Mexican Journal of Phytopathology
e-ISSN: 2007-8080

Phytopathological Note

Sensitivity of *Sclerotium rolfsii*, the causal agent of southern blight in chili pepper, to *Trichoderma* spp., plant extracts, and fungicides

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Section:
Periodical Issue

Reviewed:
March 19, 2025
Published:
August 31, 2025

Citation:
Díaz Nájera JF, Terrones Salgado J, Ayvar Serna S, Vargas Hernández M, *et al*. 2025. Sensitivity of *Sclerotium rolfsii*, the causal agent of southern blight in chili pepper, to *Trichoderma* spp., plant extracts, and fungicides. Revista Mexicana de Fitopatología 43(3): 88. https://doi.org/10.18781/R.MEX.FIT.2209-1

ABSTRACT

Background/Objective. Chili pepper (*Capsicum annuum*) are widely grown in Mexico. They are affected by facultative soil pathogens, among which the fungus *Sclerotium rolfsii* can be critical. The objectives of this research were to identify culturally, morphologically, and molecularly the causal agent of southern blight in Guerrero native chili pepper and to evaluate the *in vitro* antibiosis of *Trichoderma* spp., as well as the sensitivity of the causal agent to fungicides and plant extracts.

Materials and Methods. At CEP-CSAEGRO, plants of three types of local chili pepper genotypes with symptoms of southern blight were collected from which 20 fungal isolates were isolated, purified, and characterized. One isolate was selected for molecular identification. DNA was extracted using a commercial kit and amplified by PCR, and the internal transcribed spacer (ITS) region was subsequently sequenced. The sequences were edited, and a BLAST analysis and comparison with the NCBI database were performed. To confirm pathogenicity, Koch's postulates were performed in duplicate on chili seedlings of a local genotype. Using a completely randomized experimental design, the *in vitro* antibiotic activity of *Trichoderma* spp. against the causal agent was evaluated, as well as its *in vitro* sensitivity to fungicides and plant extracts.

Results. Cultural, morphological, and molecular characterization (PV569903, NCBI accession number) identified *Agroathelia rolfsii* (*S. rolfsii*) as the causal agent of southern blight on native chili peppers. Pathogenicity tests showed symptoms identical to those identified in the field. In antibiosis trials, the highest mycelial inhibition of *S. rolfsii* was obtained with *T. virens* (65.18%), *Trichoderma* sp. (65.18%), and *T. asperellum* (66%). In the *in vitro* fungicide sensitivity, complete mycelial inhibition (100%) was obtained with copper sulfate, chlorothalonil, quintozene, benomyl, and pyraclostrobin. Commercial plant extracts from *Reynoutria sachalinensis, Lippia graveolens* + *L. berlanderi,* and *A. indica* + *Cinnamomum verum* completely inhibited the mycelial development of *S. rolfsii* (100%).

Conclusion. *S. rolfsii* is the pathogen that causes southern blight in native chili peppers in Guerrero. *Trichoderma asperellum* (native to Cocula), *Trichoderma* sp. (native to Iguala), and *T. virens* (PHC® ROOTMATE) inhibited the growth of *S. rolfsii*, as did some commercial fungicides and plant extracts. These are suggested as promising alternatives, subject to *in vivo* validation in the field, for the management of this fungal pathogen under a sustainable integrated approach.

Keywords: *Capsicum*, *Agroathelia rolfsii*, *T. virens*, biorationals, benomyl

INTRODUCTION

Chili peppers (*Capsicum annuum*) are one of the most important vegetables in the world. In Mexico, it is produced throughout the country. In 2024, 157186.45 ha were reported as planted, with a production of 3,129877.88 t. The main producing states, in order of importance, are Sinaloa, Chihuahua, Zacatecas, San Luis Potosí, and Sonora (SADER, 2025).

