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In vitro antagonism of *Trichoderma* against *Sclerotium rolfsii* from potato (*Solanum tuberosum*)

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RESUMEN

Background/Objective. *Trichoderma* constitutes a viable alternative for reducing the destructive potential of soft rot (*Sclerotium rolfsii*) in potato crops. The objectives of this research were to determine the *in vitro* antagonistic effectiveness of *Trichoderma asperellum*, *T. asperelloides*, *T. afroharzianum*, and *T. azevedoi* against *S. rolfsii* (Scr4 and Scr17) and to determine the hyphal interactions of the antagonists, on addition to determinate the inhibition of mycelial growth of *S. rolfsii* (Scr4) by volatile metabolites produced by *Trichoderma* species.

Materials and Methods. The *in vitro* biological effectiveness of 16 *T. asperellum* isolates, five *T. asperelloides* isolates, four *T. afroharzianum* isolates, and one *T. azevedoi* isolate in inhibiting *S. rolfsii* (Scr4 and Scr17) mycelial growth was studied. The type of hyphal interaction between the same *Trichoderma* spp. isolates and *S. rolfsii* (Scr4) was also determined. The effect of volatile metabolites produced by *Trichoderma* spp. isolates on the inhibition of mycelial growth of *S. rolfsii* (Scr4) was evaluated. Data on mycelial growth inhibition were analyzed using nonparametric statistical analysis (Kruskal-Wallis), and the mean separation was performed using the procedures of Conover (1999) with ($P \leq 0.05$).

Results. In dual confrontations, *Trichoderma* species showed mycelial growth inhibitions of 21.0 to 75.4% and 23.6 to 77.1% in *S. rolfsii* isolates Scr4 and Scr17, respectively. Hyphal interactions of the same *Trichoderma* species consisted of vacuolization, granulation, coiling, adhesion, lysis, and penetration into the pathogen (Scr4); all four *Trichoderma* species inhibited mycelial growth (26.0 to 81.4%) of *S. rolfsii*. *T. azevedoi* stood out among the isolates for showing greater antagonistic capacity in all tests.

Conclusion. The results indicate that *Trichoderma* species, especially *T. azevedoi* (TAI73), inhibited the growth of S. rolfsii Scr4 (75.4%) and Scr17 (77.1%). T. azevedoi

(TAI73) caused vacuolization, granulation, coiling, adhesion, and lysis of hyphae in the Scr4 isolate.

Keywords: Antagonist, Biological Control, Mycelial Inhibition, Metabolites

INTRODUCTION

Sclerotium rolfsii is a soilborne phytopathogenic fungus considered the causal agent of potato (*Solanum tuberosum*) tuber rot. It also causes stem, root, petiole, leaf, and fruit rot in various crops. The fungus has caused losses of up to 15% in potato production in Italy (Garibaldi *et al*., 2007). Symptoms begin with dry rot at the base of the stem, along with vascular tissue necrosis; infected plants show leaf chlorosis, and as the disease progresses, the plants wilt and the tubers rot. The fungus produces white, fan-shaped mycelium on the affected tissue, which, when mature, forms sclerotia. These sclerotia are the main source of primary inoculum and spread through water, plant debris, soil, and animals (Kator *et al*., 2015; Roca *et al*., 2016). In Sinaloa, Mexico, the disease has been underestimated by potato growers, but it is estimated to have caused up to 20% production losses in fields with high-moisture-retaining soils (clay soils) and during periods of increased ambient temperatures above 30 °C. Damage also increases when harvest is delayed, allowing the fungus to cause greater injury to the tubers that remain in the soil.

Synthetic fungicides are used to combat various species of phytopathogenic fungi; however, excessive pesticide application has caused environmental and human health problems (Brauer *et al*., 2019). Alternatively, other ecologically focused methods have been used to control *S. rolfsii*, such as the use of biocontrol agents, which represent a promising alternative (Mishra *et al*., 2015; Khan *et al*., 2020). In this regard, different *Trichoderma* species are the most widely used biocontrol agents, as they exhibit various mechanisms of action (substrate competition, mycoparasitism and antibiosis) that allow the management of several diseases caused by phytopathogens (Infante *et al*., 2009; Adnan *et al*., 2019; Garrido and Vilela, 2019; Andrade-Hoyos *et al*., 2020; Pérez *et al*., 2020; Asad, 2022). Although no *in vitro* antagonism studies between *Trichoderma* species and *S. rolfsii* in potato have been conducted, other studies on different hosts have reported good control using *Trichoderma* spp. as antagonists against *S. rolfsii* isolated from tomato (*Solanum lycopersicum*), bean (*Phaseolus vulgaris*), cabbage (*Brassica oleracea*), betel (*Piper betle*), lentil (*Lens esculenta*), chickpea (*Cicer arietinum*), squash (*Cucurbita pepo*), among others (Díaz-Nájera *et al*., 2018; Kushwaha *et al*., 2018; Chandra-Sekhar *et al*., 2020; Kamel *et al*., 2020; Andrade-Hoyos *et al*., 2023). *T. hamatum*, *T. harzianum*, *T. viride*, *T. virens*, and *T. asperellum* produce different secondary metabolites that activate defense mechanisms, promote plant growth and development, and inhibit pathogen development (Lee *et al*., 2016; Chandra *et al*., 2020; Vineela *et al*., 2020; Safari *et al*., 2022; Guzmán *et al*., 2023). These metabolites may be volatile or non-volatile; volatile metabolites include acetic acid, 2-pentylfuran, 6-pentyl-2H-pyran-2-one, and 2-methyl-1-butanol, among others, which inhibit *S. rolfsii* (Phoka *et al*., 2020; Sridharan *et al*., 2020).

