Revista Colombiana de Investigaciones Agroindustriales
Volumen 8 (1). Enero – Junio. [p. 11-22]
https://doi.org/10.23850/24220582.4064

Selección de cepas de *Trichoderma* para el control biológico de *Fusarium nygamai* en sorgo (*Sorghum bicolor* L. Moench)

Selection of *Trichoderma* strains for biological control of *Fusarium nygamai* in sorghum (*Sorghum bicolor* L. Moench)

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Recibido: 25/10/2021 Aceptado: 20/10/2021

Resumen Uno de los principales problemas que afectan el cultivo de sorgo (*Sorghum bicolor*) en Uruguay es la enfermedad ocasionada por *Fusarium nygamai*, responsable de pérdidas en el rendimiento en los cultivos. Además, es capaz de producir micotoxinas como fumonicina, moniliformina y beauvericina, lo que constituye un riesgo para la alimentación animal. El objetivo de este trabajo fue identificar cepas de *Trichoderma* spp., con potencial de control de *F. nygamai* en sorgo. Se identificaron dos cepas de *Trichoderma asperellum*, cinco cepas de *Trichoderma atroviride*, dos cepas de *Trichoderma virens*, una cepa de *Trichoderma longibrachiatum* y dos cepas de *Trichoderma* sp., aisladas de suelo en plantaciones comerciales de sorgo. Se realizaron cultivos duales y evaluación de metabolitos volátiles *in vitro* para seleccionar las cepas con la mayor actividad antagonista contra *F. nygamai.* Las cepas de *Trichoderma* spp. seleccionadas se evaluaron en ensayos de protección vegetal en plántulas de sorgo inoculadas con *F. nygamai. T. asperellum* (T6) y *T. atroviride* (T21) fueron antagonistas efectivos de *F. nygamai*. Todas las plántulas de sorgo inoculadas con *F. nygamai* mostraron síntomas de la enfermedad, mientras que el 50 % de las plántulas inoculadas con *F. nygamai,* pero tratadas con *T. asperellum* (T6) permanecieron sanas, donde se recuperó la cepa de los tejidos internos de la raíz, el tallo y las hojas. Estos resultados son promisorios para el desarrollo de una formulación comercial de tratamiento de semillas para el control de *F. nygamai* en cultivos de sorgo.

Palabras clave: agente de biocontrol; fitopatógeno; micotoxinas; cultivos; *Trichoderma*; *Fusarium*

Abstract One of the main problems affecting sorghum (*Sorghum bicolor*) production in Uruguay is the disease caused by *Fusarium nygamai*, which is responsible for crop losses and can also produce mycotoxins such as fumonicin, moniliformin, and beauvericin. This constitutes a risk for animal feed. The aim of this work was to identify isolates of *Trichoderma* spp., with *F. nygamai* control potential in *S. bicolor*. We identified two strains of *Trichoderma asperellum*, five strains of *Trichoderma atroviride*, two strains of *Trichoderma virens*, one strain of *Trichoderma longibrachiatum* and two strains of *Trichoderma* sp., isolated from soils of sorghum commercial plantation. Dual cultures and evaluation of volatile metabolites were performed *in vitro* to select those strains with the highest antagonistic activity against *F. nygamai.* The selected s trains of *Trichoderma* spp. were evaluated in plant protection assays in sorghum seedlings inoculated with *F. nygamai*. *Trichoderma asperellum* (T6) and *T. atroviride* (T21) were effective antagonists of *F. nygamai*. All sorghum seedlings only inoculated with *F. nygamai* under laboratory conditions evidenced symptoms of disease, while 50 % of seedlings inoculated with *F. nygamai* but treated with *T. asperellum* (T6) remained healthy. This strain was also recovered from internal tissues of root, stem, and leaves. These results are promising for the development of a commercial formulation of seed treatment for the control of *F. nygamai* in sorghum crops.

Keywords: biocontrol agent; phytopathogen; mycotoxins; crops; *Trichoderma*; *Fusarium.*

Introduction

Sorghum (*Sorghum bicolor* L. Moench) is one of the most important cultivated cereals in the world. Sweet sorghum is a C4 metabolism plant, adapted to semiarid or subtropical regions, showing low water loss and high photosynthetic efficiency. Due to its resistance to drought and heat, it can be cultivated in relatively poor soils and used in crop rotation (Reddy, 2017).

Sorghum is a multipurpose crop used in the food industry to produce malted and distilled beverages, sorghum meal, sorghum rice, couscous, injera, leavened bread, and, togwa as feed for beef and dairy cattle, fodder and fuel production. The stem can also be used for building materials, firewood, waxes, dyes, and vegetable oil (Rao *et al.,* 2016).

