	1.15 a	0.86 a	1.15 a	0.84 b
T. koningiopsis CEN1513	1.19 a	0.88 a	1.22 a	0.84 b
T. asperelloides CEN1559	1.19 a	0.86 a	1.19 a	0.84 b
T. afroharzianum CEN234	1.19 a	0.86 a	1.15 a	0.81 b
C1	1.19 a	0.80 a	1.22 a	0.89 a
C2	1.22 a	0.90 a	1.19 a	0.89 a
C3	1.15 a	0.86 a	1.22 a	0.81 b
CV** (%)	5.80	4.00	5.19	3.55

C1 = vermiculite + ground rice uncolonized; C2 = vermiculite alone; and C3 = vermiculite + ground rice colonized with *F. crassistipitatum* or *Fusarium* sp. *Values followed by the same letter in the column do not differ from each other using the Scott-Knott test at the 0.05% level. **Coefficient of variation

Regarding the number of internodes and pods, it was observed that *T. afroharzianum* (CEN1546) had no significant effect compared to the control in any of the studied periods. In the 2020-2021 season, this was also observed with *T. afroharzianum* (CEN287), *T. azevedoi* (CEN1242), and *T. koningiopsis* (CEN1513), as shown in Table 4. In the latter season, no influence of any of the strains on the number of pods was detected.

Based on the dry weight of grains per treatment, at harvest conducted at 120 DAP, the yield was estimated in kilograms and sacks per hectare, and from these data, the increase in sacks per hectare compared to the control treatment was calculated (Table 5).

Table 4 - Mean values of internode and pod numbers per plant treated with *Trichoderma* strains and untreated (Control), seasons 2019-2020 and 2020-2021

Treatments	Season 2019-2020		Season 2020-2021	
	Internodes	Pods	Internodes	Pods
T. afroharzianum CEN1546	15 a*	51 a	13 a	48 a
T. afroharzianum CEN234	13 b	44 b	12 b	63 a
T. azevedoi CEN1242	13 b	45 b	13 a	45 a
T. lentiforme CEN1294	13 b	45 b	11 b	56 a
T. asperelloides CEN162	13 c	43 b	12 b	64 a
T. asperelloides CEN1277	12 c	35 c	12 b	75 a
T. asperelloides CEN1533	12 c	41 b	12 b	57 a
T. afroharzianum CEN287	12 c	42 b	14 a	53 a
T. koningiopsis CEN1513	11 c	33 c	14 a	59 a
T. asperelloides CEN1532	11 c	34 c	11 b	59 a
Control	14 a	55 a	13 a	50 a
CV** (%)	14.29	32.68	8.00	27.22

*Values followed by the same letter in the column do not differ from each other using the Scott-Knott test at the 0.05% level. **Coefficient of variation

Table 5 - Dry weight of grains (g), estimated yield (Kg ha^{-1} and Sc ha^{-1}), and increase/decrease (Sc ha^{-1}), in yield, observed in field-applied treatments compared to the control, seasons 2019-2020 and 2020-2021