In Mexico, *S. rolfsii* has shown high incidence levels in commercial potato fields in Sonora and Sinaloa. However, the biological effectiveness of *Trichoderma* species against this phytopathogen under controlled, greenhouse, and field conditions has not yet been evaluated. Therefore, it is important to develop ecological alternatives for managing this fungus in the short term. Based on this, the objectives of this study were to determine the *in vitro* antagonistic capacity of 26 *Trichoderma* isolates through dual confrontations, to identify hyphal interactions, and to evaluate the effect of volatile metabolites on the mycelial growth of *S. rolfsii* isolates obtained from potato-cultivated soils in the states of Sonora and Sinaloa, Mexico.

MATERIALS AND METHODS

Pathogen Isolation. The study used 16 isolates of *T. asperellum* (TAM), five of *T. asperelloides* (TES), four of *T. afroharzianum* (TAF), one of *T. azevedoi* (TAI), and two of *S. rolfsii* (Scr4 and Scr17). These isolates were provided by the microbiological bank of the Phytosanitary Diagnostic Laboratory of the Local Plant Health Board of El Fuerte Velley. The isolates were collected from the county of Altar and Caborca in the state of Sonora, and Ahome, Guasave, El Fuerte, and Sinaloa de Leyva in the state of Sinaloa, during the 2019–2021 growing seasons. The sequences of the isolates were deposited in GenBank under the accession numbers OR521159–OR521184 for *Trichoderma* and OR514113 and OR514124 for *Sclerotium rolfsii* (Table 1).

***In vitro* inhibitory effect of 26 *Trichoderma* spp. isolates against *Sclerotium rolfsii*.** The *in vitro* antagonism of 26 isolates from four *Trichoderma* species, grown for three days, was evaluated against two seven-day-old *S. rolfsii* isolates (Scr4 and Scr17) on Potato Dextrose Agar (PDA) medium (BD Bioxon, Becton, Dickinson de México, S.A. de C.V.). The evaluation was carried out using the dual culture technique (Dennis and Webster, 1971). Mycelial discs 5 mm in diameter were obtained from both the pure colonies of the antagonistic isolates and the pathogen. The *S. rolfsii* discs were placed on the periphery of 90 mm Petri dishes, and those of *Trichoderma* spp. on the opposite side. Treatments were arranged in a completely randomized design with four replicates (four Petri dishes) per *S. rolfsii*-*Trichoderma* combination. The experiment was conducted twice.

Petri dishes with PDA containing individual cultures of both *S. rolfsii* and *Trichoderma* served as controls (Pacheco *et al*., 2016). The Petri dishes were sealed with Parafilm and incubated at 25 ± 1 °C in constant darkness in a Thermo Scientific bioclimatic chamber. To determine the antagonistic capacity of *Trichoderma* spp., mycelial growth was measured every 24 hours after inoculation and ended when *S. rolfsii* filled the Petri dishes in the control group. The percentage of mycelial growth inhibition of the test pathogen (I) was calculated using the following equation: Inhibition percentage: $I = (C - T) / C \times 100$, where C = growth of the control pathogen and T = growth of the pathogen in treatment (Kotasthane *et al*., 2014). The biological effectiveness of *Trichoderma* species in inhibiting *Sclerotium rolfsii* was determined using the 1-to-5 rating scale by Bell *et al*. (1982), where: class 1) *Trichoderma* completely covered the culture medium surface, class 2) *Trichoderma* covered at least 65% of the medium, class 3) *Trichoderma* and *S. rolfsii* each grew on 50% of the medium, class 4) *S. rolfsii* covered at least 65% of the medium, and class 5) *S. rolfsii* completely covered the culture medium surface.

Table 1. Origin and identification of *Sclerotium rolfsii* and *Trichoderma* species isolates.

