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Original Article

In vitro effect of native *Trichoderma spp.* strains on *Sclerotium sp.* and *Fusarium sp.*

Efecto de cepas nativas de *Trichoderma spp.* sobre *Sclerotium sp.* y *Fusarium sp.* evaluadas *in vitro*

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Abstract

The search for biological control alternatives against plant pathogens has increased in recent years. This study aimed to determine the biological control efficiency of six native strains of *Trichoderma* spp. isolated from different ecosystems, against *Sclerotium* sp. and *Fusarium* sp. Strains of *Trichoderma* spp. were isolated from *Persea americana*, *Coffea arabica*, *Passiflora edulis*, *Mangifera indica*, *Saccharum officinarum*, *Citrus* spp., while two pathogens, *Sclerotium sp.* and *Fusarium* sp., were extracted from *Solanum lycopersicum*. The isolates were cultured on PDA medium and incubated at 27 °C. Fungal identification was performed using taxonomic keys. The experiment followed a completely randomized design with six treatments and five replications, where the radial growth, antagonism and antibiosis of *Trichoderma* spp. on pathogens were quantified. Data were analyzed using analysis of variance and Tukey's mean separation test ($p < 0.05$).
The results showed that the native *Trichoderma* spp. strain (TCHN-22) exhibited the highest radial growth. However, strains TIAN-21, TBLN-21 and TCRN-20 were found to inhibit the growth of *Sclerotium* sp., while strains TCHN-22 and TCPN-22 exhibited enhanced control of *Fusarium sp.* Regarding antagonistic activity, strains TIAN-21 and TCSN-22 showed higher levels, whereas the strains TCPN-22 and TBLN-21 stood out in terms of antibiosis. These findings suggest that native strains represent a viable alternative for the biological control of both pathogens.

Keywords: antagonism, antibiosis, biological control, native isolates, radial growth.

Resumen

La búsqueda de alternativas de control biológico sobre organismos plaga es cada vez más constante. El objetivo de este estudio fue determinar la eficiencia de control biológico de seis cepas nativas de *Trichoderma* spp. aisladas de diferentes ecosistemas contra *Sclerotium* sp. y *Fusarium* sp. Se aislaron cepas de *Trichoderma* spp. provenientes de *Persea americana, Coffea arabica, Passiflora edulis, Mangifera indica, Saccharum officinarum, Citrus* spp. y dos patógenos *Sclerotium* sp. *y Fusarium* sp. extraídos de *Solanum lycopersicum;* fueron colocados en medios PDA, incubados a 27 °C. Los hongos se identificaron empleando claves taxonómicas. Se empleó un diseño completamente al azar con seis tratamientos y cinco repeticiones. Se cuantificó el crecimiento radial, antagonismo y antibiosis de *Trichoderma* sp. sobre los patógenos; se empleó análisis de varianza y separación de medias de Tukey (0.05). Se encontró que la

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cepa nativa de *Trichoderma* spp. (TCHN-22) mostró el mayor crecimiento radial, no obstante, las cepas TIAN-21, TBLN-21 y TCRN-20, inhibieron el crecimiento de *Sclerotium* sp.; en cambio, las cepas TCHN-22 y TCPN-22 controlaron mejor a *Fusarium* sp. En el grado de antagonismo sobresale la cepa TIAN-21 y TCSN-22; al analizar la antibiosis destacan las cepas TCPN-22 y TBLN-21, lo cual indica que las cepas nativas representan una alternativa para ser empleadas en el control biológico de ambos patógenos.

Palabras clave: aislados nativas, antagonismo, antibiosis, crecimiento radial, control biológico.

1. Introduction

Nowadays, food production and environmental security are threatened by the emergence of phytopathogens, which cause plant diseases (Zin & Badaluddin, 2020). The production of vegetables, such as *Solanum lycopersicum*, is among the most affected by fungal pathogens. *Fusarium oxysporum* is an example of pathogenic fungi that can cause up to 60% production losses in tomato crops (Martínez et al., 2019).

