microbiology research

MDPI

Article

Biological Control of *Sclerotinia sclerotiorum* on Greenhouse Lettuce Using *Trichoderma koningiopsis* Agg

Snježana Topolovec-Pintarić [1,*], Ana Maria Kovaček [1], Olga Malev [2,*], Ivana Kušan [3], Neven Matočec [3], Ana Pošta [3], Lucia Pole [3] and Armin Mešić [3]

[1] Department of Plant Pathology, Faculty of Agriculture, University of Zagreb, 10000 Zagreb, Croatia; ana.maria.kovacek98@gmail.com
[2] Laboratory for Aquaculture Biotechnology, Ruđer Bošković Institute, 10000 Zagreb, Croatia
[3] Laboratory for Biological Diversity, Ruđer Bošković Institute, 10000 Zagreb, Croatia; ikusan@irb.hr (I.K.); nmatocec@irb.hr (N.M.); aposta@irb.hr (A.P.); lpole@irb.hr (L.P.); amesic@irb.hr (A.M.)
* Correspondence: tpintaric@agr.hr (S.T.-P.); omalev@irb.hr (O.M.)

Abstract: The lettuce drop or white mold is an economically important disease as the causal fungus *Sclerotinia sclerotiorum* can infect the lettuce at any stage of plant development. Polyphagous nature of *S. sclerotiorum*, the longevity of soil-borne sclerotia and air-borne ascospores makes the control difficult. Chemical fungicides are available only for foliar application against infections by ascospores so, the development of bio-control is of great importance. We tested antagonism of native isolate *T. koningiopsis* agg. (Hypocreales) (STP8) under laboratory and greenhouse environments. In vitro tests showed excellent STP8 antagonisms to *S. sclerotiorum* evidencing hyperparasitic activity on mycelia and sclerotia as well as antibiosis. The sclerotia were completely degraded after two months. In the greenhouse, infection of lettuce with *S. sclerotiorum* was reduced by treating the seedlings with an STP8 spore suspension. Uninfected plants treated with STP8 were of the best quality based on morphological parameters, confirming the ability of STP8 to promote lettuce growth. Even the infected lettuce treated with STP8 were healthier and in better condition than the control lettuce, suggesting that STP8 was also enhancing plant defense system.

Keywords: antagonism; Ascomycota; beneficial fungi; *Lactuca sativa* L.; mycoparasitism; white mold

Academic Editor: Mireille Fouillaud

Received: 10 December 2024
Revised: 13 January 2025
Accepted: 22 January 2025
Published: 31 January 2025

Citation: Topolovec-Pintarić, S.; Kovaček, A.M.; Malev, O.; Kušan, I.; Matočec, N.; Pošta, A.; Pole, L.; Mešić, A. Biological Control of *Sclerotinia sclerotiorum* on Greenhouse Lettuce Using *Trichoderma koningiopsis* Agg. *Microbiol. Res.* **2025**, *16*, 35. https://doi.org/10.3390/microbiolres16020035

1. Introduction

Lettuce (*Lactuca sativa*) is one of the most consumed vegetables among the Croatian population with a cultivated area of 241 hectares and a yearly production rate of 6.479 tons [1]. Diseases that affect lettuce include lettuce drop or white mold, which is a major concern for growers and producers. Lettuce drop occurrence results in severe damage and yield loss up to 70% because the causal pathogen can infect all lettuce parts at any stage of plant development [2,3]. The causal pathogen of the lettuce drop is the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, which belongs to the phylum Ascomycota (Helotiales, Sclerotiniaceae). It is a facultative soilborne pathogen which produces sclerotia, the primary long-term survival structures that remains viable in soil for up to 10 years [4]. The fungus *S. sclerotiorum* parasitize approximately 500 species of plants worldwide, mostly dicotyledonous, belonging to the families Solanaceae, Brassicaceae, Apiaceae, Asteraceae, Chenopodiaceae, and Fabaceae [5,6] as well as some very aggressive weed species such as *Abutilon theophrasti* Medick., *Ambrosia artemisifolia* L., and *Amaranthus retroflexus* L. [7].

Recently, one of the newly discovered hosts of *S. sclerotiorum* is *Nasturtium officinale* W.P. Aiton (Brassicaceae) [8].