Species/isolate	Year of collection	County	Code in Gen Bank
Sclerotium rolfsii /Scr4	2019	Ahome, Sinaloa	OR514113
Sclerotium rolfsii /Scr17	2019	Ahome, Sinaloa	OR514124
T. asperelloides /TES19	2020	Caborca, Sonora	OR521159
T. asperelloides /TES20	2020	Altar, Sonora	OR521160
T. afroharzianum /TAF21	2020	Altar, Sonora	OR521161
T. asperellum /TAM22	2020	Caborca, Sonora	OR521162
T. asperelloides /TES23	2020	Caborca, Sonora	OR521163
T. asperelloides /TES24	2020	Caborca, Sonora	OR521164
T. asperelloides /TES26	2020	Caborca, Sonora	OR521165
T. asperellum/TAM27	2019	Guasave, Sinaloa	OR521166
T. asperellum/TAM30	2019	El Fuerte, Sinaloa	OR521167
T. asperellum/TAM31	2019	Guasave, Sinaloa	OR521168
T. afroharzianum /TAF33	2020	Sinaloa de Leyva, Sinaloa	OR521169
T. asperellum/TAM35	2020	Ahome, Sinaloa	OR521170
T. asperellum/TAM37	2020	Guasave, Sinaloa	OR521171
*T. afroharzianum/*TAF38	2020	Guasave, Sinaloa	OR521172
T. asperellum/TAM57	2021	Ahome, Sinaloa	OR521173
T. asperellum/TAM59	2021	Guasave, Sinaloa	OR521174
T. asperellum/TAM64	2021	Ahome, Sinaloa	OR521175
*T. asperelloides/*TES65	2021	Ahome, Sinaloa	OR521176
T. asperellum/TAM67	2021	Ahome, Sinaloa	OR521177
T. asperellum/TAM68	2021	Ahome, Sinaloa	OR521178
T. asperellum/TAM69	2021	Ahome, Sinaloa	OR521179
T. asperellum/TAM70	2021	Ahome, Sinaloa	OR521180
T. azevedoi /TAI73	2021	Ahome, Sinaloa	OR521181
T. asperellum/TAM74	2021	Ahome, Sinaloa	OR521182
T. asperellum/TAF75	2021	Ahome, Sinaloa	OR521183
T. asperellum/TAM76	2020	Caborca, Sonora	OR521184

Hyphal interaction of *Trichoderma* spp. against *S. rolfsii*. The hyphal interaction of 26 *Trichoderma* spp. isolates against *S. rolfsii* (Scr4) was evaluated using the dual culture method (Dennis and Webster, 1971). The interaction was observed at the point of contact between the fungi, from which three samples were taken from the interaction zone. A piece of transparent adhesive tape was placed over the hyphae of the fungus and *Trichoderma* spp., then the tape was placed over a drop of lactophenol cotton blue on a microscope slide and observed under a light microscope to identify vacuolization, granulation, coiling, adhesion, lysis, or penetration caused by the *Trichoderma* spp. isolates (Martínez *et al*., 2018).

Effect of volatile metabolites from *Trichoderma* species with antifungal activity against *S. rolfsii*. To determine the inhibitory effect of volatile metabolites from the 26 *Trichoderma* spp. isolates against a *S. rolfsii* isolate (Scr4), both were cultured on PDA and incubated at 25 ± 2 °C for three and seven days, respectively. Afterwards, 5 mm diameter discs of the same medium with active mycelium of *S. rolfsii* and the 26 *Trichoderma* spp. isolates were transferred to the center of PDA Petri dishes. The *Trichoderma* inoculum was transferred 24 hours before that of *S. rolfsii*. For the confrontation, the bases of the Petri dishes were assembled and sealed with Parafilm (Rajani *et al*., 2021). *Trichoderma* was placed in the bottom dish and *S. rolfsii* in the top dish (Dennis and Webster, 1971; Mokhtar and Dehimat, 2012). Treatments were arranged in a completely randomized design with four replicates per *S. rolfsii*-*Trichoderma* combination. The experiment was conducted twice. Petri dishes with PDA containing individual cultures of *S. rolfsii* and *Trichoderma* were included as controls and incubated at 25 ± 2 °C. The inhibitory effect of *Trichoderma* spp. was determined once *S. rolfsii* had covered the culture medium in the control treatment without *Trichoderma*, using the inhibition percentage formula described by Kotasthane *et al*. (2014), where: Inhibition percentage: $I = (C - T) / C \times 100$, with C = growth of the control pathogen and T = growth of the pathogen in treatment.

Statistical Data Analysis. Data on *in vitro* mycelial inhibition through dual culture confrontations between the 26 *Trichoderma* isolates and two *S. rolfsii* isolates, as well as data on the inhibition of mycelial growth of one *S. rolfsii* isolate by volatile metabolites from 26 *Trichoderma* spp. isolates, were analyzed using non-parametric statistics (Kruskal-Wallis). Mean separation for mycelial growth inhibition was performed using the procedures of Conover (1999) with ($P \leq 0.05$).

RESULTS

***In vitro* inhibition of *Trichoderma* spp. isolates on *S. rolfsii*.** The results showed significant differences ($P \leq 0.05$) in the *in vitro* inhibitory effect of *S. rolfsii* among the 26 *Trichoderma* isolates evaluated, as they inhibited both *S. rolfsii* isolates (Scr4 and Scr17), with inhibition percentages ranging from 21.0 to 75.4% for Scr4 and from 23.6 to 77.1% for Scr17.