In Uruguay, 67.8 thousand ha of *S. bicolor* were planted in 2020, producing 255.000 tons (Oficina de Programación y Política Agropecuaria [OPYPA], 2020). One of the main problems affecting sorghum production is the leaf spots and stalk rot produced by *Fusarium nygamai* L. Burgess and Trimboli (del Palacio; Mionetto; Bettucci; Pan, 2016) In sorghum, it produces leaf spots and stalk rot, also decreasing the sugar content and reducing crop yield (Petrovic; Walsh; Burgess; Summerell, 2009). In addition, *F. nygamai* can produce mycotoxins, where fumonisins, moniliformines, and beauvericins are the most important ones (Hussien; Carlobos-Lopez; Cumagun; Yli-Mattila, 2017; Logrieco; Mulè; Moretti; Bottalico, 2002; Glenn, 2007). In order to control the disease, farmers usually apply synthetic chemical fungicides that have many hazardous effects. Besides, biological control can provide effective solutions for managing *F. nygamai* diseases as an eco-friendly alternative method.

Nowadays, many microorganisms have been used as biocontrol agents of plant disease, being several species of *Trichoderma* Bissett the most widely used against a large range of plant pathogens (Köhl; Kolnaar; Ravensberg, 2019). They act against plant pathogens directly by mycoparasitism, volatile, and diffusible metabolites production, and indirectly by nutrient and space competition, or triggering plant defense mechanisms (Ben-Amira *et al.,* 2017; Pascale *et al.,* 2017). Studies about biocontrol agents against *F. nygamai in vitro* or *in vivo* are infrequently performed (Parizi; Ansaria; Elaminejad, 2012). One method for obtaining effective biocontrol agents is to select *Trichoderma* species where temperature, moisture, nutrient availability, and microbial composition are similar to those where plant disease is naturally found.

Consequently, the main purpose of this work was to identify isolates of *Trichoderma* present in the soil of sorghum crops to evaluate the outcome of their interactions with *F. nygamai* in dual cultures and to determine the reduction of infection by *F. nygamai* in seedlings previously treated with the most active *Trichoderma* isolates.

Materials and methods

Trichoderma isolation and identification

Soil samples were collected of different sorghum crops from Uruguayan plantations (34°26'03,8'' S; 57°16'37,1'' W; 33°32'54,0'' S, 58°14'25,4'' W; 30°16'08,7'' S, 57°35'50,8'' W). Fungal isolation was performed by using the dilution plate method. A 500 µL aliquot of soil suspension was plated on potato dextrose agar (OxoidTM PDA) containing chloramphenicol (100 mg L-1) and cultures were incubated at 25 ºC until fungal colonies were observed. Five replicates were performed for each sample. Microscopic characteristics were recorded and pure cultures on 2 % PDA were obtained from those fungi corresponding with *Trichoderma* genus.

Trichoderma species were identified based on colony characteristics, growth rates and morphological features (Samuels, 2006).

Genomic DNA was extracted from fresh mycelia, according to Lee and Taylor (1990), for molecular identification. Sequence amplification of the ITS region, including both internal transcribed spacers (ITS1 and ITS2) and the 5.8 S gene from rDNA, were made using the fungal specific primers ITS4 and ITS5 (White; Burns; Lee; Taylor, 1990).

Polymerase chain reaction (PCR) was conducted according to the following cyclic conditions: initial denaturation at 94 °C for 3 min; followed by 35 cycles consisting of 94 °C for 1 min, 50 °C for 45 seconds and 72 °C for 1 minute; and a final extension step of 72 °C for 5 min, and then held at 4 °C. PCR amplicons were purified and sequenced directionally (Macrogen Inc., Seoul, Korea). Sequences were aligned by ClustalW (Thompson; Higgins; Gibson, 1994), together with sequences of strains obtained from the GenBank database using MEGA 6.06 software (Tamura; O´Donnell, 2013). Phylogenetic analysis was performed using MEGA 6.06 software (Tamura; O´Donnell, 2013) by Maximum Parsimony method. The robustness of the trees was evaluated by 1000 bootstrap replicates. All characters were considered of equivalent weight and gaps were excluded from the analysis.

Fusarium isolation and identification

Diseased plants of *S. bicolor* were characterized by symptoms of chlorosis and necrosis of leaves, punctured lesions, twisted leaves, reduction of the total leaf area, death of the top of the plant, and reddish stalk rot. *Fusarium* isolates were obtained from symptomatic leaf tissues of ten sorghum plants. Leaf fragments were plated on a culture medium (CM) and incubation in moist chambers (MCH).

A hundred surface-disinfected segments from different plants (10 segments per Petri dish) were plated in PDA containing chloramphenicol (100 mg L-1). Plates were then incubated at 25 °C for one week, and emerging colonies were transferred to a fresh medium of PDA. For each colony, monosporic isolation was performed and transferred for identification on PDA and Carnation Leaf Agar, CLA (pieces of sterile dry carnation leaves, agar 20 g L-1).

For MCH, leaves were surface-disinfected and incubated in sterile moist chambers for two weeks. When sporulation was evident, the surfaces of the leaves were tape-lifted and mounted on slides for direct microscopic observation to confirm the presence of *Fusarium* sp. Isolation and morphological identification were made.