In the north of Guerrero state, local chili genotypes, known as criollos, are grown. These are very popular locally and regionally and are only grown during the rainy season, which means they are more exposed to phytosanitary problems (Ayvar *et al*., 2007). Facultative soil fungi can be a serious problem, such as *Sclerotium rolfsii,* currently known as *Agroathelia rolfsii* (Sacc.) (Redhead & Mullineux, Index Fungorum 554: 1 (2023) [MB#901174]). This fungus forms abundant sclerotia that can survive in the soil for more than a year; in chili pepper, the induced symptoms are seedling blight, damping-off, wilting, and root and neck rot, which can cause significant losses at any phenological stage of the crop (Huang *et al*., 2023). Optimal management of the disease requires effective diagnosis of the causal agent. *Sclerotium rolfsii* is identified based on its cultural, morphological, morphometric, pathogenic, and molecular characteristics (Díaz *et al.*, 2018; Terrones *et al.*, 2023). Due to the importance of this disease and the ability of the inoculum to survive in the soil, it is essential to carry out effective preventive control of the pathogen through integrated management, favoring the use of different methods, such as cultural practices, the use of beneficial microorganisms, and the application of synthetic fungicides (Díaz *et al.*, 2018; Michel *et al.*, 2013). Some of the most widely used chemical fungicides are carboxin, tebuconazole, captan, tridemorph, propiconazole, mancozeb, hinosan, thiram, propineb, benlate, mancozeb, thiram, quintozene, carbendazim, benomyl, oxycarboxin, and triadimenol (Akarapisan *et al*., 2017), some of which have compromised effectiveness and/or restricted use, such as carbendazim (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances/details/506).

In integrated disease management, it is suggested to incorporate alternative control methods to chemical molecules with the aim of gradually reducing their use under a sustainable vision. The application of fungi of the genus *Trichoderma*, widely known as excellent biocontrol agents, is a promising alternative for phytopathogenic fungi suppression (Díaz *et al*., 2018). Several species of *Trichoderma* have been successfully applied in the management of diseases caused by *S. rolfsii* in chili peppers, beans, cucumbers, tomatoes, peanuts, beet, soybean, rice, wheat, sugarcane, cotton, banana, castor bean, sunflower, ginger, cardamom, black pepper, coconut, and tobacco against different pathogens (Kotasthane *et al.*, 2015; Rao *et al.*, 2015).

The potential of *Trichoderma* against this and other pathogenic fungi has been widely documented in Mexico (e.g., Michel *et al*., 2009; 2013; Díaz *et al*., 2014; 2015). In addition, the use of plant oils and extracts for disease management has recently been promoted due to their low or zero level of contamination and high biological effectiveness. Numerous studies have successfully documented the use of plant oils against pathogens that induce various diseases. For example, *R. sachalinensis* extract has been used *in vitro* against *Podosphaera xanthii* (Margaritopoulou *et al.,* 2020) and *Fusarium oxysporum* (Santos-Esteban *et al.,* 2021); this extract and *Lippia graveolens* vs. *Colletotrichum truncatum* (Terrones *et al.*, 2025); and foliar applications of *R. sachalinensis* in the management of *Podosphaera physocarpi* (Baysal-Gurel and Bika, 2021). In this context, the objectives of this research were to characterize pathogenically, culturally, morphologically, and molecularly the causal agent of southern blight of chili pepper, as well as to develop *in vitro* studies to determine the sensitivity of *S. rolfsii* to different commercial and native strains of *Trichoderma* spp., plant extracts, and commercial chemical fungicides.

