Mexican Journal of Phytopathology
ISSN: 2007-8080

Crossref
Open access

Scientific Article

In vitro sensitivity of *Sclerotium rolfsii* and four species of *Trichoderma* to common-use fungicides on the potato (*Solanum tuberosum*) crop

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Section:
Periodical Issue

Received:
30 May, 2024
Accepted:
27 March, 2025
Published:
14 April, 2025

Citation:
Herrera-Rodríguez G, Antonio Lugo-García GA, Irazoqui-Acosta MB, Lara-Flores JJ, Armenta-López SE, *et al*. 2025. *In vitro* sensitivity of *Sclerotium rolfsii* and four species of *Trichoderma* to common-use fungicides on the potato (*Solanum tuberosum*) crop. Mexican Journal of Phytopathology 43(2): 62. https://doi.org/10.18781/R.MEX.FIT.2405-12

ABSTRACT

Background/Objective. Soft rot of potato tubers, caused by *Sclerotium rolfsii*, is a disease that occurs in soils with high levels of humidity and temperatures above 30 °C. Synthetic fungicides are primarily used for its control. The objectives of this study were to determine the biological efficacy of synthetic fungicides at different concentrations against the pathogen and the sensitivity of four species of *Trichoderma* to commonly used fungicides in potato in Sinaloa.

Materials and Methods. The *in vitro* efficacy of nine fungicides at concentrations of 0.01, 0.1, 1, 10 and 100 ppm on mycelial growth inhibition and sclerotia formation of *S. rolfsii* was determined. Furthermore, the *in vitro* sensitivity of *T. afroharzianum*, *T. asperelloides*, *T. asperellum*, and *T. azevedoi* to 10 fungicides at concentrations of 100, 500, 1000, and 2000 ppm was studied. The experiment was conducted twice. Treatments were distributed in a completely randomized design and data were subjected to ANOVA. Means were compared with the Tukey test ($p < 0.05$).

Results. Thifluzamide and Propineb at the 0.01 ppm concentration inhibited mycelial growth *S. rolfsii* by 32.7 and 12.2%, respectively. On the other hand, *S. rolfsii* produced 157, 164, and 164 sclerotia per Petri dish on PDA supplemented with the fungicides. Thifluzamide, Propineb and prochloraz, respectively, at the same concentration. In contrast, Propineb at a concentration of 100 ppm inhibited the mycelial growth of *T. azevedoi*, *T. afroharzianum*, *T. asperellum* and *T. asperelloides* by 0, 0, 0 and 54.9%, respectively; while the inhibition of mycelial growth by Thifluzamide at the same concentration in *T. azevedoi*, *T. afroharzianum*, *T. asperellum* and *T. asperelloides* ranged from 0 to 63%. The results indicate that the four *Trichoderma* species are compatible with both fungicides.

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Conclusion. The effect of thifluzamide and propineb on mycelial growth inhibition and sclerotia formation of *S. rolfsii*, as well as their compatibility with the four species *Trichoderma*, indicates that the combination of *Trichoderma* spp. and the fungicides has potential use for controlling soft rot of potato tubers under field conditions.

Keywords: Production, Quality, Sclerotia, Management.

INTRODUCTION

The potato (*Solanum tuberosum*) is the sixth most widely produced crop in the world, with 470,409,159 t, obtained from 23,514,508 h. The main producing countries of this tuber are China, India, Ukraine, Russia and the United States (FAO, 2023). Mexico is in35th place, with an annual production of 1,986,198 t, with the highest-producing states are Sonora, with 612,600 t, and Sinaloa with 427,587 t a year, representing 52.4% of the national production, with a production valued in 17,426,448,000 pesos (SIAP, 2023). The crop is affected by diseases that limit its production and quality (Torrance and Talianksy, 2020; Singh *et al*., 2021). Soft rot stands out for its importance and is caused by *Sclerotium rolfsii*, which affects around 500 plant species, which are grouped into 100 different families, with tomato (*Solanum lycopersicum*), cucumber (*Cucumis sativus*), peanut (*Arachis hypogaea*), potato and others standing out (Kator *et al*., 2015; Roca *et al*., 2016; Kumar *et al*., 2018; Paparu *et al*., 2020; Meena *et al*., 2023). In the potato, soft tuber rot affects production by 20%. The fungus presents saprophytic habits and in the soil it colonizes crop residues or it can survive in the form of sclerotia, which are dispersed through water, machinery, plant residues, soil and animales (Kator *et al*., 2015; Roca *et al*., 2016).

The traditional management of tuber soft rot with synthetic fungicides contaminates the environment and affects living beings (Widmer, 2019), and its continuous use produces resistance in phytopathogens, which leads to the increase in concentrations for their control (Ferreira *et al*., 2020).

In recent years, advances have emerged regarding the use of environmentally friendly strategies for disease management in various crops, with the use of fungi as biological control agents standing out (Rubayet and Bhuiyan 2016; Yassin *et al*., 2021; Kim *et al*., 2023)

The fungi genus of the *Trichoderma* are the most widely studied and used as biological agents, due to the good results in the control of fungal diseases. This is due to the diversity of action mechanisms they have (competition for space and nutrients, mycoparasitism and antibiosis). In addition, this type of antagonistics produce secondary metabolites that promote plant growth and activate the defense mechanisms of plants, as well as increasing crop production and quality (Yao *et al*., 2023). Treatments with this fungus are directed to seed, seedlings, irrigation water and foliage; on the other hand, there are studies in which the compatibility of *Trichoderma* with commercial synthetic fungicides has been proven for use in mixtures in the control of fungal diseases in diverse crops (Gonzalez *et al*., 2020; Manandhar *et al*., 2020; Arain *et al*., 2022; Dinkwar *et al*., 2023).