Specific edaphoclimatic conditions, as well as the manipulation of contaminated tools or plant material, determine soil infestation. The phytopathogen can persist for many years in the soil and infect crop roots, causing significant damage (Sanoubar & Barbanti, 2017). According to Duarte Leal et al. (2020), *Sclerotium rolfsii* is another fungus that causes damping-off disease in tomato, showing resistance structures and a source of inoculum called sclerotia that can withstand temperature changes, floods or fungicides. Under light stress, it can also increase mycelial biomass and produce sclerotia (Zazueta Torres et al., 2021).

Mahato (2017) and Adhikari et al. (2022) mention that diverse pathogenic organisms affecting foliar tissue and roots in crops are parasitized by fungal species of *Trichoderma*, reducing the damage. This shows that *Trichoderma* fungi could be used as biocontrol agents. Moreover, they contribute to enhancing soil fertility, growth and production (Cai et al., 2015; Zhu et al., 2022).

Some *Trichoderma* species are used in agriculture due to their capacity to parasitize pathogenic fungi, such as the case of *Botritis* spp., *Fusarium* spp., *Pythium* spp., *Rhizoctonia* spp., *Verticillium* spp. and *Sclerotinia* spp (Ahluwalia et al., 2015). The damage caused by the fungi is explained by the production of metabolites that work as biofungicides with fast dispersal, which inhibit the pathogenic organism (El-Komy et al., 2015).

Dennis & Webster (1971a; 1971b) isolated many *Trichoderma* spp. strains from wood, bark, weeds and soil to evaluate the antagonism level and antibiosis against phytopathogens. Stefanova et al. (1999) confirmed the use of *Trichoderma* spp. biopreparations. These produce metabolites capable of combating fungi in different crops, where some filtrates of *Trichoderma* spp. showed the presence of lytic enzymes, carboxymethylcellulose, chitinase, and β-1,3-glucanase. This study shows that evaluated metabolites cause vacuolation, granulation, coagulation, disintegration, and lysis at the cellular level.

In Nicaragua, vegetable farmers from different departments have been affected by phytopathogens that damage roots and stems, such as *Sclerotium* spp. and *Fusarium* spp. The damage caused by these pathogens can lead to 30 to 40% of plant loss, urging the rotation of crops or stopping planting in contaminated soil, changing the sector's productivity. The present study aims to determine the efficiency of the biological control activity of six native strains of *Trichoderma* spp., isolated from different ecosystems, against *Sclerotium* sp. and *Fusarium* sp.

2. Materials and Methods

2.1. Location

The study was carried out in Managua, Nicaragua. The experiments were performed in the laboratory of Microbiology of the Universidad Nacional Agraria [UNA], located at 12 ½ km of the Carretera Norte, at the coordinates 12° 08' 51.05" N and 86° 09' 50.69" W, during February and November of 2023.

2.2. Isolation and preservation of antagonistic microorganisms

Six strains of the beneficial fungus *Trichoderma* spp. were isolated from different farms and crops (Table 1). They were isolated from decaying branch bark, sugar cane leaves (*Saccharum officinarum* L.), soils with passion fruit (*Passiflora edulis)* residues, healthy roots from avocado plants (*Persea americana)* and coffee crops (*Coffea arabica* L.).

The isolates from avocado roots and coffee were treated with common water. They were submerged in sodium hypochlorite at 1% for 30 seconds, decanted twice with sterile distilled water, and covered with sterilized paper towels. Afterwards, the roots were fragmented into 1 cm pieces and placed horizontally in a Petri dish with potato dextrose agar [PDA] (Andrade-Hoyos et al., 2020).

Soil antagonistic fungi were isolated using the suspension plate method (1 ml) of multiple dilutions (10^{-1}, 10^{-2} and 10^{-3}), and placed in Petri dishes, which were kept in the shadow at 27 °C for five days during their growth. There were five replicates per sample (Tang et al., 2022). The *Trichoderma* spp. from bark and leaves were isolated directly with a sterile handle, inoculating the mycelium and conidia in the culture medium. All the isolates from antagonistic fungi were stored at 27 ^{0}C in glass test tubes containing silica gel.