The methodology of this research was implemented on chili pepper (*Capsicum annuum*) grown under experimental conditions at the Centro de Estudios Profesionales del Colegio Superior Agropecuario del Estado de Guerrero (CEP-CSAEGRO) located at km 14.5 of the Iguala-Cocula highway, Gro., between coordinates 18°15'56.97" N and 99°38'52.49" W, at 639 meters above sea level. Random sampling was carried out on plants of the Ancho Liso, Carricillo, and Chino local varieties with putative symptoms of southern blight. The plants were placed individually in paper bags, which were labeled, sealed, and transported in a cooler to the CEP-CSAEGRO Phytopathology Laboratory, where they were stored at 4 °C. Fragments (1 cm^2 tissue) cut from the root rot area were disinfested with a 1% sodium hypochlorite solution for 1 min, then washed twice with sterile distilled water and left to dry at room temperature on sterile paper towels. Subsequently, the symptomatic tissue pieces were placed in Petri dishes with solid PDA culture medium 39 g $L^{-1}$of water (Potato Dextrose Agar, Bioxon, PDA) supplemented with lactic acid (0.1% v/v) and incubated under laboratory conditions (28 ± 1°C). Once mycelial growth with the *S. rolfsii* morphotype was observed, approximately three days later, it was purified using the hyphal tip technique and incubated for eight days under the conditions described above.

For pathogenicity testing, in accordance with Koch's postulates, 45-day-old plants of a local chili germplasm were inoculated. These plants were grown in previously sterilized river sand in 10×7×8.5 cm plastic pots under greenhouse conditions and watered and fertilized regularly. The purified fungus was grown on sterile crushed corn cob (*Zea mays)* substrate, which was inoculated with 5 cm diameter discs of PDA medium containing five-day-old fungal mycelium. The substrate was incubated under laboratory conditions (28 ± 1 °C) until approximately 100% colonization 12 days after inoculation (d.a.i.). Subsequently, a mixture of 15 g of inoculated corn cob (approximately 1500 CFU) plus 150 g of sterile sand was prepared and placed in 10×7×8.5 cm pots. Seedlings with previously disinfected bare roots were transplanted into the center of the pot and incubated under greenhouse conditions (28 ± 1 °C). The experiment consisted of five replicates and five controls, which were sprayed with sterile distilled water. Symptoms were observed four days after inoculation. Re-isolations were performed by individual lesion to complete the pathogenicity tests, which were carried out in duplicate.

Cultural and morphological characterization was performed using an Olympus BX 41 compound microscope with an Olympus U-CMAD3 T2, U-TV1X-2 T2 camera (Tokyo, Japan). The taxonomic characteristics observed, such as color, presence of septa, hyphal branching, growth rate, and presence of sclerotia, served as the basis for identifying the fungus using the taxonomic keys of Barnett and Hunter (2000) and Watanabe (2002). This information was used to group the isolates by similarity or morphotypes.

For molecular characterization, DNA was extracted from *S. rolfsii* morphotypes from seven-day-old mycelium using the DNeasyMR kit (QIAGEN®, Hilden, Germany) according to the manufacturer's instructions. The internal transcribed spacer (ITS) regions were amplified using the ITS1/ITS4 primers, combined with the thermocycling program proposed by White *et al.* (1990). The amplified products were purified and sequenced in both directions using the Sanger method by Macrogen Inc. (http://dna.macrogen, South Korea). The resulting sequences were analyzed by DNASTAR (2001) and Sequencher

(2014). The alignment was performed with Clustal W in MEGA 6.0 (Tamura *et al.*, 2013). The sequences were compared using the NCBI BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi). For diagnostic purposes, a comparative analysis was performed with ITS sequences from NCBI and an ITS sequence from a selected isolate from a total of 20 obtained in this research. The maximum likelihood method was used in MEGA 11, with Bootstrap values estimated from 1000 replicates.