In Mexico there are no studies on the compatibility of *Trichoderma* species in mixtures with fungicides aimed at the control of soft potato tuber rot. For this reason,

the aim of this study was to determine the *in vitro* biological effectiveness of different concentrations of synthetic fungicides on the inhibition of mycelial growth and formation of *S. rolfsii* sclerotia, as well as the *in vitro* effect of the fungicides on the mycelial growth of *Trichoderma afroharzianum, T. asperelloides, T. asperellum* and *T. azevedoi.*

MATERIALS AND METHODS

Origen of isolates. *S. rolfsii* (Scr4) *Trichoderma afroharzianum* (TES24), *T. asperelloides* (TAM74), *T. asperellum* (TAF75) and *T. azevedoi* (TAI73) were provided by the microbiological bank of the Phytosanitary Diagnostics Laboratory of the Local Plant Health Board of the El Fuerte Velley. The isolates were obtained from soil in commercial fields in which potato was grown in previous agricultural cycles in the Municipalities of Ahome, Sinaloa and, Altar and Caborca in Sonora, Mexico in 2019, 2020 and 2021 (Table 1).

Table 1. Species and isolates of *Sclerotium rolfsii* and *Trichoderma*, site and year of collection and code in the GeneBank.

Species/isolate	Locality	Year of collection	Code in GeneBank
Sclerotium rolfsii /Scr4	Ahome, Sinaloa/ 25.701944 -109.043333	2019	OR514113
T. asperelloides /TES24	Caborca, Sonora/31.06666 -112.338333	2020	OR521164
T. azevedoi /TAI73	Ahome, Sinaloa/25.818885 -108.956014	2021	OR521181
T. afroharzianum/TAF75	Ahome, Sinaloa/25.491445 -108.571659	2021	OR521183
T. asperellum/TAM74	Ahome, Sinaloa/ 25.491445 -108.571659	2021	OR521182

***In vitro* sensitivity of *Sclerotium rolfsii* to nine synthetic fungicides.** This study evaluated the *in vitro* sensitivity of *S. rolfsii* (isolate Scr4) to the following fungicides: Thifluzamide (Summit Agro Mexico), Thiabendazole (Arysta LifeScience Mexico), Mancozeb (Arysta LifeScience Mexico), Propineb (Bayer Crop Science), Prochloraz (Adama, Mexico), Propiconazole (Syngenta Agro), Tolclofos-methyl (Valent), Carboxin+Captan (Arysta LifeScience) and Thiophanate-methyl (Arysta LifeScience), against the mycelial growth of the fungal isolate.

The tests were performed using the poisoned food technique (Dinkwar *et al*., 2023), in Petri dishes, 90 mm in diameter, with 20 mL of the Potato Dextrose Agar (PDA) culture medium, supplemented with the test substances at the concentrations of 0.01, 0.1, 1.0, 10 and 100 ppm. PDA discs (5 mm in diameter) with a three-day active mycelial growth of *S. rolfsii* were used and transferred to the center of the Petri dish. The treatments were distributed in a completely randomized design with a 9x5 factorial arrangement, in which the first factor of the arrangement was each one of the nine fungicides and the second factor consisted of the five concentrations. The dishes were sealed with parafilm and incubated at 25 ±2 °C. The control consisted of five Petri dishes with PDA and fungal mycelium without fungicide.

The biological effectiveness of the fungicides on the mycelial growth of *S. rolfsii* was determined by measuring radial growth of the fungus every 24 h, after its transfer and ended when *S. rolfsii* filled the Petri dishes without any fungicide.

The percentage of inhibition of the mycelial growth of *S. rolfsii* was calculated according to the equation I= C-T/C X 100, where I=Percentage of inhibition, C=Growth of the control pathogen, T=Growth of the pathogen under treatment (Vincent, 1947). The experiment was performed twice.

Effect of synthetic fungicides on the formation of sclerotia by *S. rolfsii*. PDA discs, 5 mm in diameter with an active *S. rolfsii* mycelial growth were transfered onto PDA Petri dishes containing fungicides in the same concentrations described in the previous section. The design of the treatments was completely at random with a 9x5 factorial arrangements, with five replicates, in which the first factor of the arrangement was each one of the nine fungicides and the second factor was the five concentrations. The control treatment consisted of five Petri dishes with PDA with *S. rolfsii* mycelial growth without any fungicide. The number of sclerotia was determined 21 days of incubatiton. The experiment was performed twice.