For molecular identification, the fungal DNA was extracted from pure cultures grown on PDA according to Lee and Taylor (1990). Analysis of the partial sequence of the elongation factor 1-alpha gene (EF-1α) was made using the primers EF1 and EF2 described by O´Donnell and Cigelnik (1997). The same PCR cycling parameters as in the ITs amplification were considered. Amplicons were sequenced (Macrogen Inc., Seoul, Korea) and then compared using the Basic Local Alignment Search Tool (BLAST) network services of the National Centre for Biotechnology Information (NCBI), with the Basic Local Alignment Search Tool (BLAST) network services of the National Centre for Biotechnology Information (NCBI).

Interspecific interactions

Dual cultures technique

In order to evaluate the antagonistic ability of 12 isolates of *Trichoderma* spp., against two isolates of *F. nygamai*, experimental pairings were carried out according to Dennis and Webster (1971).

An agar disc (5 mm in diameter) of the antagonist *Trichoderma* isolate was placed 5 cm apart of a same-sized agar disc of *F. nygamai* on PDA Petri dishes. *Fusarium nygamai* was placed alone on a fresh PDA dish as control. The mycelium discs were taken from the growth margin of 3-day-old cultures. All treatments were carried out in triplicate and incubated at 25 ºC in darkness. Growth inhibition was evaluated measuring two perpendicular radii

of a *Fusarium* colony daily for 3 days. The percentage of growth inhibition was calculated by the formula I: 100-(Tx100/C), where T was the radius of *F. nygamai* in dual culture and C the mean radius of *F. nygamai* in control. For the evaluation of the hyphal interaction, the mycelia of *Trichoderma* and *F. nygamai* were observed under the microscope. In addition, 5 mm disks were cut from the margin on each side of the contact zone and transferred to a fresh medium to determine the viability of the hyphae in the interaction zone. Plates were incubated at 25 °C for one week.

Effect of volatile metabolites

An antifungal volatile compounds assay was conducted in dual plates according to Dennis and Webster (1971). An agar disc (5 mm in diameter) of each fungus (the *Trichoderma* isolate and the pathogen isolate) was placed in the center of a PDA Petri dish. The mycelium discs were taken from the growth margin of 3-day-old cultures. The *F. nygamai* plates were inoculated 24 h before the *Trichoderma* spp. plates. Both plates faced without their covers, sealed with parafilm, and incubated at 25 ºC. Control was carried out by placing a disk of *F. nygamai* in the center of a Petri dish with PDA medium. The growth rate of *F. nygamai* was measured by recording the growth radius of the colony daily up to 5 days. Inhibition percentage was calculated. Three replicates were made.

Biocontrol activity of Trichoderma spp. against F. nygamai on S. bicolor seedlings

Trying to simulate the natural process of sorghum seedlings infection with *F. nygamai* and the potential control by isolates of *Trichoderma*, an experimental trial in vitro was performed. Three *Trichoderma* isolates, *T. asperellum* T6, *T. atroviride* T8 and T21, were selected and used as antagonists of *F. nygamai* in dual culture. Previously, surface disinfection of sweet sorghum seeds was carried out by submerging in alcohol 80 % for 1 min, and sodium hypochlorite 4 % for 2 min, followed by two washes with sterile distilled water, dried on sterile filter paper. The percentage of seeds germination was calculated.

Pelletizing was performed by mixing seeds with the commercial adherent A. D. Cell (Lage y Cía) and suspensions of $1x10^6$ conidia mL^{-1} of each *Trichoderma* isolate. Seeds were placed in tubes containing agar-water 2 % and 100 µl of a suspension of $1x10^5$ spores mL^{-1} of *F. nygamai* were added. Untreated seeds, seeds with the commercial adherent A. D. Cell and seeds with adherent and *Trichoderma* were introduced in tubes separately as controls. Finally, other seeds were only treated with *F. nygamai*. Ten replicates were performed per treatment. All tubes were incubated at 25 ºC for 20 days.

At 21 days, the presence of symptoms in seedlings was analyzed. Each seedling was cut into segments of 5 mm long and immersed (separately) in alcohol 70 % for 1 min, and sodium hypochlorite 0.4 % for 2 min, rinsed with sterile distilled water, and dried on sterile filter paper. Segments of each plant were plated on Petri dishes containing PDA and incubated for 72 hours at 25 °C, in order to reisolate *F. nygamai* and/or *Trichoderma* sp. Incidence was calculated as (number of infected plants with *F. nygamai*/ total number of sorghum plant treated) x 100.

Data analyses

Dunnett's multiple test was performed to compare each treatment against control in dual cultures and volatile inhibitors bioassay, and statistical significance was determined at $p = 0.05$. Differences among treatments regarding the incidence of *F. nygamai* on sorghum plants were evaluated by Fisher's test (significance judged at $p < 0.05$). All statistical analyses were performed using SigmaStat 3.5 software (Fox; Shotton; Ulrich, 1995).