The TAI73 isolate of *T. azevedoi* exhibited the greatest *in vitro* inhibitory effect on the mycelial growth of the Scr4 isolate of *S. rolfsii* (Figure 1A), with an inhibition percentage of 75.4%, followed by isolates TAM74 (68.9%), TAM30 (66.6%), TAF75 (66.0%), TES65 (65.0%), TES24 (64.9%), TAM68 (64.9%), and TAM37 (64.8%). All inhibition levels were classified in group 2, with no significant differences among them ($P \geq 0.05$), but they differed significantly from the other *Trichoderma* isolates. Meanwhile, the TAF33 isolate showed the lowest inhibition percentage (21.0%) (Figure 1B) and was classified in group 4, followed by TAM70 (56.7%), TAF21 (57.2%), TAM76 (57.3%), TAM57 (57.3%), and TAM31 (59.0%), which all belonged to group 3, with no significant differences between treatments ($P \geq 0.05$) (Table 2).

On the other hand, the *Trichoderma* isolate TAI73 exhibited the highest level of inhibition in the mycelial growth of Scr17, with 77.1% (group 2), followed by TAM69 (68.8%), TES24 (67.9%) (Figure 1C), TAM30 (67.9%), TES65 (67.3%), TAF75 (67.0%), TAM74 (66.4%), TAM35 (66.2%), and TAM37 (66.1%) all in group 2, with no significant differences among them (P ≥ 0.05) (Table 2). In contrast, the *Trichoderma* isolate TAF33 (Figure 1D) (group 4) showed the lowest inhibition percentage (23.6%) against Scr17, followed by TAF38 (52.8%), TAM31 (57.4%), TAM68 (59.8%), TAM59 (60.1%), TAM64 (60.3%), and TES20 (60.3%) all in group 3, with no significant differences among them (P ≥ 0.05) (Table 2).