2.3. Isolation of pathogenic microorganisms

Sclerotium sp. and *Fusarium* sp. strains were isolated from the stem base of tomato plants (*Solanum lycopersicum* L.) and placed on PDA culture media. The purification was performed five days later. The stem was washed off with drinking water and cut into short tissue pieces (4 x 4 mm). These pieces were immersed in a 1% NaClO solution for one minute, washed with sterile water, and placed in Petri dishes with 20 mL of PDA medium without antibiotics. They were then incubated at 27 °C for five days. The isolates were purified and stored at 4 °C (Aguilar-Anccota et al., 2021).

2.4. Identification of fungal genus

Samples of purified fungi were taken using a sterile stick. The samples were placed on slides using a stain with Lactophenol at 60X to make observations under the microscope (Olympus BX41) and identify the fungal genus. The colonies were characterized macroscopically by observing the color of the mycelium, radial growth, the presence or absence of concentric rings, sporulation, pigmentation, and the presence or absence of odor. Taxonomic keys for the genus *Trichoderma* by Barnett and Hunter were consulted for identification (del Carmen H. Rodríguez et al., 2021; Guedez et al., 2012).

2.5. Radial growth of *Trichoderma* spp. and phytopathogens

Fungal samples in PDA medium were purified using lactic acid and incubated at 27 ± 1°C. Fungal growth was quantified after four days of inoculation for the genera *Trichoderma* spp. and *Sclerotium* sp., and after seven days of inoculation for *Fusarium* sp. Petri dishes were marked using a permanent marker and a ruler on the reverse. Fungal discs of 5 mm were drawn and placed at the center of the dish, and then sealed with parafilm paper for incubation (Figure 1). The measurements were carried out every 24 hours and stopped when complete fungal expansion occurred on the culture media. The average growth rate was calculated by dividing the total growth by the time (French & Herbert, 1982).

2.6. Antagonism tests of *Trichoderma* spp.

Antagonism tests were performed using dual cultures. Discs of 5 mm with mycelium were cut and isolated from each fungal sample. The discs were placed on opposite sides of Petri dishes with 20 mL of PDA at a distance of 7.5 cm between the two fungi: antagonistic-phytopathogen (Figure 2). All the plates were sealed with parafilm and incubated at 27 ± 1°C for 3 to 10 days. The growth of radial fungal colonies was daily measured using a ruler. These tests were per-

Table 1. Location, sampling and fungal isolation.

Code	Strains	Crop	Source	Farm	Department	Coordinates
TIAN-21	*Trichoderma* spp.	*Persea americana*	Root	Santa Isabel	Carazo	11^0 51`40.9¨N 86$^0$ 10`34.9¨W
TCNN-20		*Coffea arabica*	Root	Chelol	Carazo	11^0 49`55.8¨N 86 ^{0} 12¨11.0¨W
TBLN-21		*Passiflora edulis*	Soil	El Balsamo	Masaya	12^{0}06`29.1¨N 86$^0$00`55.4¨W
TCPN-22		*Mangifera indica*	Bark	Constancia	Managua	12^{0}06`59.8¨N 86$^0$07`38.1¨W
TCHN-22		*Saccharum officinarum*	Leaf	Constancia	Managua	12^{0}06`59.8¨N 86$^0$07`38.1¨W
TCSN-22		*Citrus* spp.	Soil	Constancia	Managua	12^{0}06`59.8¨N 86$^0$07`38.1¨W
SGTN-23	*Sclerotium* sp.	*Solanum lycopersicum*	Stem	Doña Gloria	Masaya	12^{0}00`10.3¨N 86$^0$06`11.6¨W
FMBM-23	*Fusarium sp.*	*Solanum lycopersicum*	Stem	Ronyana	Nandaime	11°45’17.5”N 86°02’30.8”W

formed with five replicates (Sánchez-Montesinos et al., 2021). The inhibition percentage of radial growth of the pathogen's mycelium was calculated with the radial growth of the pathogen in the control dish and the radial growth of the pathogen in the presence of *Trichoderma* spp (Matroudi & Motallebi, 2009).

$$IPRG\ (\%) = \frac{C-T}{C} \times 100 \qquad [1]$$

Where IPRG is the inhibition percentage of radial growth of the pathogen's mycelium, C is the radial growth of the pathogen in the control plate, and T is the radial growth of the pathogen in the presence of *Trichoderma spp.*

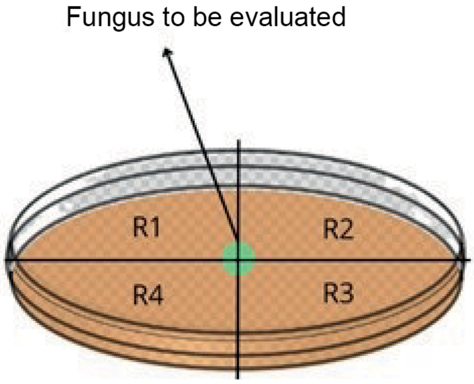

Figure 1. Diagram of radial growth measurement.