Results

Fungal identification

Twelve *Trichoderma* isolates were identified: two *T. asperellum* Samuels, Lieckf. and Nirenberg (T6, T48), five *T. atroviride* P. Karst (T3, T8, T21, T31, T38), two *Trichoderma* sp. (T40, T51), one *T. longibrachiatum* Rifai (T47) and two *T. virens* (J. H. Mill., Giddens & A. A. Foster) Arx. (T15, T49) (Table 1; Figure 1).

From segments of *S. bicolor* leaves incubated in CH and on PDA, several isolates of *Fusarium* species were present, two of them corresponded to *F. nygamai*.

Antagonistic activity in dual culture assay

All *Trichoderma* spp. isolates had the ability to significantly reduce the growth rate of *F. nygamai* (Figure 2). *Fusarium* control grew almost twice on those treated with *Trichoderma* spp., *F. nygamai* F5 strain was more susceptible.

T. atroviride (T21) was the most effective isolate against both strains of *F. nygamai* evaluated, producing 73.1 % and 62.7 % of growth inhibition, respectively. The percentage of growth inhibition ranged from 39% to 73 %, being the lowest for the isolate T49 vs. F4, and the highest for isolate T21 vs. F5.

The overgrowth of *T. asperellum* (T6), *T. atroviride* (T8, T21 T31, T38) and *Trichoderma* sp. (T40) producing abundant conidia and chlamydospores over *F. nygamai* colony was observed (data not shown). From the overgrowth zone, only *T. asperellum* (T6) and *T. atroviride* (T21) were recovered. In dual cultures with other strains of *T. atroviride* (T8, T31, T38) and *Trichoderma* sp. (T40), both fungi were reisolated. *Trichoderma atroviride* (T3), *Trichoderma* sp. (T51), *T. asperellum* (T48) and *T. longibrachiatum* (T47) partially surrounded and developed under *F. nygamai* (F5, F4) colony (Figure 3c). Mycelia of *F. nygamai* and *Trichoderma* were intermingled showing physical contact but no obvious mycoparasitism. Deadlock was observed between *T. virens* (T15, T49) and *F. nygamai* (F5, F4), both fungi were reisolated from the interaction interface (Figure 3d).

Table 1. *Trichoderma isolates recovered in this study included in the phylogenetic analyses and GenBank numbers*

Fungal species	Isolate	GenBank accession number ITS rDNA
Trichoderma atroviride	T3	MK619481
Trichoderma asperellum	T6	MK619478
Trichoderma atroviride	T8	MK619485
Trichoderma virens	T15	MK619479
Trichoderma atroviride	T21	MK619480
Trichoderma atroviride	T31	MK619482
Trichoderma atroviride	T38	MK619483
Trichoderma sp.	T40	MK619474
Trichoderma longibrachiatum	T47	MK619475
Trichoderma asperellum	T48	MK619484
Trichoderma virens	T49	MK619476
Trichoderma sp.	T51	MK619477

Source: own elaboration.

Antifungal volatile compounds assay

Most *Trichoderma* isolates produced volatile metabolites with a low or absent growth inhibition activity of *F. nygamai* (F5, F4). *T. atroviride*, isolate T21, showed the highest inhibition (19.5 % and 20.6 %) on both *F. nygamai* strains. In the other treatments, percentage of growth inhibition ranged from 0 % to 16.3 %, being the lowest for isolates T48 and T51 vs. F5 (Figure 4).

Antagonistic activity of Trichoderma spp. inoculated in S. bicolor seeds contaminated with F. nygamai

T. asperellum (T6), *T. atroviride* (T8), and *T. atroviride* (T21) were selected since they were effective for the growth control of *F. nygamai* (F5 and F4) in dual culture. The percentage of seeds germination was high after all treatments (> 70 %), varying between 70 % in seeds with *F. nygamai* and 90 % in control seeds without treatment.