In the evaluation of *in vitro* antibiotic activity of *Trichoderma* species against *S. rolfsii*, seven *Trichoderma* products/strains were used, three commercial and four native, which were characterized culturally and morphologically in a previous study (Michel *et al*., 2013). The treatments evaluated are described in Table 1. The antibiosis of *Trichoderma* strains was evaluated using the cellophane technique in 9 cm diameter Petri dishes with PDA (Patil *et al.*, 2014). Pre-sterilized 9 cm diameter circles of cellophane paper were placed in these dishes; subsequently, for each treatment, a 5 mm diameter disc with active *Trichoderma* mycelium was placed in the center of the dish to stimulate the production and diffusion of secondary metabolites in the culture medium. After 24 hours of incubation, the cellophane paper with the *Trichoderma* disc was removed and PDA discs with five-day-old active *S. rolfsii* mycelium were immediately placed in the center of the dishes. Incubation was carried out at 25 ± 1 °C with a 12-hour photoperiod and 40% relative humidity (Michel *et al.*, 2009). This was done for each *Trichoderma* product/strain.

To estimate the sensitivity of *S. rolfsii* to fungicides and plant extracts, sterile PDA medium was used, to which the doses of commercial fungicides and plant extracts indicated in Table 2 were added independently. The addition to the medium was carried out directly in the PDA preparation flask prior to solidification at 40 °C. The mixture was poured into 90 mm diameter Petri dishes and left to stand in the dark for 24 hours before use. The prepared dishes were inoculated with a 5 mm diameter disc of PDA with active mycelium growth taken from the periphery of five-day-old S. *rolfsii* colonies. Once inoculated, the Petri dishes were incubated for five days under the conditions described above.

The diameter of colony growth was measured with a digital vernier caliper (Truper® , Mexico) every 24 hours for three days to estimate the antibiotic activity of *Trichoderma* spp. and every four days for the sensitivity of S. *rolfsii* to plant extracts and fungicides until the mycelium of the control fungus (PDA plates without metabolites and without additives) filled the Petri dish in two perpendicular directions. These measurements were used to generate the percentage of mycelial growth inhibition estimated per treatment relative to the control.

The experimental design for both experiments was completely randomized. For the antibiotic activity of *Trichoderma* spp., there were eight treatments, five replicates, and 40 experimental units. For the sensitivity of plant extracts and fungicides, there were 11 treatments, five replicates, and 55 experimental units, each experimental unit consisting of a 90 × 15 mm Petri dish. Two replicates of the experiment were performed.

The data obtained were subjected to normality tests using the Shapiro-Wilk test. For homogeneity of variance, the Levene test was used with a significance level of $p = 0.05$. Analysis of variance and Tukey's test were performed with the variable percentage of inhibition of *S. rolfsii* mycelial growth with $p = 0.05$. The analyses were performed in SAS V.9.1 for Windows.

The results of this research confirmed the association of *S. rolfsii* with the three native chili peppers studied. A total of 20 isolates with the fungal morphotype were obtained from the roots of plants with putative southern blight symptoms. The fungal mycelium was abundant and cottony, with the presence of round, dark yellowish sclerotia less than 2 mm in size, typical of *Sclerotium.*

In pathogenicity tests**,** symptoms of soft rot appeared four days after inoculation. Initially, loss of turgidity in leaves and stems, reduced seedling vigor, chlorosis, and generalized wilting were observed. Abundant white, cottony, watery aerial mycelium was detected at the base of plants, with eventual formation of sclerotia. The symptoms and signs were like those observed in the field. No symptoms or signs of the fungus were observed in the control seedlings. The re-isolated fungus showed the same cultural and morphological characteristics as the one initially inoculated. These results are analogous to other studies with ranges in the appearance of symptoms from 5 to 7 d.i. (Remesal *et al.*, 2010; Sun *et al.* (2019). The variation in the appearance of symptoms, although low, may be due to pathogenic variability, experimental conditions, or host effect (Gopalakrishnan *et al.*, 2005).