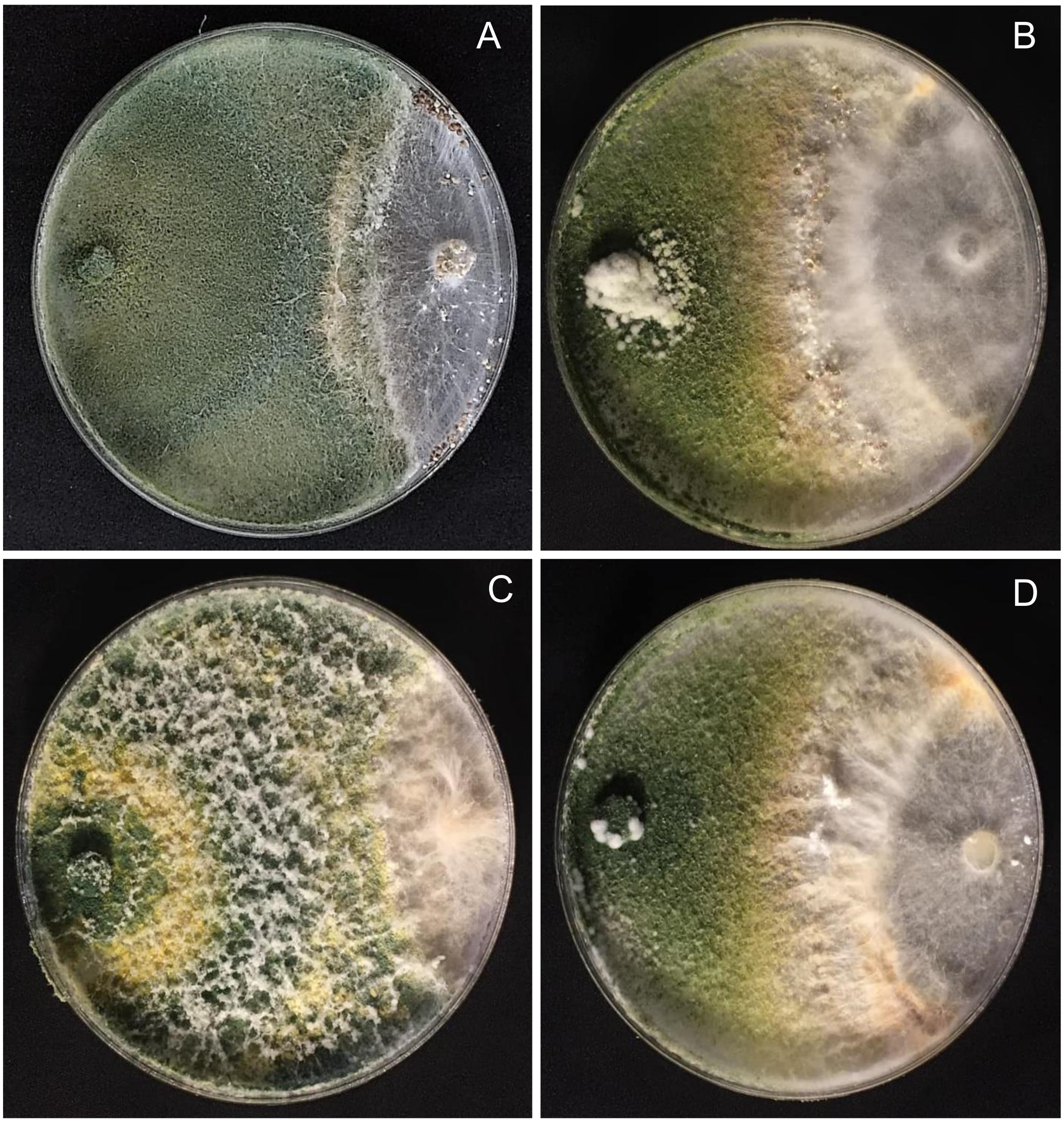

Figure 1. *In vitro* antagonism of *Trichoderma* spp. (left) against *S. rolfsii* (right). (A) *Trichoderma* isolate TAI73 against *S. rolfsii* Scr4, (B) *Trichoderma* TAF33 against *S. rolfsii* Scr4, (C) *Trichoderma* TES24 against *S. rolfsii* Scr17 and (D) *Trichoderma* TAF33 against *S. rolfsii* Scr17.

The *T. azevedoi* isolate (TAI73) showed the highest percentage of *in vitro* inhibition of the mycelial growth of the *S. rolfsii* isolate Scr4, with 75.4% inhibition (Table 2); while the same *Trichoderma* isolate exerted 77.1% inhibition on the *S. rolfsii* isolate Scr17. In contrast, *T. afroharzianum* (TAF33) had the lowest inhibitory effect on both *S. rolfsii* isolates (Table 2). A marked variation was observed in the effect of the *Trichoderma* isolates on the inhibition of *S. rolfsii*, with significant differences among them (P ≤ 0.05) (Table 2).

Table 2. *In vitro* antagonism of 26 *Trichoderma* spp. isolates against two *Sclerotium rolfsii* isolates.

Scr4 isolate from *S. rolfsii*			**Scr17 isolate from *S. rolfsii***		
***Trichoderma* isolate**	***Trichoderma* isolate**	**Scale**	***Trichoderma* isolate**	***Trichoderma* isolate**	**Scale**
Control	0.0 a[z]	5	Control	0.0 a*	5
TAF33	21.0 ab	4	TAF33	23.6 ab	4
TAM70	56.7 abc	3	TAF38	52.8 ab	3
TAF21	57.2 abcd	3	TAM31	57.4 abc	3
TAM76	57.3 abcd	3	TAM68	59.8 abc	3
TAM57	57.3 abcd	3	TAM59	60.1 abcd	3
TAM31	59.0 abcde	3	TAM64	60.3 abcd	3
TES26	60.3 bcdef	2	TES20	60.3 abcde	3
TAM67	60.5 cdefg	2	TAF21	60.4 bcde	2
TAF38	60.5 cdefg	2	TES26	60.5 bcde	2
TAM64	60.5 cdefg	2	TAM57	61.9 cdef	2
TAM59	60.9 defgh	2	TAM23	61.9 cdef	2
TAM23	61.9 efghi	2	TAM70	62.6 defg	2
TAM27	62.0 efghi	2	TAM67	63.0 defg	2
TAM22	62.7 fghij	2	TAM22	63.4 efgh	2
TAM69	63.2 fghij	2	TAM27	63.4 efgh	2
TES20	63.3 fghij	2	TAM76	64.7 fghi	2
TES19	63.4 ghijk	2	TES19	65.0 fghi	2
TAM35	63.6 hijk	2	TAM37	66.1 ghij	2
TAM37	64.8 ijkl	2	TAM35	66.2 ghij	2
TAM68	64.9 ijkl	2	TAM74	66.4 ghij	2
TES24	64.9 ijkl	2	TAF75	67.0 hij	2
TES65	65.0 jkl	2	TES65	67.3 hij	2
TAF75	66.0 jkl	2	TAM30	67.9 ij	2
TAM30	66.6 kl	2	TES24	67.9 ij	2
TAM74	68.9 l	2	TAM69	68.8 j	2
TAI73	75.4 l	2	TAI73	77.1 j	2

[z] Means with a common letter are not significantly different (P ≤ 0.05), Kruskal-Wallis.

Determination of hyphal interactions between *Trichoderma* spp. and *S. rolfsii*. The hyphal contact zone between *Trichoderma* spp. isolates and *S. rolfsii* (Scr4) showed several types of interaction. Of the isolates, 23 of the antagonist caused lysis (Figure 2A), 20 produced granulation (Figure 2B), 20 showed adhesion (Figure 2C), 19 exhibited coiling (Figure 2D), 13 induced vacuolization (Figure 2E), and one demonstrated penetration (Figure 2F). The TES65 isolate of *T. asperelloides* was the only one that penetrated the hyphae of *S. rolfsii* (Table 3, Figure 2F). The *Trichoderma* spp. isolates TAM37, TAM59, TES65, TAI73, and TAM76 showed up to five interactions when confronted with *S. rolfsii*. Meanwhile, the isolates TES20, TAF21, TAM23, TES24, TES26, TAM30, TAM31, TAF33, TAM35, TAM57, TAM68, TAM70, and TAF75 of the antagonist fungus exhibited three interactions (Table 3).