A scale with a 1-5 score was used to evaluate the level of antagonism (Table 2) and the control capacity of *Trichoderma* spp. A strain was considered highly antagonistic when the average value for a given comparison was equal to or greater than class 2, and it was not considered highly antagonistic if it was equal to or less than class 3 (Bell et al., 1980).

2.7. Antibiosis Tests

Six *Trichoderma* spp. strains were used to evaluate the activity of metabolites against *Sclerotium* sp. and *Fusarium* sp., and the *in vitro* antagonistic capacity when inhibiting or slowing down mycelium growth in *Fusarium* sp. and *Sclerotium* sp. A sterile cellophane paper membrane was used to cover the PDA medium in Petri dishes (Figura 3). The five replicates of the *Trichoderma* spp. strain were inoculated with 5 mm agar plugs taken from a three-day-old pure culture.

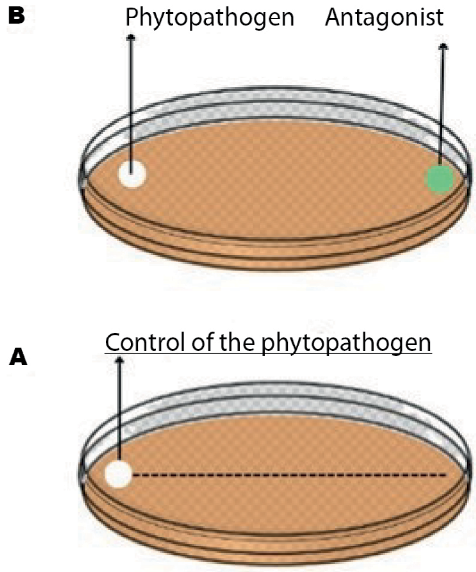

Figure 2. Diagram of the antagonism assay setup.

Every Petri dish was marked on its reverse to measure the pathogen growth and labelled with a unique code. The *Trichoderma* spp. discs were placed on cellophane paper at the center of the Petri dish, and sealed with parafilm for incubation for 48 hours at 27 °C in the dark. During this time, the metabolites are spread through the medium, preventing conidia or fungal mycelium from passing through. The controls were prepared in the same way but without *Trichoderma* spp. growth on the membranes before inoculation (Álvarez-García et al., 2020).

After 48 hours of incubation, the membranes were removed from the plates over the culture medium, and 5 mm diameter mycelial discs of the *Sclerotium* sp. and *Fusarium* sp. strains were placed at the same time. As a control, the two pathogen isolates were cultured on PDA medium. The results were expressed as the percentage of radial growth inhibition [PGI] of the

Tabla 2. Scale used to measure the degree of antagonism (biological control) of Trichoderma spp. strains against pathogens Fusarium sp. and Sclerotium sp.

Class	Description
1	The antagonist grows completely over the pathogenic colony and covers the entire surface of the culture medium.
2	The antagonist covers at least two-thirds of the surface of the culture medium.
3	The antagonist and the pathogen cover approximately half of the surface of the culture medium.
4	The pathogen covers at least two-thirds of the culture medium, limiting the antagonistic growth.
5	The pathogen grows over the antagonistic colony and covers all the surface of the culture medium.

Sclerotium sp. and *Fusarium* sp. strains (Cubilla-Ríos et al., 2019).