Cultural and morphological characteristics allowed us to identify *Agroathelia rolfsii* (Sacc.) Redhead & Mullineux (Syn: *Sclerotium rolfsii* Sacc). A fast radial growth rate of the fungal mycelium was observed (3 cm day^{-1}). Colonies were white, cottony, with dense aerial mycelium. It produced septate, hyaline hyphae, branched at an angle of 65° with thin walls, with clamp connections at each septum of the mycelium (fibules); when mature, it presented abundant sclerotia, spherical, white at the beginning and dark when mature, with a smooth, shiny, and compact surface measuring 1.2 to 1.9 mm in diameter (n=100) after 10 days; the number of sclerotia produced per Petri dish ranged from 83 to 431 (n=50). These results are consistent with previous reports for *S. rolfsii* (Le *et al.*, 2019; Mahadevakumar *et al.*, 2015; Sun *et al.*, 2019; Tang *et al.*, 2010; Terrones *et al.*, 2023; You *et al.*, 2015). Based on the similarity of morphological and cultural characteristics, all fungal isolates corresponded to a single group, from which one isolate was selected for molecular identification.

The sequence of the isolate CEP-ChSr7 was deposited in GenBank with the accession number PV569903. BLAST analysis of the partial ITS sequence (674 bp) showed 100% similarity with isolates of *Agroathelia rolfsii* (*Sclerotium rolfsii)* (GenBank: OM647806, OM729592) (Balamurugan *et al.*, 2022; Sun *et al.*, 2023). The diagnostic/phylogenetic analysis is shown in Figure 1. The isolate selected from this research identified as *Agroathelia rolfsii* CEP-ChSr7 is indicated with a black circle and is grouped in the *A. rolfsii* clade*. S. cepivorum* was used as an outgroup.

In the *in vitro* evaluation of the antibiotic activity of *Trichoderma* spp. against *Sclerotium rolfsii*, on the first two evaluations of *S. rolfsii* colony growth, the values showed highly significant differences (P < 0.0001). On the first evaluation, the treatments *Trichoderma* sp. *(*FITHAN®), *T. virens* (PHC® ROOTMATE), *T. asperellum* (native to Cocula), and *T. asperellum* (native to Santa Teresa) completely inhibited (100%) the growth of S. *rolfsii* (Table 1). In the second and third experiments, *T. asperellum* (native to Cocula) inhibited the mycelial growth of *S. rolfsii* by 94.45% and 65.88%, respectively (Table 1). At the end of the experiment, *T. asperellum* (native to Cocula), *Trichoderma* sp. (native to Iguala), and *T. virens* (PHC® ROOTMATE) were the ones that successfully inhibited the growth of *S. rolfsii.* In general, *Trichoderma* sp. (FHITAN®), *T. virens* (PHC® ROOTMATE), *T. asperellum* (native to Cocula), and *T. asperellum* (native to Santa Teresa) showed a fungistatic effect, as they controlled 100% of the fungus's growth in the first evaluation. However, in subsequent evaluations, reactivation of *S. rolfsii* mycelium growth was observed.