Treatments	Season 2019-2020				Season 2020-2021			
	Dry Weight (g)	Yield		Increase/Decrease	Dry Weight (g)	Yield		Increase/Decrease
		Kg ha^{-1}	Sc ha^{-1}	Sc ha^{-1}		Kg ha^{-1}	Sc ha^{-1}	Sc ha^{-1}
T. afroharzianum CEN287	1038.9 a*	2985.47	49.8	-3.1	4993.4 a	4993.4	83.2	14.8
T. azevedoi CEN1242	1195.4 a	3434.94	57.2	4.3	4990.2 a	4990.2	83.2	14.8
T. koningiopsis CEN1513	1172.6 a	3369.42	56.2	3.3	4640.7 a	4640.7	77.3	9.0
T. asperelloides CEN162	1073.5 a	3084.70	51.4	-1.5	4483.7 a	4483.7	74.7	6.3
T. asperelloides CEN1533	1058.9 a	3042.91	50.7	-2.2	3887.6 b	3887.6	64.8	-3.6
T. afroharzianum CEN1546	1350.6 a	3881.15	64.7	11.8	3853.8 b	3853.8	64.2	-4.2
T. *asperelloides* CEN1532	1159.7 a	3332.46	55.5	2.6	3617.1 b	3617.1	60.3	-8.1
T. lentiforme CEN1294	1088.2 a	3126.90	52.1	-0.8	3568.3 b	3568.3	59.5	-8.9
T. afroharzianum CEN234	1031.6 a	2964.27	49.4	-3.5	3478.7 b	3478.7	58.0	-10.4
T. asperelloides CEN1277	1242.2 a	3569.60	59.5	6.6	3090.0 b	3090.0	51.5	-16.9
Control	1104.6 a	3174.27	52.9	0.0	4103.3 b	4103.3	68.4	0.0
CV** (%)	18.63	-	-	-	11.71	-	-	-

*Values followed by the same letter in the column do not differ from each other using the Scott-Knott test at the 0.05% level. **Coefficient of variation

In the 2019-2020 season, there was no significant difference between the treatments regarding the dry weight of grains. However, compared to the control treatment, there were increases or decreases in production, as shown in Table 5. For example, for strains CEN1546 (*T. afroharzianum*), an increase of 11.8 sacks/ha is estimated. This data, although not significant in terms of dry weight of grains, represents a substantial increase in soybean production.

In the 2020-2021 season, statistical differences were observed regarding the dry weight of grains. Plants treated with *Trichoderma* showed increases or decreases in production compared to the control treatment (Table 5). Strains CEN287 (*T. afroharzianum*), CEN1242 (*T. azevedoi*), CEN1513 (*T. koningiopsis*), and CEN162 (*T. asperelloides*) stood out, differing statistically from the control treatment, and providing, in estimation, 14.8, 14.8, 9.0, and 6.3 sacks per hectare, respectively.

Strains CEN1242 and CEN1513 showed estimated increases in productivity in both seasons, unlike strains CEN1546, whose estimated productivity increase was not confirmed in the 2020-2021 season. On the other hand, strains CEN287 and CEN162 showed estimated decreases in the 2019-2020 season and increases in the following season (Table 5). The inconsistency in the results can be attributed to the use of biological material not formulated in the study. Developing formulations is crucial for stabilizing the strains against climatic variations, particularly.

The average increase of 11.3 sacks per hectare represents a substantial financial gain for the producer, and it is of utmost importance to stabilize these strains through appropriate formulations that ensure consistent results.

The dual culture method is routinely used to initiate the selection of *Trichoderma* strains for biological control (Korkom; Yildiz, 2022; Stracquadanio *et al*., 2020). The results obtained here demonstrate that the inhibition of mycelial growth *in vitro* may reflect the mechanisms of action employed by different strains of biocontrol agents against a specific phytopathogen. These mechanisms of action are diverse, such as parasitism, antibiosis and competition for space, light and nutrients. This is because it may be related to the growth rate and the ability to compete for space of both colonies or the antagonist's ability to parasitize the hyphae and survival structures of the pathogen (Harman *et al*., 2004; Rabinal; Bhat, 2020; Silva *et al*., 2022b).

Several studies aimed at selecting biological control agents begin with a significant number of antagonist strains, and after the dual culture test, only a small percentage of the total is taken to the greenhouse and/or field. In this context, some strains that did not show good results in the laboratory are excluded from in vivo tests, but it may happen that the results obtained in the laboratory do not repeat in the field or vice versa. Therefore, in this study, despite statistical differences between *Trichoderma* spp. strains in inhibiting the mycelial growth of *S. sclerotiorum* and *S. rolfsii* in dual culture, it was decided to test all 60 strains in the greenhouse. Additionally, the fact that the various mechanisms of action used by *Trichoderma* spp., such as induced resistance and parasitism, can act together in the interaction with the target pathogen (Ramírez-Valdespino *et al*., 2018), adds support to this decision.