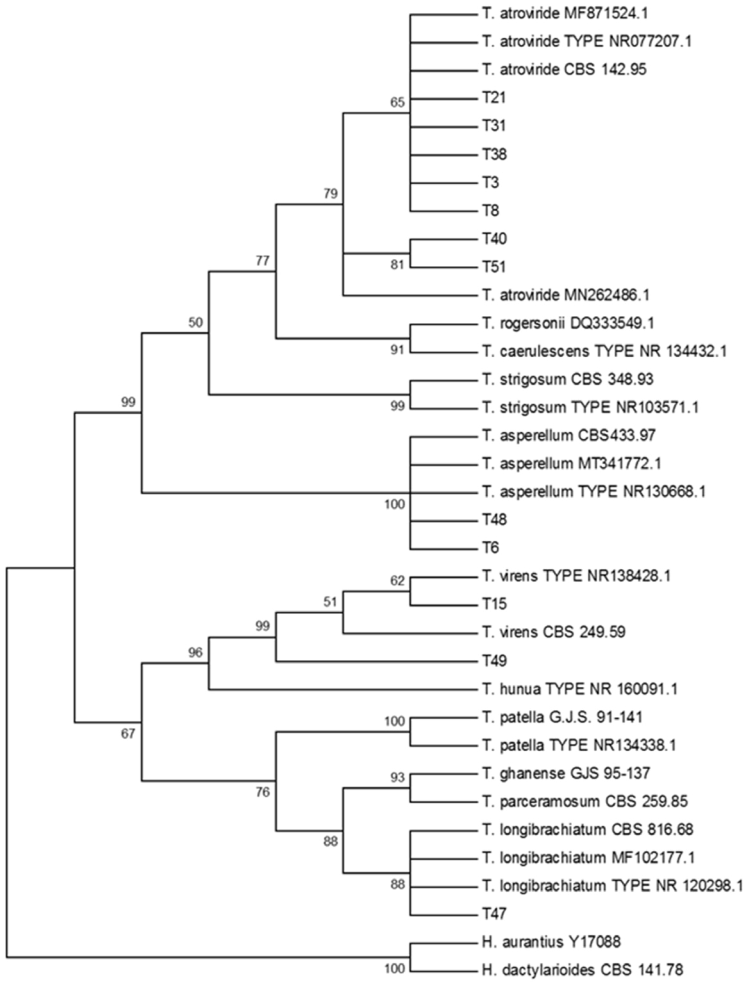

Figure 1. *Phylogenetic tree constructed by maximum parsimony from ITS region sequences of Trichoderma spp.*
Note. Isolates and GenBank sequences. Boostrap support values based on 1000 replicates are shown in the nodes. *Hypomyces aurantius* and *H. dactylarioides* were used as an external group.
Source: own elaboration.

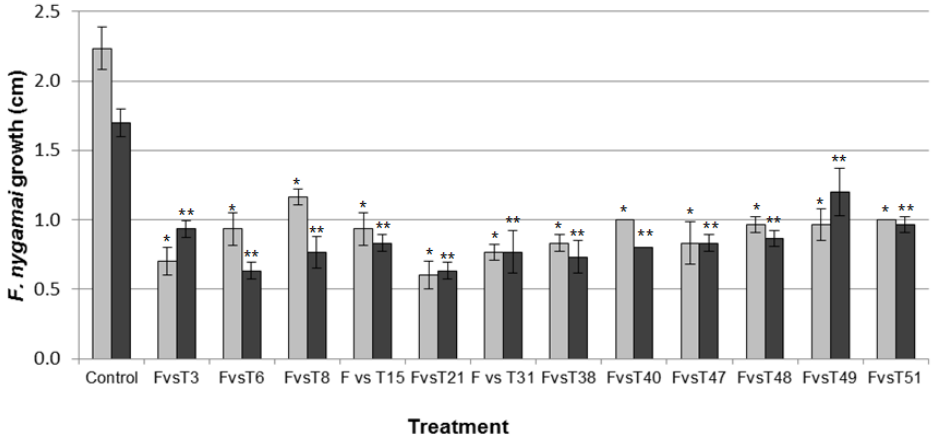

Figure 2. Growth of *F. nygamai* in dual cultures faced against 12 isolates of *Trichoderma* sp.
Note. T6, T48: *T. asperellum*, T3, T8, T21, T31, T38: *T. atroviride*, T40, T51: *Trichoderma* sp. T47: *T. longibrachiatum* and T15, T49: *T. virens*. Gray color strain F5 and black color strain F4 of *F. nygamai*. Treatments with an asterisk (F5 * or F4 **) are significantly different from the control ($p < 0.05$) according to Dunnett's multiple test.
Source: own elaboration.