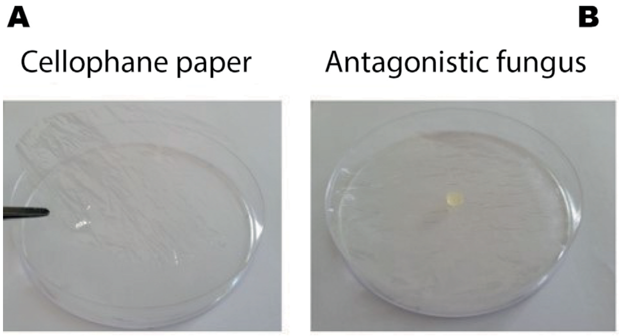

Figure 3. Antibiosis test.

2.8. Statistical Analysis

A randomized experimental design with five replicates per treatment was used. The results were analyzed using RStudio program (4.2.3). Normality assumptions were first confirmed, and then an analysis of variance [ANOVA] was carried out. The comparison between the samples' media was estimated by Tukey's rank test ($p < 0.05$). Differences were considered significant at a 95% level.

3. Results and Discussion

A total of six native *Trichoderma* spp. strains (Figure 4) from different substrates were obtained, one *Sclerotium* sp. strain and one *Fusarium* sp. Similar *Trichoderma* isolates were reported by Andrade Hoyos et al. (2019), who confirmed the purification of three *Trichoderma* spp. species in PDA medium. The sporulation of the isolates was stimulated by exposure to white light for 2 days, which was determined by the aggressiveness of the *Trichoderma* genus that grows on different substrates and sporulates under stress conditions.

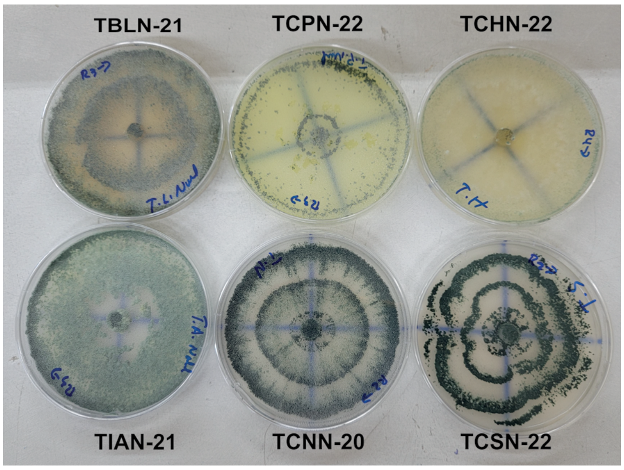

Figure 4. Native Trichoderma spp. strains isolated in this study

3.1 Radial growth of *Trichoderma spp.* strains

The highest radial growth percentage was observed for the strain TCHN-22 with 25.92 mm, followed by TCPN-22 (22.88 mm h^{-1}), which shows a faster growth rate for these strains (Figure 5). The increased growth rate of these two strains could be because they were isolated from decomposing material. Similar results were published by Duarte-Leal et al. (2018), who observed that *Trichoderma* strains showed higher growth when incubated at temperatures higher than 25 °C.

3.2 Antagonism tests of *Trichoderma* ssp.

An inhibition effect on the growth of *Sclerotium* sp. was observed after comparing *Trichoderma* spp. strains at 48 hours. The strains TIAN-21, TBLN-21 and TCRN-2 showed the greatest growth and reduced the pathogen's advance on the culture medium (Figure 6). This behaviour could be because TIAN-21 and TBLN-21 are endophytes of roots, and might be producing a volatile metabolite that delays the growth of *Sclerotium* sp. The inhibition percentage of fungi from the genus *Trichoderma* over *Sclerotium rolfsii*

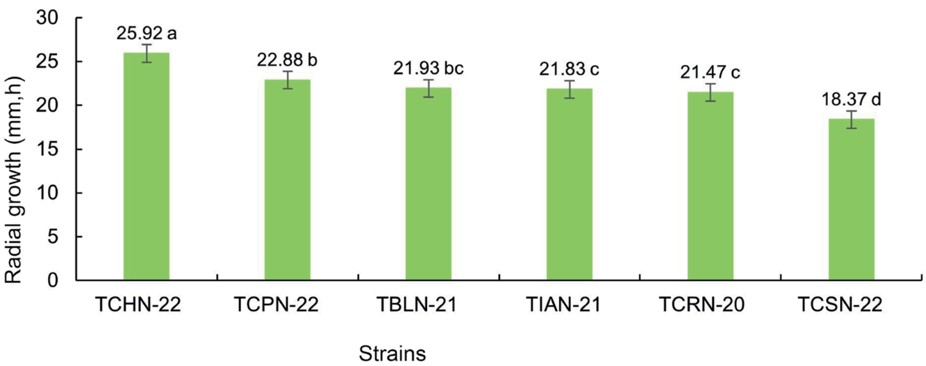

Figure 5. Radial growth of six native *Trichoderma spp*. strains.
(Different letters indicate significant differences according to Tukey's Test, p=0.05).