The results of the present study regarding *S. sclerotiorum* are consistent with Karimi and Altinok (2019), Elnhas *et al*. (2020), and Hou *et al*. (2021), who obtained mean mycelial inhibition rates of *S. sclerotiorum* in the dual culture of 40.56%, 45.97%, and 57.41%, respectively. Fungi of the genus *Trichoderma* can produce various cell wall degradation enzymes, including chitinase, cellulase, xylanase, glucanase, and protease, to break down and dissolve the mycelium of the target pathogen (Gruber; Seidl-Seiboth, 2012). In addition to this mechanism of direct attack on the pathogen (hyperparasitism), other mechanisms may act alone or together, such as antibiosis and competition, for example, for physical space, water, and nutrients.

When comparing the results of dual culture with *S. sclerotiorum* and the variable "live plants" in the greenhouse experiment, it was found that only *T. koningiopsis* CEN1513 remained in the best statistical grouping in both experiments (Tables 1 and 2). For *S. rolfsii*, a larger group of strains showed superior performance in both the *in vitro* and in vivo tests: CEN144, CEN197, CEN209, CEN221, CEN230, CEN234, CEN235, CEN254, CEN281, CEN287, CEN288, CEN1282, CEN1293, CEN1294, CEN1336, CEN1533, CEN1542, and CEN1546 (Tables 1 and 2). Additionally, *T. ghanense* CEN1550 was the least efficient strains in the *in vitro* test with *S. sclerotiorum*, but in the in vivo experiment, it was in the superior grouping for the variable of live plants (Tables 1 and 2). The ability of *Trichoderma* strains to suppress or reduce the severity and symptoms of white mold in soybean has been previously demonstrated. Sumida *et al*. (2018) showed in field conditions that *T. asperelloides* (T25 and T42) and *T. harzianum* (commercial product) reduced white mold incidence by 37.6%, 32.4%, and 22% respectively, compared to the untreated control, and disease severity by 37.9%, 36.3%, and 37.6%, respectively. Another commercial product based on *T. harzianum* also showed a significant effect only on disease severity. These authors suggest that the biocontrol ability of *Trichoderma* may vary depending on the strain of *S. sclerotiorum* used, linked to the geographical location from where this pathogen was strains, and with the types of metabolites and lytic enzymes produced by *Trichoderma* strains.

Similar to the results obtained by Sridharan *et al*. (2020) and Rajani *et al*. (2021), the present study also demonstrated the ability of *Trichoderma* spp. to inhibit the mycelial growth of *S. rolfsii*. In studies conducted by Hua *et al*. (2021) to elucidate the antagonistic mechanism of *T. virens* T23 in dual culture with *S. rolfsii*, a mutant of T23 deficient in gliotoxin was used, constructed via *Agrobacterium tumefaciens*. This resulted in gliotoxin deficiency and attenuation of the antagonistic effect against *S. rolfsii*, indicating that gliotoxin is an important antifungal metabolite of *T. virens*. The same authors also inferred that the low growth rate of the T23 strain suggests that competition is probably not the main factor for its antagonistic effect in inhibiting the mycelial growth of the pathogen.

The data obtained in this study are consistent with the results obtained by Mishra *et al*. (2011), in greenhouse experiments with soil contaminated by *S. rolfsii*, where the authors demonstrated that a mixed formulation of *T. harzianum* and the bacterium *Pseudomonas fluorescens* resulted in lower soybean seedling mortality, with an average of 16.15% compared to 67.30% in the control treatment. The individual treatment with *T. harzianum* showed a mortality rate of 28.14%. In the study conducted by Auler, Carvalho and Mello (2013), some strains of *T. harzianum* were able to ensure 100% survival of soybean plants after 15 days of inoculation with *S. rolfsii*. Therefore, the efficiency of *Trichoderma* in disease control may be related to different factors, such as temperature, humidity, nutrients, soil type, microbiota, aeration, pH, and organic matter content, which influence the survival of *Trichoderma* in soil or substrate (Howell, 2003).