In vitro sensitivity of *Trichoderma* to 10 synthetic fungicides. This study evaluated the *in vitro* sensitivity of *T. afroharzianum*, *T. asperelloides*, *T. asperellum* and *T. azevedoi* to the fungicides 1) Thifluzamide (Summi Agro Mexico), 2) Thiabendazole (Arysta LifeScience Mexico), 3) Mancozeb (Arysta LifeScience Mexico), 4) Propineb (Bayer Crop Science), 5) Prochloraz (Adama, Mexico), 6) Propiconazole (Syngenta Agro), 7) Carboxin+Captan (Arysta LifeScience Mexico), 8) Thiophanate-methyl (Arysta LifeScience), 9) Fluazinam (Syngenta Agro) and 10) TCMTB (Summit Agro Mexico).

The trial was performed based on procedures described by Dinkwar *et al*. (2023), and for this purpose, Petri dishes with a diameter of 90 mm and 20 mL of PDA containing the test substances were used in the following concentrations: 100, 500, 1000 and 2000 ppm. PDA discs, 5 mm in diameter with an active mycelial growth of each species of *Trichoderma* were used, which were placed in the center of the Petri dish. The treatments were distributed in a completely randomized experimental design and with a 4x10x4 factorial arrangement, with four repetitions, in which the first factor of the arrangement corresponded to each one of the *Trichoderma* species, the second one was the 10 fungicides and the third one, the four concentrations of fungicides. The dishes were sealed with parafilm and incubated at 25±2 °C. The control consisted on four Petri dishes with PDA with the *Trichoderma* species without fungicide. The experiment was performed twice.

The biological effectiveness of the fungicides on the mycelial growth of the four *Trichoderma* species was determined by measuring their radial growth every 24 h after during the experimental period and culminated when each of the *Trichoderma* species filled the Petri dishes without fungicide.

The percentage of inhibition of the mycelial growth of the *Trichoderma* species was calculated according to the formula I= C-T/C X 100, where I=Percentage of inhibition, C=Growth of the control antagonist, T=Growth of the antagonist under treatment (Vincent, 1947).

Statistical analysis of data. Considering that the data of the experiments 1 and 2 on the inhibition of the radial growth and the production of sclerotia by *S. rolfsii* in the different concentrations of the fungicides did not display significant differences

between them, in addition to their variances being homogenous, the data of both experiments were combined, analyzed as a single one. The data were subjected to an ANOVA in the statistical package (SAS 9.0, SAS Institute Inc., North Carolina, USA). The comparison of means was performed using Tukey's test (P=0.05) (Little and Hills, 1973). The data on the inhibition of mycelial growth of the four *Trichoderma* species by the fungicides were subjected to the same statistical analyses.

RESULTS

Biological *in vitro* effectiveness of synthetic fungicides in the inhibition of mycelial growth in *Sclerotium rolfsii.* The factorial variance analysis between fungicides displayed significant differences (F=65687.3, P≤ 0.0001). Significant differences (F=141381, P≤ 0.0001) were also reflected between fungicidal concentrations. The interaction of fungicides and their concentrations was also significant (F=9142.35, P≤ 0.0001), which implies a differential sensitivity of *S. rolfsii* to the concentrations of the fungicides under study (Table 2).

Table 2. Factorial analysis of variance of the percentage of *in vitro* inhibition of the mycelial growth in *S. rolfsii* to four concentrations of nine fungicides.

Source	df	Sum of squares	F values
Fungicides	8	174055.4360[z]	65687.3[b]
Concentrations of fungicides	4	187313.2096	141381
Fungicides X concentrations of fungicides	32	96900.0220	9142.35

[z]The analysis of variance was performed with data transformed into the Arc $\sin^{-1}$ form, using Type III sum of squares from SAS's generalized linear model (GLM) procedures. b= Significant effect at P≤ 0.0001.

The inhibition of the mycelial growth of *S. rolfsii* by the fungicides Tifluzamide, Propineb and Propiconazole at the concentration of 0.01 ppm varied between 5.7 and 32.7%, with significant differences (P = 0.05) between treatments, whereas the rest of the fungicides at the same concentrations exerted no inhibition in the mycelial growth of the fungus, while the inhibition exerted by the fungicides Thifluzamide, Carboxin+Captan, Propiconazole, Propineb, Prochloraz and Thiabendazole at 0.1 ppm concentrations varied between 6.8 and 78.1%, with significant differences (P=0.05) between them. In turn, the treatments displayed differences with the rest of the fungicides, where there was no inhibition of the mycelial growth of the fungus (Table 3).

The fungicides Thifluzamide, Propineb, Propiconazole, Carboxin+Captan, Tolclofos-methyl, Prochloraz and Thiabendazole, at a concentration of 1 ppm, inhibited the mycelial growth by 7.1-93.1%, with significant differences (P=0.05) between

treatments. Thifluzamide and Carboxin+Captan stand out for their biological effectiveness, whereas Mancozeb and Thiophanate methyl did not inhibit the mycelial growth of the fungus (Table 3).