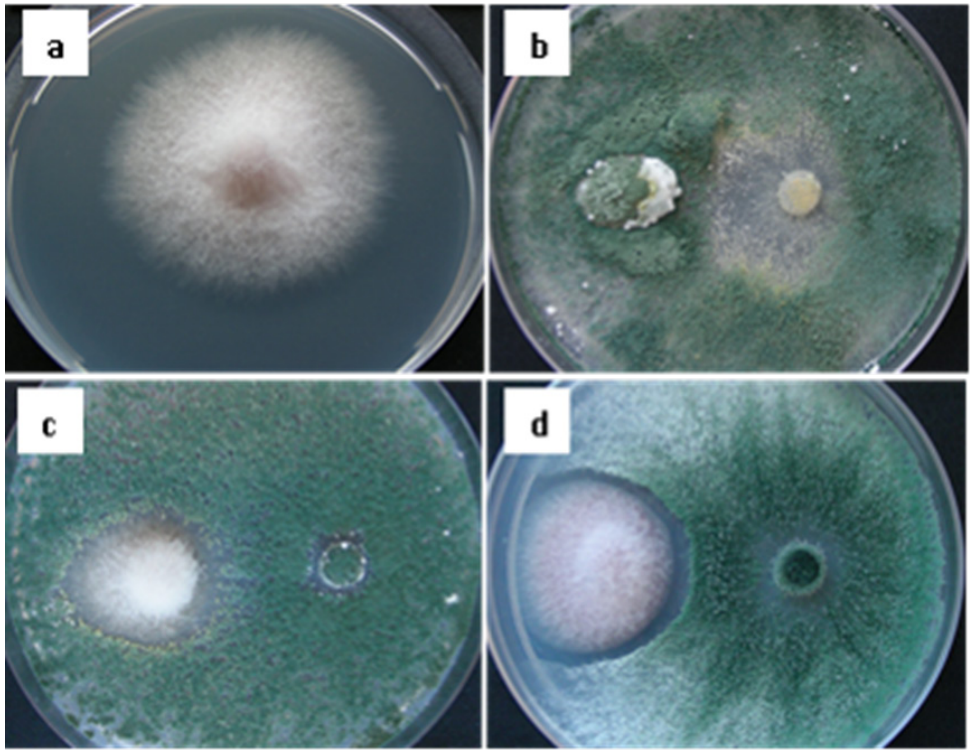

Figure 3. *Interaction between Trichoderma spp. and F. nygamai*
Note. a: *Fusarium nygamai* (F5) control, b: *T. atroviride* (T21) overgrowing *F. nygamai* F4, c: *T. atroviride* (T3) surrounding the mycelium of *F. nygamai* (F5) and d: *T. virens* (T49), and *F. nygamai* (F4) inhibiting each other's "deadlock".
Source: own elaboration.

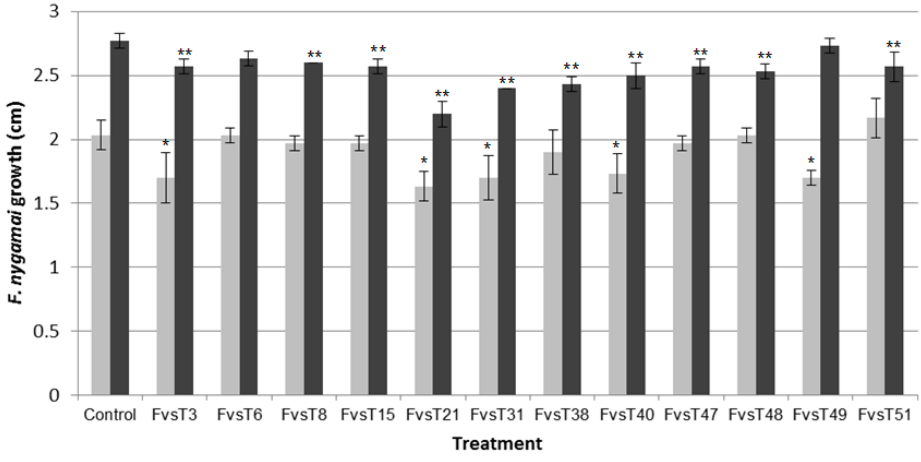

Figure 4. *Growth of F. nygamai in presence of volatile inhibitors of the 12 isolates of Trichoderma and control*
Note. T6, T48: *T. asperellum*, T3, T8, T21, T31, T38: *T. atroviride*, T40, T51: *Trichoderma* sp., T47: *T. longibrachiatum* and T15, T49: *T. virens*. Gray color strain F5 and black color strain F4 of *F. nygamai*. Treatments with an asterisk are significantly different from the control (F5 * or F4 **) ($p <$ 0. 05) according to Dunnett's multiple test.
Source: own elaboration.

Most seedlings that had been infected with *F. nygamai* (F5, F4) evidenced symptoms of disease, except those that were treated with *T. asperellum* (T6) that protected 50 % of seedlings (Figure 5) showing significant differences ($p <$ 0.05) with other isolates of *Trichoderma*, according to Fisher test (Figure 6). In these seedlings, the *Trichoderma* strain, inoculated as an antagonist, was recovered from internal tissues of root, stem, and leaves.

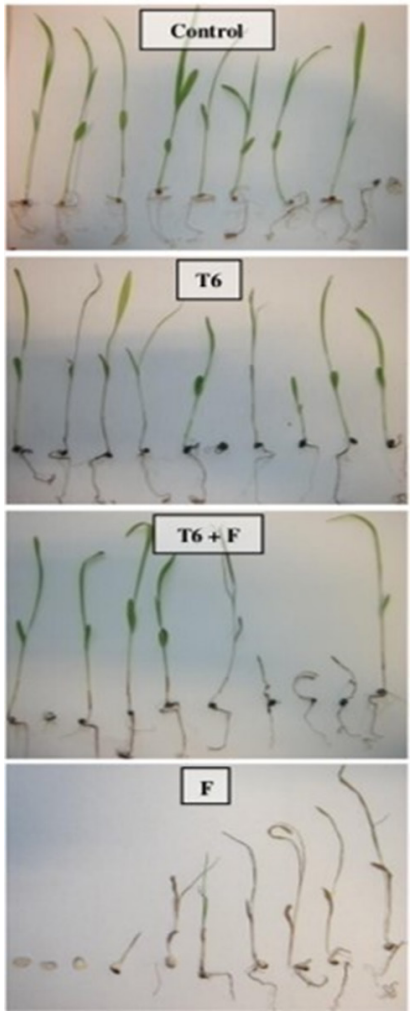

Figure 5. *Seedlings with different treatment.*
Note. Control: without fungal treatment; T6: treatment with *T. asperellum*; T6 + F: treatment with *T. asperellum* and *F. nygamai*; F: treatment with *F. nygamai.*
Source: own elaboration.