The antagonism of *Trichoderma* spp. against *F. crassistipitatum* in dual culture occurred with all tested *Trichoderma* strains, with inhibition of the pathogen's mycelial growth reaching up to 69.17%. Perveen and Bokhari (2012) tested strains of *T. viride* and *T. harzianum* against another *Fusarium* species (*F. oxysporum*) and found average values of mycelial growth inhibition ranging from 66.3 to 56.43%. In addition to mycelial inhibition, Barreto *et al*. (2021) reported the ability of *Trichoderma* spp. to reduce the sporulation of *Fusarium* spp. The physical action of *T. harzianum* was also observed in interaction with *F. chlamydosporum* by Yassin, Mostafa and Al-Askar (2021). These authors observed adherence and coiling of the antagonist on the pathogen's mycelium, as well as penetration into the mycelium using an appressorium-like structure. Enzymatic action was demonstrated by the degradation of *F. chlamydosporum* mycelium. The increase in secretion or expression of chitinases and α-1,3-glucanase during interactions of *Trichoderma* spp. with *F. oxysporum* and *T. harzianum* KMISO2-2-19A with *F. virguliforme* suggests that these enzymes are involved in mycoparasitic activity against this pathogen (Ojha; Chatterjee, 2011; Pimentel *et al*., 2020). Studies of this nature should be conducted with *Trichoderma* strains in confrontation with the *F. crassistipitatum* pathogen to clarify the mechanism of action involved.

Despite the promising *in vitro* results against *F. crassistipitatum*, it was not possible to evaluate the biocontrol capacity of *Trichoderma* spp. against *Fusarium* spp. in greenhouse experiments up to 10 days after emergence due to the absence of symptoms caused by the pathogen. The authors Zhang *et al*. (2017), observed that *T. harzianum* T-soja reduced disease severity in soybean plants caused by *F. oxysporum* to 16.0% compared to 34.5% in control plants on the 7th day after inoculation, and to 26.8% on the 20 th day. Treatment with T-soja also significantly reduced infection, with average biocontrol efficiency of 53.6% and 39.2% on the 7 th and 20 th day, respectively. Another study demonstrated the ability of *Trichoderma* spp. to reduce the amount of *F. virguliforme* present in soybean roots by over 50% under greenhouse conditions (Pimentel *et al*., 2020). The divergence between the results may be related to the methodologies used in each study and indicates the need to extend the observation period, as *Fusarium* species can attack at more advanced stages of the crop cycle.

In the study by Zhang *et al*. (2017), soybean roots aged 10 days were pierced with a sterile needle for subsequent inoculation. The opening of wounds may have favored the pathogen, thus enabling the evaluation of infection rates and severity in the seedling phase. On the other hand, the methodology of Pimentel *et al*. (2020) resembles that used in the present study; however, the pathogen inoculum was obtained from cultivation on sorghum grains. The evaluation was conducted solely by quantifying the pathogen in soybean roots through quantitative PCR (q-PCR) with specific primers for *F. virguliforme*, thus not depending on the presence of symptoms. As demonstrated, the only difference observed in this study was for the ESI variable with *Fusarium* sp. CEN1631. This may indicate that the presence of the pathogen, although not affecting the initial stand of the crop, may interfere with the initial stand establishment, and the pathogen may present problems at any time throughout the crop cycle.