Table 3. Average percentage of *in vitro* inhibition of the radial growth of *Sclerotium rolfsii*, caused by various synthetic fungicides.

ppm	Percent of inhibition of mycelial growth								
	Tif[z]	Prop	Prp	C+C	Prc	Tia	Tol	Man	Tio
0.01	32.7* a**	12.2 b	5.7 c	0.0 d	0.0 d	0.0 d	0.0 d	0.0 d	0.0 d
0.1	78.1 a	15.0 d	18.2 c	64.2 b	14.0 d	6.8 e	0.0 f	0.0 f	0.0 f
1.0	90.7 b	16.0 e	48.0 d	93.1 a	15.8 e	7.1 f	61.4 c	0.0 g	0.0 g
10	92.4 b	26.9 d	100 a	100 a	33.0 c	17.5 f	100 a	20.7 e	6.6 g
100	93.0 b	41.1 d	100 a	100 a	100 a	20.9 e	100 a	75.0 c	8.1 f

[z]Tif =Thifluzamide, Pro = Propineb, Prp= Propiconazole, C+C = Carboxin+Captan, Prc = Prochloraz, Tia = Thiabendazole, Tol = Tolclofos-methyl, Man = Mancozeb and Tio =Thiophanate-methyl.
*The number in the row expresses the average percentage of mycelial growth inhibition in *S. rolfsii* for each one of the fungicides. **Means with a common letter in the rows are not significantly different (P=0.05) when placed under Tukey's test.

The concentration of 10 ppm in all fungicides inhibited the mycelial growth of the fungus, with Propiconazole, Carboxin+Captan and Tolclofos-methyl standing out for their efficiency, since they inhibited 100% of the mycelial growth with no significant differences (P=0.05) between these treatments, although there were differences compared to the other fungicides, where mycelial growth ranged from 6.6 to 92.4%, with Thifluzamide showing the greatest inhibiting effect (Table 3).

The inhibition of the mycelial growth of *S. rolfsii* by fungicides Tifluzamide, Propiconazole, Carboxin+Captan, Prochloraz y Tolclofos-methyl at a concentration of 100 ppm was evident, with inhibitions of the mycelial growth of 93.0 to 100%, with significant differences (P=0.05) among these treatments. The mycelial inhibition by the fungicides Propineb, Tiabendazol, Mancozeb and Thiophanate-methyl varied from 8.1 to 75.0% with significant differences (P=0.05) among them and also with the rest of the fungicides (Table 3)

***In vitro* effect of synthetic fungicides on the *in vitro* formation of *Sclerotium rolfsii* sclerotia.** Significant differences (F=2524.74, P≤ 0.0001) were reflected in the production of *S. rolfsii* sclerotia in PDA with the different chemical fungicides. The five concentrations also displayed significant differences (F=10146.5, P≤ 0.0001) in the productions of the fungal resistance structures. The interaction between the fungicides and their concentrations was also significant (F=642.22, P≤ 0.0001) (Table 4).

The production of sclerotia varied between 157 and 180 per Petri dish in PDA with the different fungicides in the concentration of 0.01 ppm. The lowest number of slcerotia occurred in the culture medium with Thifluzamide, Propineb and Prochloraz, whcih displayed no significant differences (P=0.05) between them, but there were differences with the rest of the fungicides and the control without fungicide, where 182 sclerotia were counted for each Petri dish (Table 5).

Table 4. Factorial analysis of variance of sclerotia production by *S. rolfsii* in PDA with five concentrations of nine fungicides.

Source	df	Sum of squares	F values
Fungicides	9	386.840642*	2524.74[b]
Concentrations of fungicides	4	1554.645170*	10146.5
Fungicides X concentrations of fungicides	36	98.400870*	642.22

*The analysis of variance was carried out with data transformed to the square root form using type III of the sum of squares in SAS's GLM procedures. b= Significant effect at P≤ 0.0001.

The concentration of 0.1 ppm of Thifluzamide and Carboxin+Captan exerted the greatest effect in the inhibition of the formation of sclerotia with 147 and 148, respectively for every Petri dish. In the rest of the treatments, the production of sclerotia varied between 161 and 171, with significant differences (P = 0.05) between them.

Sclerotium rolfsii formed no sclerotia in the concentration of 1 ppm of Thifluzamide, although the number of sclerotia for each Petri dish varied between 69 and 162 in the culture medium with the same concentration in the different fungicides. There were significant differences (P = 0.05) between these treatments and with the control treatment, where 182 sclerotia were produced per Petri dish.

No sclerotia were formed in PDA with the concentration of 10 ppm of Thifluzamide, Propineb and Carboxin+Captan, but they did form in the medium with the rest of the fungicides at the same concentration, with significant differences (P = 0.05) between treatments, where the number of sclerotia varied between 22 and 146 per Petri dish. The lowest efficiency was displayed by Thiabendazole, since this treatment displayed the lowest number of sclerotia.

Table 5. Production of *Sclerotium rolfsii* sclerotia per Petri dish with PDA added with the different concentrations of nine fungicides.

ppm	Fungicides/number of sclerrotia									
	Tif[y]	Prc	Pro	Tol	Prp	C+C	Tia	Man	Tio	Tes
0.01	157 c*	164 cb	164 cb	171 a	173 ab	174 ab	174 ab	178 a	180 a	182 a
0.1	147c	162 cb	163 abc	164 abc	171 ab	148 c	167 ab	161 cb	162 cb	182 a
1	0 e	92 c	69 d	148 b	162 b	93 c	161 b	78 d	148 b	182 a
10	0g	75 d	0 g	30 e	129 c	0 g	146 b	22 f	131 c	182 a
100	0 d	0 d	0 d	9 c	0 d	0 d	132 b	0 d	129 b	182 a

[y]Tif =Tifluzamide, Prc = Prochloraz, Pro = Propineb, Tol = Tolclofos-methyl, Prp= Propiconazole, C+C = Carboxin+Captan, TC = TCMTB, Tia = Thiabendazole, Man = Mancozeb, Flu = Fluazinam, Tio =Thiophanate-methyl and control without fungicide. [z]Sclerotia in PDA with different concentrations of fungicides 21 days of incubation. *Means with a common letter in the rows are not significantly different (P=0.05) according to Tukey's procedure.