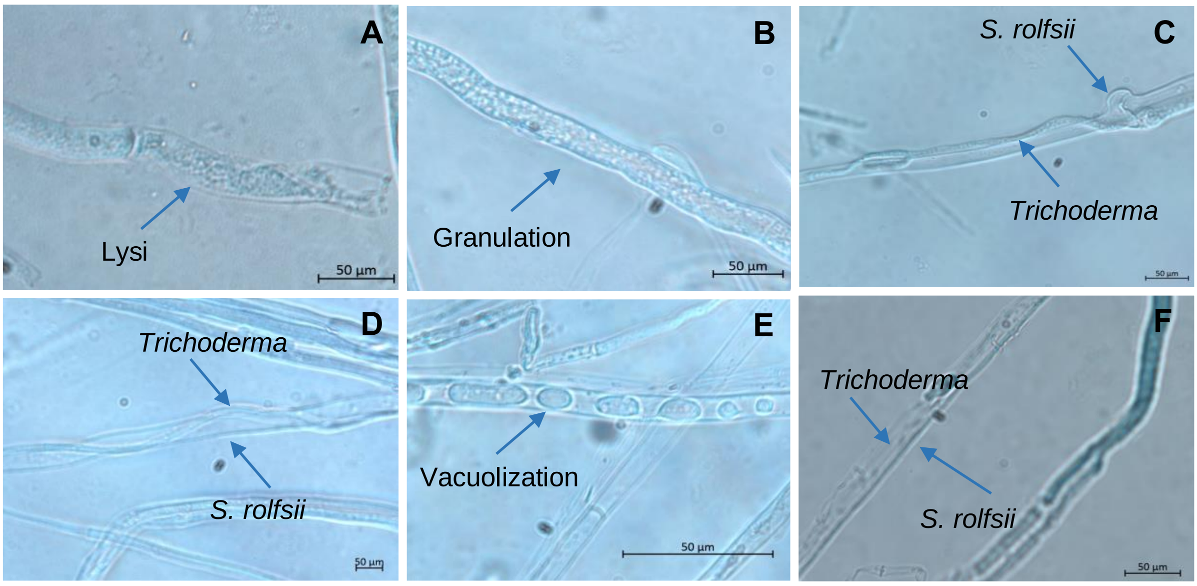

Figure 2. Hyphal interactions between *Trichoderma* spp. isolates and *Sclerotium rolfsii*. (A) Lysis (TAM67), (B) granulation (TES65), (C) adhesion (TAM69), (D) coiling (TAM59), (E) vacuolization (TAM76), and (F) penetration (TES65).

Table 3. *In vitro* hyphal interaction of 26 *Trichoderma* spp. isolates and *Sclerotium rolfsii* (Scr4).

***Trichoderma* isolate**	**Types of hyphal interactions**					
	Lysis	**Granulation**	**Adhesion**	**Coiling**	**Vacuolization**	**Penetration**
TES19	x		x	x	x	
TES20	x	x	x			
TAF21	x		x		x	
TAM22	x	x		x	x	
TAM23	x	x		x		
TES24		x	x	x		
TES26		x	x	x		
TAM27	x	x	x	x		
TAM30	x	x	x			
TAM31	x	x			x	
TAF33	x		x	x		
TAM35	x	x			x	
TAM37	x	x	x	x	x	
TAF38	x	x	x	x		
TAM57	x			x	x	
TAM59	x	x	x	x	x	
TAM64	x	x	x		x	
TES65	x	x	x	x		x
TAM67		x	x	x	x	
TAM68	x		x	x		
TAM69	x	x	x	x		
TAM70	x		x	x		
TAI73	x	x	x	x	x	
TAM74	x	x	x	x		
TAF75	x	x			x	
TAM76	x	x	x	x	x	

Note: The interaction zone was stained with lactophenol-blue when the fungal hyphae interacted.

In vitro* inhibition of volatile metabolites from *Trichoderma* against *S. rolfsii. The volatile metabolites from 26 *Trichoderma* spp. isolates inhibited the mycelial growth of *S. rolfsii* (Scr4) *in vitro*. The inhibition percentage ranged from 26 to 81.4%. The highest inhibition was exhibited by the TAI73 isolate of *T. azevedoi*, with 81.4%, while the inhibition percentages of the isolates TAM68 (Figure 3A), TES65, TAM74, TAM57, TAF75, TAM70, and TAM37 ranged from 78.2 to 70.9%, with no significant differences between them ($P \geq 0.05$) (Table 4). The isolates TAM59, TAM35, TES26, TES19, TAF38, TAM27, and TAM30 (Figure 3B) showed inhibition ranging from 49.6 to 57.1% against *S. rolfsii*. The TAF33 isolate of *T. afroharzianum* showed the lowest inhibition percentage, equivalent to 26%. The variation in the inhibitory effect of the *Trichoderma* isolates was reflected by significant differences between isolates ($P \leq 0.05$) (Table 4).