Regarding the effect of the 10 *Trichoderma* spp. strains tested on soybean grain yield under field conditions, strains CEN1242 and CEN1513, belonging to the species *T. azevedoi* and *T. koningiopsis*, respectively, stood out. These strains showed an estimated increase in productivity in both seasons compared to the control treatment. Recently, it was reported that *T. koningiopsis* CEN1513 promotes seedling growth and increases onion productivity in greenhouse conditions (Silva *et al*., 2022a). The ability of *Trichoderma* to promote soybean growth is

well documented (John *et al*., 2010; Kuchlan; Kuchlan; Ansari, 2019; Sarzi *et al*., 2019). According to Zhang *et al*. (2017), inoculation of soybean plants with *T. harzianum* T-soja resulted in significant increases in dry weight, fresh weight, and number of lateral roots, suggesting that the activities of urease, invertase, catalase, and cellulase significantly increased, indicating that T-soja may promote soybean growth by improving the rhizosphere environment and consequently increasing nutrient uptake by plants. Another study using *T. longibrachiatum* and *T. simmonsii* demonstrated that growth promotion in soybean may be related to increased potassium uptake by plants (Bakhshandeh *et al*., 2020). Additionally, Mishra, Singh and Arora (2017) reported that plant growth-promoting microorganisms can enhance vegetable yield by producing siderophores and hydrogen cyanide, which are known plant defense regulators against pathogens.

Obtaining *Trichoderma* spp. with the ability to control soil pathogens and promote plant growth simultaneously is a challenging objective in many studies involving fungi of this genus. After an extensive study involving 60 *Trichoderma* strains, we observed that meeting this demand is not a simple task. The results varied considerably concerning the target pathogen and the ability to improve agronomic parameters of soybean plants under field conditions. This underscores the importance of conducting studies to formulate commercial products containing multiple *Trichoderma* strains, to combine different mechanisms of action of the biocontrol agent, such as parasitism, antibiosis, and competition, along with the ability to promote plant growth.

CONCLUSIONS

1. The biocontrol capacity of *Trichoderma* strains against *S. sclerotiorum*, *S. rolfsii*, and *Fusarium* spp. varies depending on the interaction with the target pathogen;
2. Strains that showed better effects on pathogens did not always yield better results in the agronomic performance of the plants;
3. The strains that showed the best performance under *in vitro* and greenhouse conditions against *S. sclerotiorum* and *S. rolfsii* were *T. afroharzianum* CEN230, *T. koningiopsis* CEN209, and *T. rifaii* CEN288;
4. The strains *T. azevedoi* CEN1242 and *T. koningiopsis* CEN1513 significantly increased soybean final yield in field conditions;
5. The strains that showed the best effects on pathogens and those that demonstrated better agronomic performance should be tested individually and in mixtures in field trials, preferably in formulations.

ACKNOWLEDGMENTS

The authors would like to thank the National Council for Scientific and Technological Development (CNPq) for the Industrial Technological Development (DTI A) fellowship granted to the first author; the Foundation for Research Support of the Federal District (FAPDF); and the company Bionat Biological Solutions for the financial support.

REFERENCES

ALFIKY, A.; WEISSKOPF, L. Deciphering *Trichoderm*a-plant-pathogen interactions for better development of biocontrol applications. **Journal of Fungi**, v. 7, n. 1, p. 1-18, 2021.

AOKI, T. *et al*. Sudden-death syndrome of soybean is caused by two morphologically and phylogenetically distinct species within the *Fusarium solani* species complex: *F. virguliforme* in North America and *F. tucumaniae* in South America. **Mycologia**, v. 95, n. 4, p. 660-684, 2003.

AULER, A. C. V.; CARVALHO, D. D. C.; MELLO, S. C. M. Antagonismo de *Trichoderma harzianum* a *Sclerotium rolfsii* nas culturas do feijoeiro e soja. **Revista Agro@mbiente On-line**, v. 7, n. 3, p. 359-365, 2013.

BAKHSHANDEH, E. *et al*. Plant growth promoting microorganisms can improve germination, seedling growth and potassium uptake of soybean under drought and salt stress. **Plant Growth Regulation**, v. 90, p. 123-136, 2020.

BARRETO, G. G. *et al*. Antagonism of *Trichoderma* on the control of *Fusarium* spp. on *Phaseolus lunatus* L. **Acta Brasiliensis**, v. 5, n. 2, p. 57-64, 2021.