In the concentration of 100 ppm of Thifluzamide, Prochloraz, Propineb, Propiconazole, Carboxin+Captan and Mancozeb completely inhibited the formation of sclerotia, without any significant differences (P=0.05) between treatments, although

there were differences with the number of sclerotia (9 to 132) produced in the medium with the fungicides Tolclofos-methyl, Thiabendazole and Thiophanate-methyl at the same concentration.

***In vitro* sensitivity of four *Trichoderma* species to 10 synthetic fungicides.** In the factorial analysis of variance, the four *Trichoderma* species displayed significant differences (F=9576.97, P≤ 0.0001) in the percentage of inhibition of the mycelial growth. The species of the fungus displayed a differential response to the fungicides included in this study. Likewise, the 10 fungicides displayed significant differences (F=177350, P≤ 0.0001) in the inhibition of the *Trichoderma* species. There were also significant differences (F=24772.8, P≤ 0.0001) between the concentrations. The interaction between the 10 fungidides and their four respective concentrations was also significant (F=5208, P≤ 0.0001). Similarly, the interaction of the *Trichoderma* species and the 10 fungicides was also significant (F=4212.90, P≤ 0.0001). The interaction between the species of *Trichoderma* and the four concentrations of fungicide evaluated displayed significant differences (F=1173.88, P≤ 0.0001). Finally, the interaction *Trichoderma* species X fungicides X concentrations of fungicides was also significant (1211.06, P≤ 0.0001) (Table 6).

Table 6. Factorial analysis of variance of the percentage of *in vitro* inhibition of the mycelial growth of four *Trichoderma* species by four concentrations of ten fungicides.

Source	gl	Sum of squares	values
Species of *Trichoderma*	3	4044.8603*	9576.97[b]
Fungicides	9	74904.1540*	177350
Concentrations of fungicides	3	10462.8481*	24772.8
Fungicides X concentrations of fungicides	27	2199.9104*	5208.70
Species of *Trichoderma* X fungicides	27	1779.3305*	4212.90
Species of *Trichoderma* X concentrations of fungicides	9	495.7913*	1173.88
Species of *Trichoderma* X fungicides X concentrations of fungicides	81	511.4926*	1211.06

*The analysis of variance was performed with data transformed into the Arc sen^{-1} form, using Type III sum of squares from SAS's GLM procedures. b= Significant effect at P≤ 0.0001.

The fungicides Propineb and Tifluzamide at a concentration of 100 ppm did not lead to an *in vitro* inhibition of the mycelial growth of *T. azevedoi* (Figure 1 A and B); the rest of the fungicides inhibited mycelial growth from 30.8 to 100%, with significant differences between treatments (P=0.05). *T. azevedoi* displayed sensitivity to 2000 ppm, particularly in the case of Propineb. Increasing the concentrations of 500 to 2000 ppm led to an increased inhibition of the mycelial growth by Thifluzamide, but without completely inhibiting the mycelial development. By contrast, Fluazinam (Figure 1 C) and the rest of the fungicides inhibited the mycelial development of *T. azevedoi* from 88.9 to 100%, with significant differences (P=0.05) between treatments (Table 7).

Similar to *T. azevedoi*, *T. afroharzianum* displayed no sensitivity to Propineb in the concentrations of 100 (Figure 1 D), 500 and 1000 ppm, although it did display a sensitivity to 2000 ppm. The inhibition of the mycelial growth of *T. afroharzianum* increased gradually when increasing the concentrations of the fungicides Thifluzamide, Mancozeb, Caboxin+Captan and Fluazinam. The fungicides Propiconazol (Figure 1 E), Prochloraz, Thiabendazole and Thiophanate-methyl at concentrations of 100 to 2000 ppm inhibited 100% of the mycelial growth of the antagonist.