and *Rhizoctonia* was reported by Michel-Aceves et al. (2013) and Zapata-Narváez & Gómez-Marroquín (2022), who observed controls of up to 94% inhibition.

Evaluating the effect against *Fusarium* sp., it was found that the strains TCHN-22 and TCPN-22 could reduce between 84 and 85% of pathogen growth (Figure 7). It cannot be ruled out that these strains presented the highest growth rate, and that *Fusarium* sp. presents slow growth. Studies by Sánchez Miranda (2022) and Rodríguez-García & Wang-Wong (2020) showed that *Trichoderma* spp. can reduce radial growth in *Fusarium* sp. for up to 87%. Similarly, Martínez-Coca et al. (2018) reported that the antagonist fungus grows faster, covering the phytopathogenic fungus completely in a maximum period of 96 hours.

It is possible that this result is due to some metabolite produced by the strain. The conidia managed to grow on the sclerotia; however, Garrido & Vilela (2019), when evaluating the antagonistic capacity of *Trichoderma*, determined that it does not achieve dominance over the pathogen, which is consistent with the results obtained in this investigation. On the other hand, strain TCSN-22 achieved a greater antagonistic effect against *Fusarium* sp., surpassing the other *Trichoderma* strains (Figure 9). This finding may be explained by the fact that the strain originates from soils where vegetables of the Solanaceae family were cultivated. A study by Miguel-Ferrer et al. (2021) mentions that antagonistic strains of the fungus reduce or slow the growth of the phytopathogen, thereby decreasing the risk of damage to the host plant.

3.3 Antibiosis Tests

After antibiosis testing, it was determined that TCPN-22 was better than the other strains at reducing the growth of *Sclerotium* sp. and *Fusarium* sp. (Figures 10 and 11). The results from radial inhibition growth by metabolites might have depended on the strain type, the biocontrol species, and the substrate on which the fungus was isolated. A study by Lezcano Escobar et al. (2018) reported that *Trichoderma* secretes enzymes that hydrolyze fungal cell walls, such as proteases, chitinases, and glucanases.

Even if the results are promising, it is necessary to evaluate the biosynthesis of metabolites and the pathways to identify the metabolites produced by the strains under investigation (Khan et al., 2020). Another alternative is the use of secondary metabolites from *Trichoderma* produced by liquid fermentation to reduce the reproductive capacity of *Fusarium* (Hernández et al., 2014).

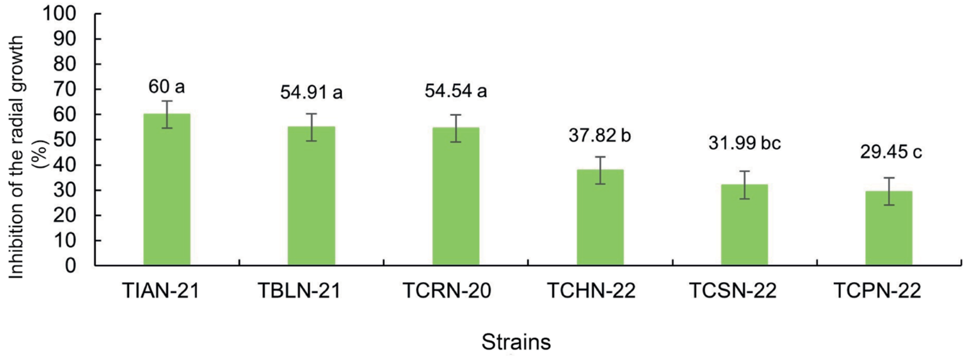

Figure 6. Growth of six native Trichoderma spp. strains against Sclerotium sp. in dual cultures. (Different letters indicate significant differences according to Tukey's test, p = 0.05).