BETTIOL, W.; MEDEIROS, F. H. V. **Como o Brasil se tornou o maior produtor e consumidor de produtos de biocontrole. 2023. Disponível em:** https://www.embrapa.br/busca-de-noticias/-/noticia/79156418/artigo-como-o-brasil-se-tornou-o-maior-produtor-e-consumidor-de-produtos-de-biocontrole**. Acesso em: 9 nov. 2023.**

CARVALHO, D. D. C. *et al*. Biological control of white mold by *Trichoderma harzianum* in common bean under field conditions. **Pesquisa Agropecuária Brasileira**, v. 50, n. 12, p. 1220-1224, 2015.

CLEMENTE, T. E.; CAHOON, E. Soybean oil: genetic approaches for modification of functionality and total content. **Plant Phyisiology**, v. 151, n. 3, p. 1030-1040, 2009.

COMPANHIA NACIONAL DE ABASTECIMENTO (CONAB). **Boletim da safra de grãos**. 2025. Disponível em: https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de-graos. Acesso em: 20 janeiro 2025.

DALL´AGNOL, A. **A Embrapa Soja no contexto do desenvolvimento da soja no Brasil**: histórico e contribuições. Londrina: Embrapa Soja, 2016. 72 p.

DENNIS, C. WEBSTER, J. Antagonistic properties of species-groups of *Trichoderma*. I. Production of non-volatile

antibiotic. **Transactions British Mycological Society**, v. 57, n. 1, p. 25-39, 1971.

ELNHAS, G. A. *et al*. Antifungal potentiality and physiological characterization of *Trichoderma* isolates from Port Said Governorate. **Egyptian Journal of Botany**, v. 60, n. 1, p. 55-69, 2020.

GRUBER, S.; SEIDL-SEIBOTH, V. Self-versus non-self: fungal cell wall degradation in *Trichoderma*. **Microbiology**, v. 158, n. 1, p. 26-34, 2012.

HAN, S. *et al*. *Bacillus subtilis* HSY21 can reduce soybean root rot and inhibit the expression of genes related to the pathogenicity of *Fusarium oxysporum*. **Pesticide Biochemistry and Physiology**, v. 178, p. 1-12, 2021.

HARMAN, G. E. *et al*. *Trichoderma* species-opportunistic, avirulent plant symbionts. **Nature Reviews Microbiology**, v. 2, p. 43-56, 2004.

HOU, X. Y. *et al*. A native *Trichoderma harzianum* strain Th62 displays antagonistic activities against phytopathogenic fungi and promotes the growth of *Celosia cristata*. **Horticulture, Environment, and Biotechnology**, v. 62, p. 169-179, 2021.

HOWELL, C. R. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. **Plant Disease**, v. 87, n. 1, p. 4-10, 2003.

HUA, L. *et al*. Gliotoxin is an important metabolite involved in suppression of *Sclerotium rolfsii* of *Trichoderma virens* T23. **Phytopathology**, v. 111, n. 10, p. 1720-1725, 2021.

JOHN, R. P. *et al*. Mycoparasitic *Trichoderma viride* as a biocontrol agent against *Fusarium oxysporum* f. sp. *adzuki* and *Pythium arrhenomanes* and as a growth promoter of soybean. **Crop Protection**, v. 29, n. 12, p. 1452-1459, 2010.

KARIMI, A. K.; ALTINOK, H. H. *In vitro* antifungal activity of *Trichoderma harzianum rifai* and PGRP strains as biocontrol agents against gray mold and white mold in eggplant. **Fresenius Environmental Bulletin**, v. 28, n. 8, p. 6166-6173, 2019.

KHAN, R. A. A. *et al*. Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic fungi. **Microorganisms**, v. 8, n. 6, p. 1-22, 2020.

KORKOM, Y.; YILDIZ, A. Evaluation of biocontrol potential of native *Trichoderma* isolates against charcoal rot of strawberry. **Journal of Plant Pathology**, v. 104, p. 671-682, 2022.