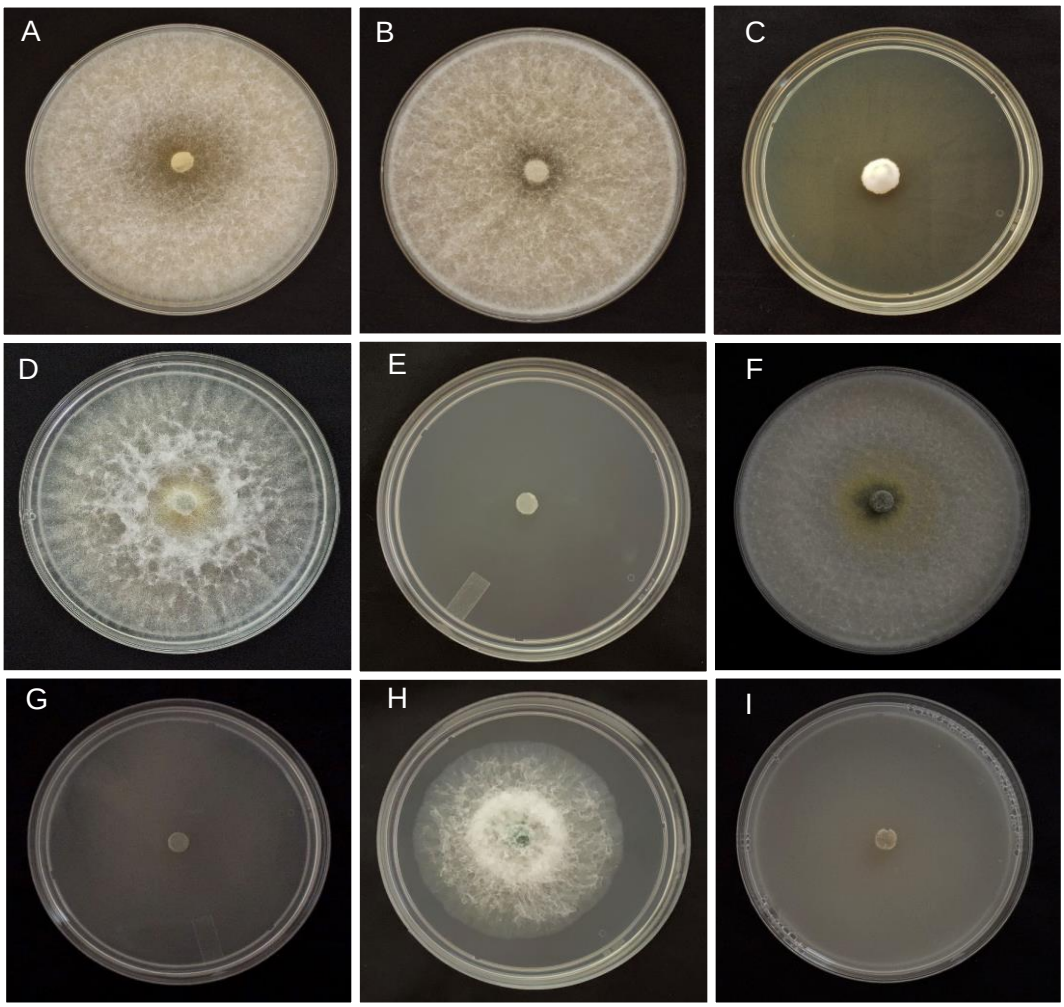

Figure 1. Inhibition of the mycelial growth of four *Trichoderma* species in PDA added with synthetic fungicides**.** A) *T. azevedoi*+Propineb 100 ppm; B) *T. azevedoi*+Tifluzamide 100 ppm; C) *T. azevedoi*+Fluazinam 100 ppm; D) *T. afroharzianum*+Propineb 100 ppm; E) *T. afroharzianum*+Propiconazole 100 ppm; F) *T. asperellum*+Propineb 100 ppm; G) *T. asperellum*+Thiabendazole 100 ppm; H) *T. asperelloides*+Tifluzamide 100 ppm; I) *T. asperelloides*+TCMTB 100 ppm.

The fungicides Propineb (Figure 1 F) and Thifluzamide at concentrations of 100 and 500 ppm ndid not exert an *in vitro* inhibiting effect on the mycelial growth of *T. asperellum*. The fungus only displayed sensitivity to the concentrations of 1000 and 2000 ppm of both fungicides, whereas Mancozeb and Carboxin+Captan inhibited mycelial growth by 36.7 to 92.2%. The rest of the fungicides exerted an *in vitro* inhibition of 95.0 to 100% (Table 7; Figure 1 G), regardless of their concentrations.

Table 7. Means of percentage of *in vitro* inhibition of the radial growth of four *Trichoderma* species, caused by various synthetic fungicides.

	ppm	Percent of inhibition of mycelial growth									
		Pro[z]	Tif	Man	C+C	Flu	TC	Prp	Prc	Tia	Tio
T. azevedoi	100	0.0 e	0.0 e	30.8 d	73.0 c	88.9 b	100 a	100 a	100 a	100 a	100 a
	500	0.0 e	33.4 e	53.0 d	90.4 c	90.7 b	100 a	100 a	100 a	100 a	100 a
	1000	0.0 e	54.8 d	56.5 c	100 a	92.2 b	100 a	100 a	100 a	100 a	100 a
	2000	58.0 e	78.9 c	66.4 d	100 a	93.9 b	100 a	100 a	100 a	100 a	100 a
T. afroharzianum	100	0.0 g	63.3 e	37.3 f	82.2 d	92.1 c	97.3 b	100 a	100 a	100 a	100 a
	500	0.0 f	69.6 d	54.2 e	85.5 c	92.9 b	100 a	100 a	100 a	100 a	100 a
	1000	0.0 f	82.0 d	57.4 e	87.8 c	94.3 b	100 a	100 a	100 a	100 a	100 a
	2000	88.6 c	87.0 c	73.4 d	97.0 b	95.5 b	100 a	100 a	100 a	100 a	100 a
T. asperellum	100	0.0 e	0.0 e	36.7 d	43.7 c	95.0 b	100 a	100 a	100 a	100 a	100 a
	500	0.0 e	0.0 e	42.8 d	78.3 c	97.0 b	100 a	100 a	100 a	100 a	100 a
	1000	36.2 f	47.4 d	45.9 e	87.6 c	97.8 b	100 a	100 a	100 a	100 a	100 a
	2000	38.5 f	73.7 d	48.2 e	92.2 c	98.5 b	100 a	100 a	100 a	100 a	100 a
T. asperelloides	100	54.9 e	58.0 c	57.3 d	23.2 f	94.6 b	100 a	100 a	100 a	100 a	100 a
	500	56.3 d	58.7 c	58.6 c	100 a	96.1 b	100 a	100 a	100 a	100 a	100 a
	1000	58.1 e	69.4 c	62.0 d	100 a	97.1 b	100 a	100 a	100 a	100 a	100 a
	2000	61.1 e	80.0 c	68.3 d	100 a	97.8 b	100 a	100 a	100 a	100 a	100 a