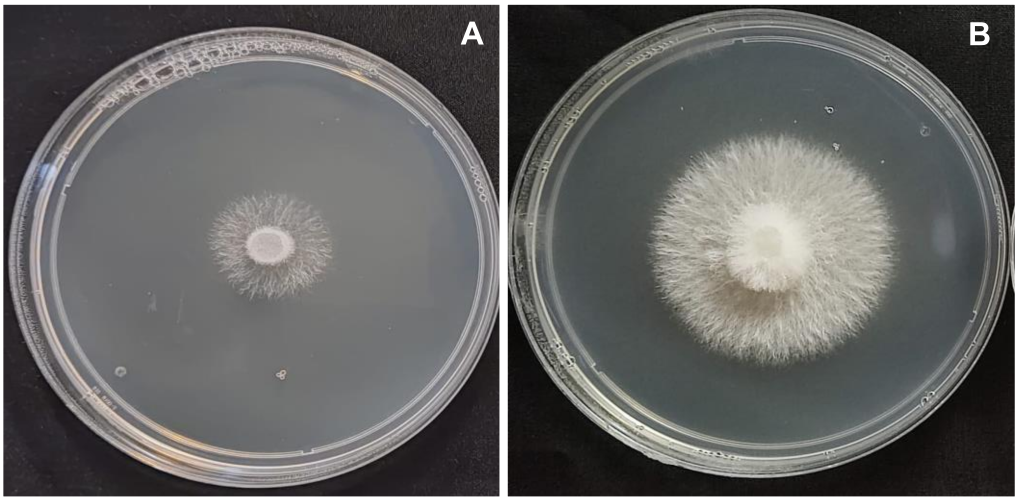

Figure 3. Inhibitory effect of volatile metabolites from *Trichoderma* spp. against *Sclerotium rolfsii*. (A) *Sclerotium rolfsii* (Scr4) isolate exposed to volatile metabolites from *Trichoderma* TAM68 and (B) *S. rolfsii* (Scr4) exposed to volatile metabolites from *Trichoderma* TAM30.

Table 4. Effect of volatile metabolites from *Trichoderma* isolates against *Sclerotium rolfsii* Scr4.

***Trichoderma* isolate**	**% inhibition**	***Trichoderma* isolate**	**% inhibition**
Control	00.0 a^z	TAM31	62.5 efghij
TAF33	26.0 a	TAM64	63.0 fghijk
TAM59	49.6 ab	TAM76	65.8 ghijk
TAM35	50.3 ab	TAM67	68.1 hijkl
TES26	54.1 abc	TAF21	69.2 ijklm
TES19	55.6 abcd	TAM37	70.9 ijklmn
TAF38	56.3 abcde	TAM70	72.0 jklmn
TAM27	56.9 abcdef	TAF75	73.5 klmn
TAM30	57.1 abcdef	TAM57	73.5 klmn
TES20	58.4 bcdefg	TAM74	76.3 lmn
TES24	58.7 bcdefg	TES65	76.3 lmn
TAM22	59.5 cdefgh	TAM68	78.2 mn
TAM69	61.4 defghi	TAI73	81.4 n
TAM23	62.2 efghij		

^{z}Means with a common letter are not significantly different (P ≤ 0.05), Kruskal-Wallis.

DISCUSSION

The use of *Trichoderma* as a biological control agent in potato cultivation is limited, as producers prefer the use of synthetic fungicides, arguing that the efficacy of these substances is well-known. In Mexico, there is no scientific evidence on the use of different *Trichoderma* species to control soft rot in potatoes.

In this study, it was found that isolates of *T. azevedoi*, *T. asperellum*, *T. asperelloides*, and *T. afroharzianum* from soils subjected to potato cultivation showed different levels of inhibition on the mycelial growth of *S. rolfsii in vitro*. *T. azevedoi* exhibited the highest inhibitory effect, with 75.4% against *S. rolfsii* (Scr4) and 77.1% against the same species (Scr17). These results are consistent with those reported by Kamel *et al*. (2020), who evaluated six *Trichoderma* species against three *S. rolfsii* isolates from bean, tomato, and cabbage crops in Egypt. In this study, *T. viride* showed the highest inhibitory effect (85.6%) against *S. rolfsii* isolated from tomato, while *T. harzianum* inhibited the pathogen by 79.3% and 77.8% from cabbage and bean isolates, respectively. Similarly, Vineela *et al*. (2020) reported that *T. hamatum* inhibited the mycelial growth of *S. rolfsii* by 75.2%, followed by *T. harzianum* (74.7%) and *T. viride* (71.9%), obtained from peanut (*Arachis hypogaea*) crops in India. On the other hand, Safari *et al.* (2022) evaluated 30 antagonistic fungi through dual culture and reported that *T. virens* showed the highest mycelial growth suppression capacity against *S. rolfsii* (90.8%) isolated from peanut crops in Iran, followed by *T. viride* with 66.8%. Similarly, Kushwaha *et al*. (2018) determined that *T. harzianum* exerted an inhibitory effect of 63.5% *in vitro*, followed by *T. virens* with 51.5% and *T. viride* with 50.8% against *S. rolfsii* isolated from lentil (Lens culinaris) crops in India. In contrast, Chandra-Sekhar *et al*. (2020) reported low *in vitro* inhibition of mycelial growth of *S. rolfsii* isolated from tomato in India, where *T. harzianum* inhibited by 50%, *T. viride* 39.7%, and *T. asperellum* 33.8%.