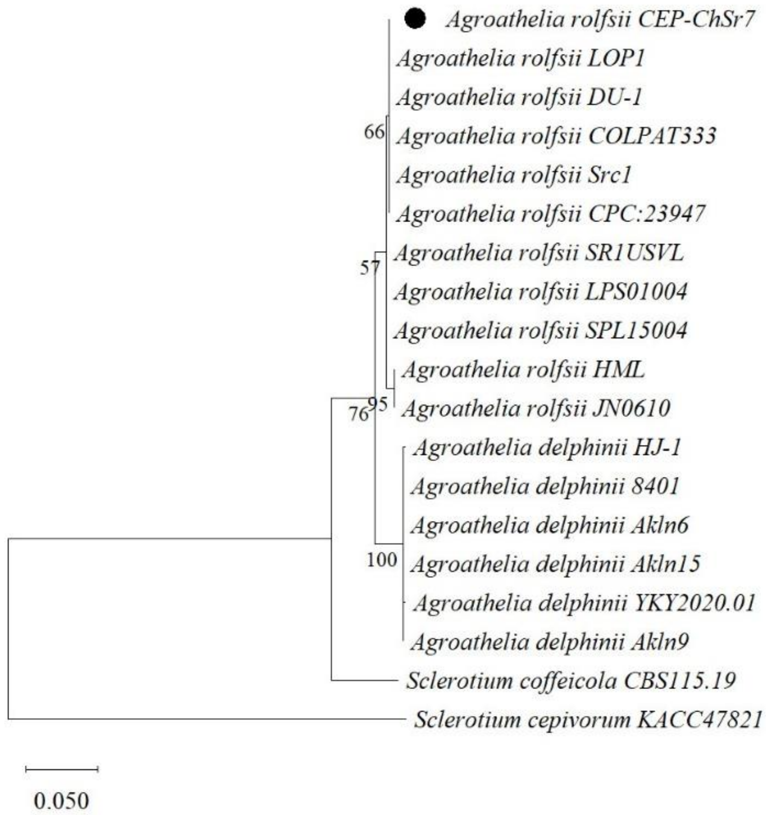

Figure 1. Phylogenetic tree constructed for diagnostic purposes from sequences of the internal transcribed space (ITS) region of *Agroathelia* spp. isolates from the NCBI and the isolate found in Guerrero *CEP-ChSr7* (in black circle). Maximum likelihood algorithm in MEGA 11, with Bootstrap a1000 simulations.

The ability of *Trichoderma* spp. to inhibit fungal growth is due to antibiosis, which depends on the production of toxic, volatile molecules and hydrolytic enzymes (Shoresh and Harman, 2008). In this regard, Hirpara *et al*. (2017) reported that hydrolytic enzymes such as chitinases, proteases, ligases, and glucanases are produced by *Trichoderma* when it detects a fungus, ideally antagonistic, to degrade its cell wall to facilitate the penetration of hyphae and absorption of the mycelial cytoplasmic content.

At the end of the evaluations, three days after sowing, the maximum percentage of inhibition of *S. rolfsii* mycelial growth was 65.9%, which was obtained with *T. asperellum* (native strain from Cocula). This value coincides with other *in vitro* reports (Rasu *et al.*, 2013). Slightly lower inhibition levels have been reported for *T. harzianum (*57.5%, 55.8%) and *T. viridae* (53.63%) (Bindu *et al.*, 2011).

T. asperellum (native to Cocula), *Trichoderma* sp. (native to Iguala), and *T. virens* (PHC® ROOTMATE), although they had the lowest mycelium growth, showed the highest percentage of inhibition of *S. rolfssi*. Hirpara *et al.* (2017) recorded inhibition in the range of 89.33%–91.13% with *T. virens.* However, Parmar *et al.* (2015) documented only 50% inhibition. The *Trichoderma* strains native to Cocula and Iguala Guerrero showed the highest percentage of *S. rolfsii* inhibition. These strains could be an important option for the management of *S. rolfsii.* Nevertheless, they must be evaluated under different field conditions for their eventual integration into a sustainable management plan (Michel *et al.*, 2009; 2013; Díaz *et al.*, 2014; 2015).

Table 1. *In vitro* antibiotic activity - PDA with seven *Trichoderma* spp. products/strains against *S. rolfsii* isolated from local Guerrero chili peppers (*Capsicum annuum*).

Treatments	Hours after *in vitro* sowing		
	24[z]	48	72
FITHAN® Trichoderma sp.	0.0 c	1.0 b	4.3 a
PH® ROOT MATE *Trichoderma virens*	0.0 c	0.9 b	3.0 a
Trichoderma asperellum (Native to Cocula)	0.0 c	0.4 b	2.9 a
BACTIVA ® *Trichoderma reesei*	0.9 b	1.7 b	3.2 a
Trichoderma asperellum (native to Santa Teresa)	0.0 c	0.9 b	4.4 a
Trichoderma asperellum (native to Chilapa)	0.2 bc	1.0 b	4.2 a
Trichoderma sp. (Native to Iguala)	0.4 bc	1.4 b	3.0 a
Control	2.9 a	6.9 a	8.5 a
DMS	0.8172	2.6917	5.9761

[z]Mean values of the colony diameter of *S. rolfsii* in cm, followed by at least one common letter in the same column, are statistically equal (Tukey α = 0.05). MSD = Minimum Significant Difference.