[z]Pro = Propineb, Tif =Thifluzamide, Man = Mancozeb, C+C = Carboxin+Captan, Flu = Fluazinam, TC = TCMTB, Prp= Propiconazole, Prc = Prochloraz, Tia = Thiabendazole and Tio =Tiophanate-methyl.
*The number in the rows expresses the mean percentage of growth inhibition in *T. azevedoi*, *T. afroharzianum*, *T. asperellum* and *T. asperelloides* for each one of the fungicides.
**Means with a common letter in the rows are not significantly different (P=0.05) according to Tukey´s procedure.

The mycelial growth of *T. asperelloides* was inhibited by the different concentrations of all fungicides. The fungicides Propineb, Thifluzamide (Figure 1 H) and Mancozeb reduced mycelial growth 54.9 to 80.0%. The mixture of Carboxin+Captan at a concentration of 100 ppm inhibited mycelial growth by 23.2%, but the same combination of fungicides at concentrations of 500, 1000 and 2000 ppm inhibited mycelial growth by 100% (Figure 1 I). Similar results were observed when the rest of the fungicides were used at the same concentrations, where the inhibition of the mycelial growth varied between 94.6 and 100 % (Table 7).

DISCUSSION

In this study Thifluzamide had a greater *in vitro* effect on the mycelial growth of *S. rolfsii*, especially in the dose of 0.1 and 0.01 ppm in the five concentrations studied: despite the lack of any reference studies on the effect of this fungicide on the *in vitro* inhibition of the mycelial growth of the fungus, studies carried out in China indicated

that the same fungicide has a preventative effect against soft rot in *Dendrobium officinale* (Yajun *et al*., 2018).

Likewise, Propineb inhibited the mycelial growth of the fungus by 41.1%, which coincides with previous studies in which the concentration of 100 ppm of this fungicide was used (Shirsole *et al*., 2019), and which inhibited 75.4% of the mycelial growth of *S. rolfsii* obtained from chickpea (*Cicer arietinum*) in India. In turn, Vikram *et al*. (2023) evaluated the same molecule at 150 ppm, and it inhibited the mycelial growth of the fungus in wheat plants (*Triticum* spp.) in India by 17.7%, which contrasts with the results from this study. On the other hand, Mancozeb displayed an inhibition of 75.0% of the phytopathogen at a concentration of 100 ppm, which coincides with Shirsole *et al*. (2019), who found that this fungicide inhibited the growth of the fungus by 100% at the same concentration, whereas Vikram *et al*. (2023) found that the fungicide, at 50 and 150 ppm, inhibited they mycelial growth of the fungus by 57 and 100%, respectively. However, Das *et al*. (2014) reported an inhibition of 20% at 100 ppm, which contrasted with Chandra *et al*. (2020), who recorded that the same fungicide at 250 ppm inhibited 6.3% of the mycelial growth of the fungus from tomato plants in India. In this study, the Carboxin+Captan mixture completely inhibited *S. rolfsii* at concentrations of 10 and 100 ppm, which contrasted with the results by Das *et al*. (2014), who determined that Carboxin mixed with Tiram inhibited the growth of the fungus by 86.4 and 100%, respectively, at the same dose, which may be due to a synergy when combining both molecules.

Propiconazole exerted an inhibitory effect, comparable to the results by Das *et al*. (2014) who evaluated at 1, 10 and 100 ppm of this molecule, which inhibited the growth of *S. rolfsii* isolated from eggplant (*Solanum melongena*) in India by 27.2, 55.5 and respectively. Similar results were obtained by Prasad *et al*. (2017) in India, who found that this fungicide inhibited the mycelial growth of the fungus obtained from tomato plants by 100% at a concentration of 150 ppm. This contrasts with the results by Das *et al*. (2014), who indicated that the fungicide did not inhibit the mycelial growth of the pathogen at 1, 10 and 50 ppm, although it did inhibit (19.4%) at a concentration of 100 ppm, which coincides with the results of this study, in which the fungicide inhibited by 100% at a concentration of 100 ppm.