KUCHLAN, P.; KUCHLAN, M. K.; ANSARI, M. M. Efficient application of *Trichoderma viride* on soybean [*Glycine max* (L.) Merrill] seed using thin layer polymer coating. **Legume Research**, v. 42, n. 2, p. 250-259, 2019.

MACENA, A. M. F. *et al*. Antagonism of *Trichoderma*-based biofungicides against brazilian and north american isolates of *Sclerotinia sclerotiorum* and growth promotion of soybean. **BioControl**, v. 65, p. 235-246, 2020.

MAHARTHA, K. A.; SUPRAPTA, D. N. Efficacy of *Enterobacter cloacae* KtB3 to control damping-off disease on soybean caused by *Sclerotium rolfsii*. **International Journal of Agriculture & Biology**, v. 20, n. 4, p. 871-876, 2018.

MARQUES, E.; MARTINS, I.; MELLO, S. C. M. Antifungal potential of crude extracts of *Trichoderma* spp. **Biota Neotropica**, v. 18, n. 1, p. 1-5, 2018.

MEENA, M. *et al*. Antagonistic assessment of *Trichoderma* spp. by producing volatile and non-volatile compounds against different fungal pathogens. **Archives of Phytopathology and Plant Protection**, v. 50, n. 13/14, p. 629-648, 2017.

MISHRA, D. S. *et al*. Combination of fungal and bacterial antagonists for management of root and stem rot disease of soybean. **Pakistan Journal of Botany**, v. 43, n. 5, p. 2569-2574, 2011.

MISHRA, J.; SINGH, R.; ARORA, N. K. Plant growth-promoting microbes: diverse roles in agriculture and environmental sustainability. *In:* MISHRA, J.; SINGH, R.; ARORA, N. K. **Probiotics and plant health**. Singapore: Springer, 2017. p. 71-111.

O'BRIEN, P. A. Biological control of plant diseases. **Australasian Plant Pathology**, v. 46, p. 293-304, 2017.

OJHA, S.; CHATTERJEE, N. C. Mycoparasitism of *Trichoderma* spp. in biocontrol of fusarial wilt of tomato. **Archives of Phytopathology and Plant Protection**, v. 44, n. 8, p. 771-782, 2011.

PERVEEN, K.; BOKHARI, N. A. Antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* isolated from soil of date palm field against *Fusarium oxysporum*. **African Journal of Microbiology Research**, v. 6, p. 3348-3353, 2012.

PIMENTEL, M. F. *et al*. Identification of differentially expressed genes in *Trichoderma koningii* IABT1252 during its interaction with *Sclerotium rolfsii*. **Current Microbiology**, v. 77, p. 396-404, 2020.

RAJANI, P. *et al*. Inhibition of plant pathogenic fungi by endophytic *Trichoderma* spp. through mycoparasitism and volatile organic compounds. **Microbiological Research**, v. 242, p. 1-12, 2021.

RAMÍREZ-VALDESPINO, C. A. *et al*. Functional characterization of TvCyt2, a member of the p450 monooxygenases from *Trichoderma virens* relevant during the association with plants and mycoparasitism. **Molecular Plant-Microbe Interactions**, v. 31, n. 3, p. 289-298, 2018.

RIVERA-MÉNDEZ, W.; BRENES-MADRIZ, J.; ALVARADO-MARCHENA, L. Effect of *Setophoma terrestris*, *Sclerotium cepivorum*, and *Trichoderma* spp. on *in vitro* onion (*Allium cepa*) root tissues and the final yield at the field. **European Journal of Plant Pathology**, v. 160, p. 53-65, 2021.

ROTH, M. G. *et al*. Integrated management of important soybean pathogens of the United States in changing climate. **Journal of Integrated Pest Management**, v. 11, n. 1, p. 1-28, 2020.

SARZI, J. S. *et al*. Biocontrole da podridão vermelha da raiz e promoção de crescimento da soja. **Nativa**, v. 7, n. 6, p. 629-635, 2019.