Significant differences were also found in the sensitivity of *S. rolfsii* to fungicides and plant extracts in the four evaluation periods (P < 0.0001). Fungicides and plant extracts inhibited the mycelial growth of *S. rolfsii* in different ways depending on the type of extract, fungicide, and dose. All treatments evaluated completely suppressed the mycelial growth of *S. rolfsii,* except for CERCOBIN® M (methyl thiophanate) (Table 2). Different studies have evaluated the sensitivity of *S. rolfsii* to different fungicides; for example, Amule *et al.* (2014) evaluated the fungicide pyraclostrobin against *S. rolfsii,* isolated from chickpea (*Cicer arietinum)*, with complete (100%) inhibition of fungal growth, analogous to the present study. This fungicide belongs to a class that mainly affects mitochondrial respiration and energy production, with lethal effects on fungi (Fillinger and Walker, 2016). The fungicidal effect of chlorothalonil, cymoxanil, chlorothalonil + benomyl has also been proven in *S. cepivorum* isolated from garlic (*Allium sativum)* (Pérez *et al.*, 2015).

In this study, methyl thiophanate was the only fungicide that did not inhibit 100% of the pathogen's growth, although it differed statistically from the control in the first 8 days. This is possibly due to the low dose used. This finding coincides with that reported by Suryawanshi *et al.* (2015), who reported 63.81% inhibition, while Díaz *et al.* (2018) reported 4.17% inhibition of *S. rolfsii* isolated from pumpkin (*Cucurbita argyrosperma)*. In contrast, quintozene controlled 100% of *S. rolfsii* growth*,* like the results of Díaz *et al.* (2018). Quintozene interferes with the synthesis of lipids and cell membranes, which directly affects the mycelial growth of fungi (Fillinger and Walker, 2016).

Most plant extracts inhibited 100% of *S. rolfsii* mycelial growth. These levels are well documented *in vitro* with extracts of garlic (*Allium sativum)*, yucca (*Schefflera gleasonii)*, matarrata (*Gliricidia sepium)*, and oregano (*Origanum vulgare)* (Flores *et al.*, 2017). Moderate levels (71.4%) have been observed with ethanolic extracts of wild oregano *Lippia origanoides* (Alvarado *et al.*, 2011). The product NEEMIX 4.5% EC, made from *Azadirachta indica,* with apparent fungistatic action (Jebaraj *et al.*, 2016), did not successfully inhibit S. *rolfsii* as it had the lowest inhibition percentage, consistent with studies by Alvarado *et al.* (2011), although in the four evaluations it contrasted significantly with the control. Interestingly, Flores *et al.* (2017) documented 100% effectiveness of neem extract *in vitro* against the causal agent of southern blight in chili peppers.

Table 2. *In vitro* sensitivity – PDA of *Sclerotium rolfsii,* isolated from Guerrero native chili pepper (*Capsicum annuum*), to commercial fungicides and plant extracts evaluated at four 4-days intervals.