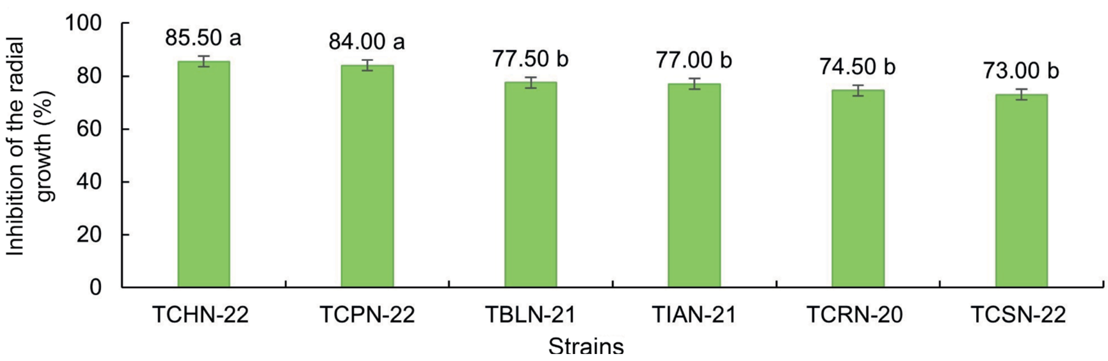

Figure 7. Growth of six native Trichoderma spp. strains against Fusarium sp. in dual cultures. (Different letters indicate significant differences according to Tukey's test, p = 0.05).

5. Conclusions

The native *Trichoderma* spp. strain TCHN-22 showed the greatest radial growth. However, the strains TIAN-21, TBLN-21 and TCRN-20 were the most effective at inhibiting the growth of *Sclerotium* sp. The strains TCHN-22 and TCPN-22 showed improved capacity to control *Fusarium* sp.

Regarding antagonism, the strains TIAN-21 and TCSN-22 showed promising effects. However, strains TCPN-22 and TBLN-21 stood out after antibiosis analyses.

These results show that native strains represent an alternative for the biological control of the pathogens *Sclerotium* sp. and *Fusarium* sp.

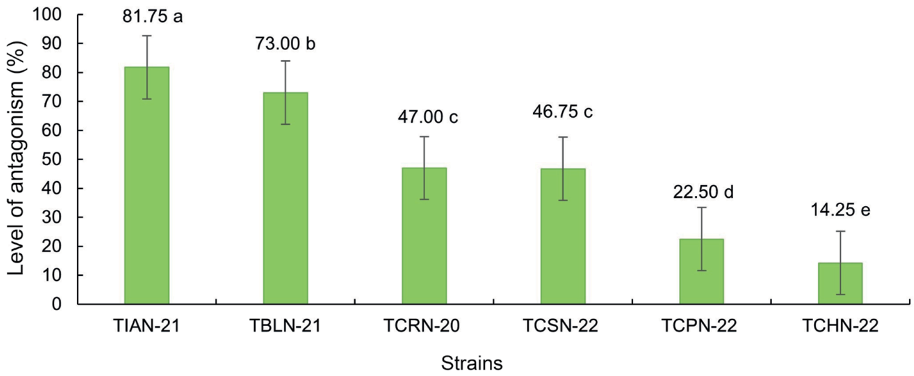

Figure 8. Degree of antagonism of native *Trichoderma spp.* strains against *Sclerotium sp.* (Different letters indicate significant differences according to Tukey's test, p = 0.05).