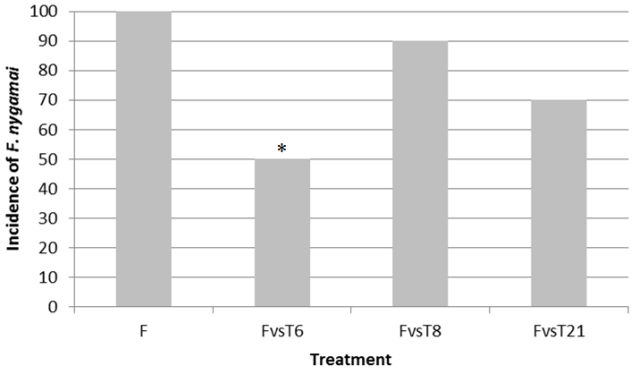

Figure 6. *Incidence of F. nygamai in sorghum plants treated with T. asperellum: T6 and T. atroviride: T8, T21.*
Note. Treatments with an asterisk (*) are significantly different from the control ($p < 0.05$) according to Fisher's test.
Source: own elaboration.

Discussion

F. nygamai is an important pathogen of sorghum, which has been frequently isolated from grains in Uruguay (del Palacio *et al.,* 2016). The infection of crops with this fungus can generate significant losses in crop yields and negatively affect animal and human health due to its ability to produce mycotoxins. There is limited information about the biological control of *F. nygamai* using *Trichoderma* spp. The antagonism between fungi is mediated by a series of mechanisms developed by the bio-controller and the pathogen. *Trichoderma* sp. has a great variety of modes of action; among the best known are mycoparasitism, antibiosis, competition for nutrients and space, release of enzymes that affect the metabolism of other microorganisms, induction of the defense response in the plant, and growth promotion (Infante; Martínez; González; Reyes, 2009). These mechanisms and their intensity vary between species and fundamentally between isolated strains.

In this study, specific *in vitro* test results showed that *Trichoderma* spp. produces some volatile compounds and some soluble ones capable of inhibiting the mycelial growth of *F. nygamai*. Furthermore, it was observed that *Trichoderma* strains were capable of overgrowing and sporulating abundantly onto the *Fusarium* mycelium. In seed trials, protection against the disease was observed. This effect could be correlated to the modes of action against pathogens such as antibiosis, mycoparasitism and induction of defensive response by the plant. The high growth rate and strong sporulation shown by *Trichoderma* strains are also very important characteristics for the selection of an antagonist, which are related to a highly competitive capacity for space and for substrate, as well as a high colonizing capacity.

It was shown that several species of *Trichoderma*, isolated from soil, had antagonistic activity against *F. nygamai*. The use of *Trichoderma* strains from sorghum soils could improve the efficiency in the establishment of

populations since strains are already adapted in sites where they will be applied. *T. atroviride* was the most frequent species of *Trichoderma* isolated, and together with *T. asperellum* were the ones that showed the highest degrees of inhibition of *F. nygamai* growth.

In dual cultures, a bioassay of 12 isolates of *Trichoderma* spp. reduced *F. nygamai* growth, but the extent of it in mycelial reduction varied among species and isolates, with different antagonist strategies observed according to the *Trichoderma* isolate. Several strains of *T. asperellum* and *T. atroviride* overgrew *F. nygamai* in a few days, since they grew faster than *F. nygamai*, allowing them to efficiently compete for nutrients and space.

Martínez-Coca, Infante, Caraballo, Duarte-Leal, and Echevarría-Hernández (2018) found strains of *T. asperellum* that showed highly competitive capacity for space and stood out for their mycoparasitism against *F. nygamai* and *Fusarium oxysporum* f. sp. *ciceri* from chickpeas. Similar results were reported by Win, Bo, Malec, Khan, and Fu (2021), where *T. asperellum* could inhibit mycelial growth and spore germination of *F. oxysporum, F. fujikuroi, F. tricinctum, F. cantenulatum* by growth competition and directly by antibiosis and mycoparasitism. Furthermore, they found that *T. asperellum* secreted chitinase and β-1,3, glucanase, able to degrade cell walls of *Fusarium* spp.

The confrontation of *F. nygamai* with *T. virens* resulted in a deadlock zone, indicating the production of growth inhibitory metabolites by both fungi. Several antifungal compounds have been identified from *T. virens* included toxins and antibiotics (Daguerre; Edel-Hermann; Steinberg, 2017; Li; Li; Zhang, 2019).