Treatments	Dose[s] (mL g^{-1})	Days after *in vitro* sowing			
		4[z]	8	12	16
REGALIA® MAXX (*Reynoutria sachalinensis*)	0.05	0.0 b	0.0 c	0.0 c	0.0 c
LIPPOIL® (*Lippia graveolens* + *L. berlanderi*)	0.30	0.0 b	0.0	0.0	0.0 c
NEEMACAR® CE (*A. indica* + *Cinnamomum verum*)	0.30	0.0 b	0.0 c	0.0 c	0.0 c
NEEMIX® 4.5% EC (***Azadirachta indica***)	0.30	0.0 b	**0.4 c**	**1.8 b**	**4.3 b**
COMET® (copper sulfate)	0.10	0.0 b	0.0	0.0 c	0.0 c
DACONIL 72 F® (chlorothalonil)	0.07	0.0 b	0.0	0.0	0.0 c
PENTACLOR 600 F® (quintozene)	0.15	0.0 b	0.0	0.0	0.0 c
CERCOBIN® M (**methyl thiophanate**)	0.02	**0.2 b**	**2.6 b**	**5.7 a**	**8.1 a**
PROMYL® e (benomyl)	0.03	0.0 b	0.0	0.0 c	0.0 c
HEADLINE® (pyraclostrobin)	0.03	0.0	0.0	0.0	0.0 c
Control	-	1.3 a	3.5 a	6.9 to	8.5 to
DMS		0.1684	0.6696	1.1427	0.7331

[y] Dose used per 20 mL of PDA in each Petri dish
[z] Mean values of the colony diameter of *S. rolfsii* in cm, with at least one letter in common within the same column are statistically equal (Tukey α = 0.05). MSD = Minimum Significant Difference. In bold, the smallest effects compared to the control.

The inhibitory effect of various extracts *in vitro* against *S. rolfsii* has been widely documented, such as *Lupinus rotundiflorus* and *L. exaltatus* (Bernal *et al*., 2005), castor bean (*Ricinus communis*), basil (*Ocimum basilicum*), cress (*Lepidium virginicum*), garlic (*Allium sativum)* (Marcano *et al.*, 2005), *Coronopus didymus* (Drakshan and Javaid, 2011), garlic (*A. sativum)* and ginger (*Zingiber officinale)* (Yasmin, 2016), garlic and onion (Banakar *et al.*, 2017). Although the use of extracts seems a promising alternative (Zapata *et al.*, 2020), field studies are needed to evaluate their effect on the primary inoculum, consisting of sclerotia, to assure the management of *S. rolfsii and* other fungi, with a preventive approach.

In conclusion, based on pathogenic, cultural, morphological, and molecular characterization (PV569903, NCBI accession number), the causal agent of southern blight isolated from native chili pepper germplasm in Cocula, Guerrero, was identified as *Agroathelia rolfsii* (*Sclerotium rolfsii*). *Trichoderma asperellum* (native to Cocula), *Trichoderma* sp. (native to Iguala), and *T. virens* (PHC® ROOTMATE) inhibited the *in vitro* growth of *S. rolfsii.* The fungus also exhibited high *in vitro* sensitivity to the fungicides copper sulfate, chlorothalonil, quintozene, benomyl, and pyraclostrobin, as well as to commercial plant extracts based on *Reynoutria sachalinensis, Lippia graveolens* + *L. berlanderi, A. indica* + *Cinnamomum verum.* It is postulated that these strains of *Trichoderma,* fungicides, and plant extracts may be a viable alternative for field validation and possible integration into a sustainable management program for southern blight of native chili peppers caused by *S. rolfsii* in Guerrero.

Limitation

Validation *in vivo* of experimental products under field conditions were not included.

Conflicts interest

All authors declare that they have no conflict of interest in relation to this research.

Funding

Institutional funding was provided by CSAEGRO and UPAEP.

Author contributions

Work design, JFDN, JTS; conceptualization, JFDN, JTS, SAS, and JLAV; methodology, JFDN, JTS, SAS, and JFAB; statistical analysis, JFDN and MVH; data collection, JFDN, JTS, SAS, JFAB; preparation of the original manuscript, review and editing, JFDN, JTS, SAS, MVH, MAB, JLAV, JFAB; obtaining funding, JFDN, JTS, SAS. All authors have read and accepted the published version of the manuscript.

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