SILVA, L. R. *et al*. Morphological and protein alterations in *Sclerotinia sclerotiorum* (Lib.) de Bary after exposure to volatile organic compounds of *Trichoderma* spp. **Biological Control**, v. 147, p. 1-7, 2020.

SILVA, L. R. *et al*. *Sclerotium rolfsii* mycelial profile analysis by MALDI-TOF related to biological control, volatile organic

compounds diversity and onion growth promotion, as influenced by *Trichoderma* spp. **Biological Control**, v. 172, p. 1-14, 2022a.

SILVA, L. R. *et al*. *Trichoderma*: suas interações e uma abordagem metodológica aplicada ao estudo de compostos orgânicos voláteis para o mofo-branco e promoção de crescimento de plantas. **Revisão Anual de Patologia de Plantas**, v. 28, p. 163-183, 2022b.

SILVA, L. R. *et al*. Volatile organic compounds emitted by *Trichoderma azevedoi* promote the growth of lettuce plants and delay the symptoms of white mold. **Biological Control**, v. 152, p. 1-10, 2021.

SMOLIŃSKA, U.; KOWALSKA, B. Biological control of the soil-borne fungal pathogen *Sclerotinia sclerotiorum*: a review. **Journal of Plant Pathology**, v. 100, p. 1-12, 2018.

SRIDHARAN, A. P. *et al*. Comprehensive profiling of the VOCs of *Trichoderma longibrachiatum* EF5 while interacting with *Sclerotium rolfsii* and *Macrophomina phaseolina*. **Microbiological Research**, v. 236, p. 1-13, 2020.

SRIVASTAVA, M. *et al*. *Trichoderma* a potential and effective bio fungicide and alternative source against notable phytopathogens: a review. **African Journal of Agricultural Research**, v. 11, n. 5, p. 310-316, 2016.

STRACQUADANIO, C. *et al*. Antifungal activity of bioactive metabolites produced by *Trichoderma asperellum* and *Trichoderma atroviride* in liquid medium. **Journal of Fungi**, v. 6, n. 4, p. 1-18, 2020.

SUMIDA, C. H. *et al*. *Trichoderma asperelloides* antagonism to nine *Sclerotinia sclerotiorum* strains and biological control of white mold disease in soybean plants. **Biocontrol Science and Technology**, v. 28, n. 2, p. 142-156, 2018.

TOLOI, M. N. V. *et al*. Development indicators and soybean production in Brazil. **Agriculture**, v. 11, n. 11, p. 1-15, 2021.

WESTRICK, N. M. *et al*. Gene regulation of *Sclerotinia sclerotiorum* during infection of *Glycine max*: on the road to pathogenesis. **BMC Genomics**, v. 20, n. 157, p. 1-22, 2019.

WIDMER, T. L. Compatibility of *Trichoderma asperellum* isolates to selected soil fungicides. **Crop Protection**, v. 120, p. 91-96, 2019.

WONGLOM, P. *et al*. Biological control of *Sclerotium* fruit rot of snake fruit and stem rot of lettuce by *Trichoderma* sp. T76-12/2 and the mechanisms involved. **Physiological and Molecular Plant Pathology**, v. 107, p. 1-7, 2019.

YASSIN, M. T.; MOSTAFA, A. A. F.; AL-ASKAR, A. A. *In vitro* antagonistic activity of *Trichoderma harzianum* and *T. viride* strains compared to carbendazim fungicide against the fungal phytopathogens of *Sorghum bicolor* (L.) Moench. **Egyptian Journal of Biological Pest Control**, v. 31, n. 118, p. 1-9, 2021.

ZHANG, F. *et al*. *Trichoderma harzianum* containing 1-aminocyclopropane-1-carboxylate deaminase and chitinase improved growth and diminished adverse effect caused by *Fusarium oxysporum* in soybean. **Journal of Plant Physiology**, v. 210, p. 84-94, 2017.