Collapsed hyphae of *F. nygamai* observed at the interaction interface, could be due to the activity of *Trichoderma* enzymes that participate in wall degradation. Metcalf and Wilson (2001) found that *T. koningii* Oudem. produced endo and exochitinases capable to degrade *Sclerotium cepivorum* Berk. cell walls.

The volatile metabolites produced by *T. atroviride* inhibited 20 % of *Fusarium* growth. This could be important in soil conditions, where fungistatic activity is largely due to the production of volatile metabolites by the fungal community. It is possible that the volatile metabolites saturate the niches of the rhizosphere allowing the suppression of pathogenic fungi even before direct contact (Reino; Guerrero; Hernández-Galán; Collado, 2008). Volatile metabolites are not only important in the soil due to their accumulation in microspaces and their ability to inhibit the growth of pathogens, but they are also very important due to their interaction with the plant roots, mediating the induction of systemic resistance (Contreras-Cornejo; Macías-Rodríguez; del-Val; Larsen, 2016). Volatile compounds can participate in various intra- and inter- kingdom interactions, for which they are of great interest. Nawrocka, Małolepsza, Szymczak, and Szczech (2018) also found that *T. atroviride* induced the resistance response in cucumber plants with the simultaneous promotion of plant growth.

All seedlings that were infected with *F. nygamai* (F4 and F5) *in vivo* under laboratory conditions evidenced wilt as disease symptoms, except those that were treated with *T. asperellum* (T6), where 50 % did not develop disease symptoms. Intana, Kheawleng, and Sunpapao (2021) found that volatile organic compounds emitted from *T. asperellum,* with phenylethyl alcohol as the principal compound, were able to inhibit mycelial growth of *F. incanatum in vitro* and smaller lesions of muskmelons were obtained in *in vivo* assays.

The protection of seeds against *Fusarium* infection and the capacity of endophytic colonization of seedling tissues by *T. asperellum* (T6) suggests that this strain is the most promising for the control of *F. nygamai*. Since *F. nygamai* can infect the plant through its root, the use of seeds pelleted with *Trichoderma* can be advantageous as a control strategy and would be a useful method for application. Assays *in vivo*, under laboratory conditions, could be a

useful tool to evaluate the inhibitory activity of biocontrol agents before performing the evaluation under greenhouse or field conditions.

Currently, several strains of the genus *Trichoderma* are being tested as alternatives to chemical fungicides (Imran; Arif; Shah; Bari, 2020; Zin; Badaluddin, 2020). Saravanakumar *et al.* (2017) found that *T. harzianum* can control *F. graminearum* in maize crops, and Al-Mekhlafi, Abdullah, Al-Helali, and Alghalibi (2019) found that *T. citrinoviride* can control *F. oxysporum* in tomato plants under greenhouse conditions. However, full-scale application of *Trichoderma* for the biological control of plant pathogens has not been widespread. According to the results obtained, *Trichoderma* spp. isolates from cultivated sorghum soils are capable of endophytic colonization and protection of sorghum plants, therefore they could be a promising source of antagonists for the biological control of *F. nygamai* disease. The pelleting seeds method was effective and could be a useful tool for field application. Yaqub and Shahzad (2008) used *Trichoderma* spp. for seed pelleting to prevent diseases caused by *Sclerotium rolfsii*, observing that pelleting of seeds with microbial antagonists increased the efficacy of these biological control agents. Coninck *et al.* (2020) suggest a specific seed coating for field crops such as maize, with the action of *T. atroviride* BC0584 strain against *F. avenaceum* and *F.culmorum* soil-borne pathogens.

Conclusions

Trichoderma asperellum (T6) *and T. atroviride* (T21) were effective antagonists of *F. nygamai in vitro*. The protection of seeds against *Fusarium* infection and the ability of endophytic colonization of seedling tissues by *Trichoderma asperellum* (T6) suggest that this strain is the most promising for the control of *F. nygamai*, considering that this fungus evidenced the ability to protect 50 % of seedlings contaminated with *F. nygamai*. The pelleting seeds method was effective and could be a useful tool for field application.

According to the results obtained, *Trichoderma* spp. isolates from cultivated sorghum soils are capable of endophytic colonization and protection of sorghum plants, therefore they could be a promising source of antagonists for the biological control of *F. nygamai* disease. It seems possible to assume that *Trichoderma* could constitute an environmentally friendly way to obtain healthy and mycotoxin-free cultures

Consequently, the incorporation of *Trichoderma* to biological formulates, in an integrated plan management for the control of *F. nygamai* in sorghum crops, could be very important.

Author contribution statement

Ana Belén Corallo Fabiano and Susana María Tiscornia Córdoba conceived research. Ana Belén Corallo Fabiano and Susana María Tiscornia Córdoba conducted experiments. Ana Belén Corallo Fabiano, Lina Julia Bettucci Rossi and Susana María Tiscornia Córdoba contributed to material. Ana Belén Corallo Fabiano, Lina Julia Bettucci Rossi and Susana María Tiscornia Córdoba wrote the manuscript. All authors read and approved the final manuscript.

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