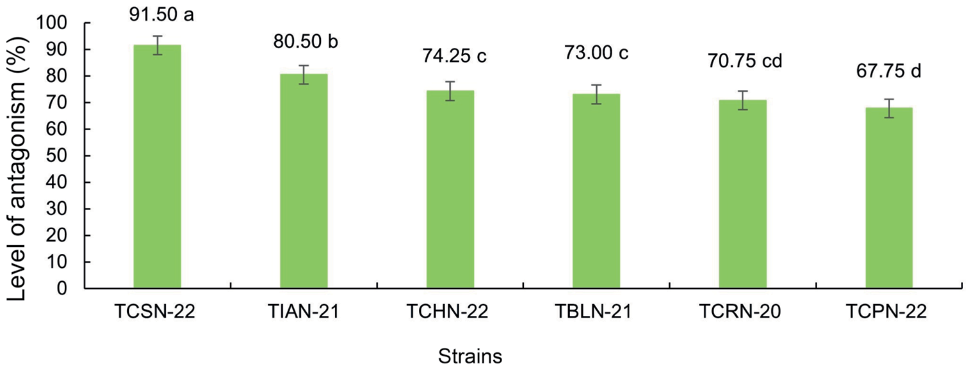

Figure 9. Degree of antagonism of native *Trichoderma spp*. strains against *Fusarium sp.* (Different letters indicate significant differences according to Tukey's test, p = 0.05).

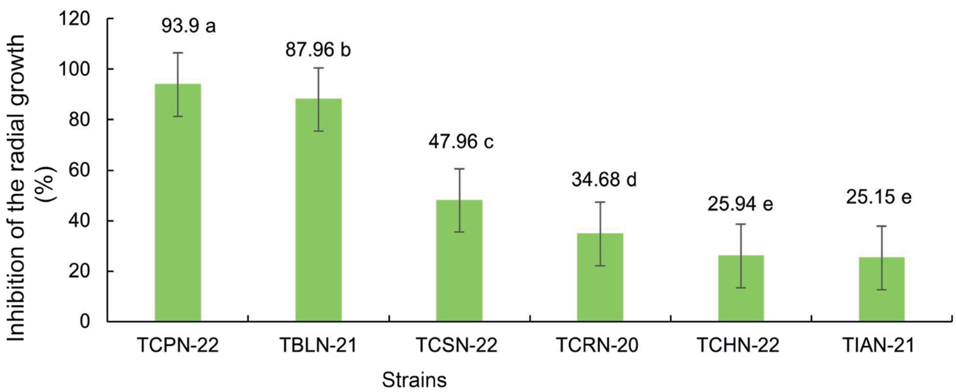

Figure 10. Antibiosis of six native *Trichoderma spp*. strains against *Sclerotium sp.* in dual culture (Different letters indicate significant differences according to Tukey's test, p = 0.05).

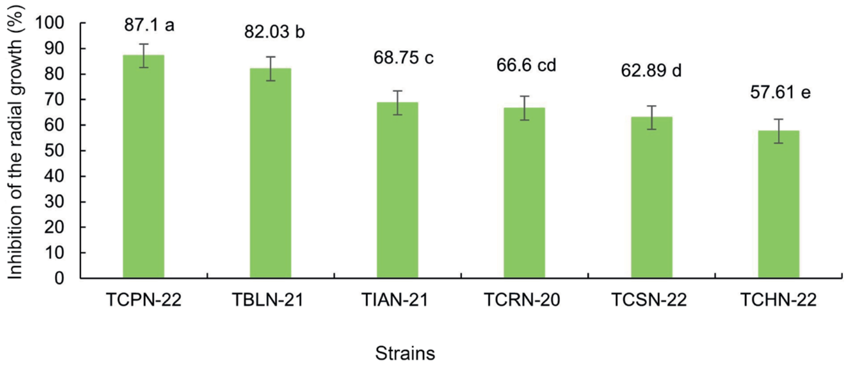

Figure 11. Antibiosis of six native Trichoderma spp. strains against Fusarium sp. in dual cultures. (Different letters indicate significant differences according to Tukey's test, p = 0.05).

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Contributor roles

- Noel Antonio Herrera Rodríguez: investigation, methodology, project administration, writing – review & editing.Markelyn José
- Rodríguez Zamora: investigation, methodology, writing – original draft.
- Juan Carlos Morán Centeno: data curation, formal analysis, writing – review & editing.
- Jorge Ulises Blandon Diaz: investigation, visualization, writing – review & editing.

Data availability

Data will be made available on request.

Use of Artificial Intelligence

The authors declare that no artificial intelligence has been used in the preparation of the manuscript.

Ethical implications

The authors declare that there are no ethical implications.

Conflict of Interest

The authors declare that they have no affiliation with any organization with a direct or indirect financial interest that could have appeared to influence the work reported.

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