In relation to the production of sclerotia by *S. rolfsii* en PDA, Thifluzamide, Prochloraz, Propineb, Propiconazole, Carboxin+Captan and Mancozeb inhibited the formation of sclerotia in the concentration of 100 ppm, which contrasted with the fungicides of the group of Benzimidazoles (Thiabendazole and Thiophanate-methyl). These eariler studies indicate that Thiabendazole at a concentration of 4,000 inhibited the formation of *S. rolfsii* sclerotia obtained from onion (*Allium cepa*), chilli pepper (*Capsicum annuum*), tomato and barley (*Hordeum vulgare*) plants (Pérez-Moreno *et al*., 2009), which coincides with the results of this investigation, since the reduction in the formation of sclerotia was related with the increase in the concentrations of the fungicide. On the other hand, additional studies indicated that the *in vitro* evaluation of Mancozeb at 2,000 ppm reduced from 545 to 272 the *S. rolfsii* sclerotia obtained from eggplant plants in Bangladesh (Siddique *et al*., 2016). These results contrast with those obtained in this study, since at 100 ppm, the production of sclerotia was completely inhibited, indicating variability in the sensitivity of the fungus to this molecule. The results of this study suggest that Thifluzamide, Prochloraz, Propineb, Propiconazole, Carboxin+Captan and Mancozeb can reduce the *in vitro* production of sclerotia,

indicating that these fungicides display potential for the reduction of these structures in the field.

The fungicides Propineb, Thifluzamide and Mancozeb displayed the lowest effect on mycelial growth in *T. azevedoi*, *T. afroharzianum*, *T. asperellum* and *T. asperelloides*, with a differential response of these species to the fungicides and their respective concentrations. In general, *T. azevedoi* and *T. afroharzianum* displayed the lowest inhibiting effect due to the different concentrations of Propineb. These results coincide with those by Manandhar *et al*. (2020), who, in Nepal, determined that *T. harzianum* and the isolation T22 *in vitro* at 100 ppm were not inhibited by Propineb, whereas *T. asperellum*, *T. viride* and the isolation T69 were inhibited by 13.1, 28.7 and 0.8%, respectively; these isolations were obtained from the rhizosphere of vegetable orchards in Nepal. These authors recorded that Mancozeb at 100 ppm inhibited *T. harzianum*, *T. viride*, *T. asperellum*, T22 and T69 by 10.8, 16.2, 28.3, 31.2 y 46.6%, respectively, which contrasts with findings by González *et al*. (2020), who recorded that *Trichoderma reesei* was not inhibited at 100 ppm.

In this study, Propiconazole inhibited the mycelial growth of the *Trichoderma* strains by 100% at a concentration of 100 ppm. By contrast, Zapata *et al*. (2023) found that, five days after the beginning of the study, the same fungicide at 1.25 mL L^{-1} inhibited the mycelial growth of *T. koningiopsis* by 31% (Tri-cotec® WG in Colombia). These authors recorded that Fluazinam inhibited by 39.6% at the dose of 1 mL L^{-1}, which contrasts with the results of this study, since the mycelial growth inhibition of the four *Trichoderma* species varied between 88.9 and 98.5%. They also recorded that Prochloraz and Thiabendazole completely inhibited the mycelial growth, which also coincides with our results.

The difference in the sensitivity of *S. rolfsii* isolates and *Trichoderma* species to the different fungicides evaluated in this study, compared to other research studies, could be due to the constant use of fungicides in various crops. The exposure of this fungus to these products varies according to the agronomic management of the plant species, and over time, the fungus adapts to high doses of synthetic fungicides for disease management (Pérez-Moreno *et al*., 2009; Chaparro *et al*., 2011).

The results of this study open new lines of research related to studies of the effectiveness of fungicides at greenhouse and field levels. In this sense, the effectiveness of the molecules that were efficient in the *in vitro* inhibition of *S. rolfsii* must be determined. The most adequate phenological stage of the crop should also be evaluated, along with the concentration and the number of applications for the management of *S. rolfsii*, which causes the soft potato tuber rot, in the field.

The combination of the *Trichoderma* species with the fungicides Propineb and Thifluzamide and the *in vitro* effect of these against *S. rolfsii* is an option for the producer in the control of soft potato tuber rot. This approach has been practiced for the control of this disease in the potato crop (Rubayet and Bhuiyan, 2016). Future projects should lean towards the reduction in the concentration of fungicides when mixed with antagonistic *Trichoderma* species, with the aim of reducing environmental contamination, but particularly the contamination of tubers.

CONCLUSIONS

The results indicate that Thifluzamide (0.01 ppm) more efficiently inhibited the mycelial growth of *S. rolfsii* (32.7%) as compared to Propineb (12.2%). Both fungicides reduced sclerotia production by the pathogen (157-164 sclerotia/Petri dish), while the mean production of these resistance structures was 182 sclerotia per Petri dishes without fungicides. In *Trichoderma* spp., Propineb (100 ppm) only affected *T. asperelloides* (54.9% mycelial growth inhibition), whereas Thifluzamide showed variable effects (0-63%), confirming its compatibility with *Trichoderma* species. These findings suggest that: 1) Thifluzamide is more effective against *S. rolfsii* but requires combination with other strategies to reduce sclerotia formation; 2) Both fungicides can be integrated with *Trichoderma* spp. in integrated management programs, as they show low toxicity toward *Trichoderma* spp.; 3) Fungicide selectivity varies according to *Trichoderma* isolate, highlighting the need for specific evaluations. It is recommended to optimize doses and formulations to improve *S. rolfsii* control without compromising biocontrol agents, prioritizing schemes that combine both approaches for sustainable management

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