In the present study, it was found that, of the 26 *Trichoderma* isolates evaluated against *S. rolfsii* (Scr4), 20 of them (73.0%) were placed in class 2 according to the Bell scale (Bell *et al*., 1982); while 17 isolates of the antagonist (73.0%) were classified in class 2 for the inhibition of mycelial growth of *S. rolfsii* (Scr17). These results suggest that their control potential should be evaluated in the field. On the other hand, Kotasthane *et al*. (2014) evaluated 20 isolates of *T. harzianum*, *T. aureoviride*, *T. viride*, and *T. virens* from rice (*Oryza sativa*) crops in India, of which 19 were placed in classes 1 and 2, while only one was classified in class 3. These results contrast with those obtained by Bell *et al.* (1982), who evaluated 77 *Trichoderma* isolates. In their study, antagonism against *S. rolfsii* was not evident, as 88.0% of the isolates were classified in classes 3, 4, and 5 with limited action, while only 1.0% were placed in class 2. It is important to emphasize that competition for space and nutrients in *Trichoderma* species during antagonistic processes against phytopathogens is a desirable characteristic, as it is associated with their rapid growth and production of antimicrobial metabolites (Chandra *et al*., 2020). Therefore, further studies are needed to identify the secondary metabolites, especially in *T. azevedoi*, which showed the greatest antagonism against the two *S. rolfsii* isolates in this work.

Regarding the hyphal interaction, the *Trichoderma* isolates included in this study caused vacuolization, granulation, coiling, adhesion, lysis, and penetration of *S. rolfsii* hyphae, leading to the disintegration of the fungal mycelium, with *T. azevedoi* standing out as it exhibited the highest number of hyphal interactions. Similar results were reported by Kamel *et al*. (2020), who observed that most *Trichoderma* species adhered to, coiled around, and caused lysis of the pathogen's hyphae in India. In our study, the action of *T. azevedoi* is notable, where the different interactions can be attributed to its ability to produce hydrolytic enzymes such as glucanase and chitinase, which affect the cellular integrity of the hyphae, leading to the collapse of *S. rolfsii* mycelium (Kotasthane *et al.*, 2014; Kamel *et al.*, 2020).

The results of this study indicate variability in the inhibition of mycelial growth of *S. rolfsii* (26.0 to 81.4%), with *T. azevedoi* standing out for its inhibitory capacity; this is consistent with the findings of Sangle *et al.* (2016), who evaluated 30 isolates of *T. harzianum*, *T. viride*, *T. hamatum*, *T. reesei*, *T. piluliferum*, and *T. virens* against *S. rolfsii* from lentil crops in India, where *T. harzianum* exhibited the highest inhibitory effect (84.5%) on the mycelial growth of the pathogen. Similarly, Amin *et al*. (2010) evaluated *T. harzianum*, *T. virens*, and *T. viride* against *S. rolfsii* from different crops in India, obtaining inhibition percentages lower than those reported in the present study (20 to 40%). On the other hand, Ruangwong *et al*. (2021) analyzed the inhibitory effect of *T. asperelloides*, from *Anthurium* flowers in Thailand, against *S. rolfsii*, which inhibited the mycelial growth by 41.2%. In their study, they identified 17 types of volatile metabolites, with 2-phenylethanol, a compound with antifungal properties, being prominent. Sridharan *et al*. (2020) found that *T. longibrachiatum*, from rice cultivation in India, inhibited the growth of *S. rolfsii* and *Macrophomina phaseolina* by 57 and 35%, respectively. Furthermore, this antagonist caused changes in the mycelial structure by reducing the mycelium thickness and decreasing the number of sclerotia compared to the pathogen in the absence of the antagonist. These results highlight the ability of volatile metabolites produced by *Trichoderma* isolates to inhibit the mycelial growth of *S. rolfsii* without direct contact between these fungi; this characteristic is important for protecting potato plants from *S. rolfsii* (Sangle *et al*., 2016). The results of this study open new avenues for research related to the identification of the molecular mechanisms involved in mycelial growth inhibition, as well as for evaluating the stability of these effects under field conditions. The findings of this study are significant, as most *Trichoderma* isolates showed antagonistic potential, with *T. azevedoi* standing out as a potential biological control agent against *S. rolfsii* in potato cultivation.

CONCLUSIONS

The TAI73 isolate of *T. azevedoi* exhibited the best antagonistic potential for the control of *S. rolfsii* in all the *in vitro* tests conducted in this study. *T. azevedoi* inhibited the mycelial growth of the *S. rolfsii* isolates Scr4 and Scr17 by 75.4% and 77.1%, respectively, and was classified in class 2 against both isolates on the Bell scale, indicating its high antagonistic capacity. This antagonist also caused vacuolization, granulation, coiling, adhesion, and lysis of the *S. rolfsii* (Scr4) hyphae. Additionally, *T. azevedoi* produced volatile metabolites that inhibited 84.1% of the mycelial growth of the fungus *in vitro*. These results open new avenues for research related to determining the biological effectiveness of *T. azevedoi* as a potential biocontrol agent in the field.

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