The production and viability of sclerotia is essential to the survival of *S. sclerotiorum* in soil and the onset of disease, as they provide a primary inoculum by myceliogenic or carpogenic germination, depending on environmental conditions [9]. They can be directly infectious to a nearby host when they germinate in the mycelium, and they can be responsible for epidemics when they develop apothecia and produce ascospores that bear anemochory in the spring. Infection can thus occur on any part of the lettuce plants where the ascospores have been deposited. The life cycle is monocyclic since no secondary inoculum is produced. The characteristic sign of *S. sclerotiorum* presence is patches of cottony-white mycelium, often accompanied by hypogenous, black, irregularly shaped sclerotia measuring 2–20 mm by 3–7 mm [3,9].

The control of lettuce drop is complex because of the nature of *S. sclerotiorum* polyphagous, coupled with the longevity of soilborne sclerotia and airborne ascospores. Therefore, the usual agricultural measure crop rotation is futile and even deep ploughing to some extent. Control measures such as solarization, dry fallow land, and flooding fields may reduce the number of sclerotia in the soil by inducing starvation, oxygen deprivation, or desiccation [5], but these measures are partially successful and are unachievable in many breeding conditions. Moreover, they are not environmentally friendly. Fungicides are available only for foliar application in vegetation while soil application is environmentally unacceptable. However, over-reliance on broad-spectrum chemical fungicides poses a serious risk to the environment, disrupts the ecology, reduces the number of beneficial microorganisms in the soil, and accelerates the development of fungicide-resistant strains of *S. sclerotiorum* [10,11]. Therefore, the development of biological control is of great significance. One type of biological control is soil suppression, which relies on the microbiological activity of native microorganisms that develop after some plants, usually Brassicaceae, are ploughed into soil [12,13]. However, true biocontrol requires the use of bio-agents, and the most successful fungal antagonists that have been characterized for the control of *S. sclerotiorum* are: *Paraphaeosphaeria minitans* (W.A. Campb.) Verkley, Göker & Stielow as *Coniothyrium minitans* W. A. Camp [14], and *Alternaria atra* (Preuss) Woudenb. & Crous as *Ulocladium atrum* Preuss [15].

In recent decades *Trichoderma* Pers. spp. have gained importance due to their antagonistic abilities against *S. sclerotiorum* in various crops [3,16,17]. They are currently marketed as biopesticides, biofertilizers, plant growth enhancers, and stimulants of plant natural resistance [18–20]. Recent biogeography and diversity studies have identified *Trichoderma* isolates from different localities around the world, confirming that their ecological specialization is modulated by climate, soil type, cropping system, and complex ecological interactions that influence their effectiveness as biocontrol agents [21]. In addition, the effectiveness of *Trichoderma*-mediated suppression of plant pathogens and growth promotion is species and strain dependent [22,23]. Given the growing concern about the impact that invasive allochthonous *Trichoderma* strains introduced by commercial products may have on the native rhizosphere community, it is important to investigate the antagonistic capabilities of native strains of *Trichoderma* species [19]. The aim of this work was to investigate the biocontrol potential of a native Croatian strain, *Trichoderma koningiopsis* agg., against *S. sclerotiorum* under laboratory and greenhouse conditions in association with lettuce plants, also considering its role as a growth and yield promoter of lettuce.

2. Materials and Methods

2.1. Fungal Strains

In this study, native strains of *Sclerotinia sclerotiorum* and *Trichoderma koningiopsis* agg. were used, both isolated from the humus soil in experimental vegetable garden site at the Faculty of Agriculture (Zagreb, Croatia). The isolation of *T. koningiopsis* agg. strain was carried out according to [24]. Axenic cultures are stored on potato dextrose agar (PDA) media in the temporary laboratory collection of the first author under the codes STP8 (*Trichoderma*) and SS (*S. sclerotiorum*). Fungal isolates are taxonomically determined by molecular methods, with available phenetic characters (sclerotia of *Sclerotinia*) used as additional data for species identification.

2.2. DNA Isolation, PCR, Sequencing, Sequence Alignment and Phylogenetic Analysis

Total genomic DNA of SS was extracted from mycelium and STP8 from anamorphic structures, both from axenic cultures, using the Quick-DNA Fungal/Bacterial Miniprep kit (Zymo Research, Irvine, CA, USA) following the manufacturer's instructions. The ITS region was amplified using primer pair ITS4/ITS5, *rpb*2 gene region using fRPB2-5f/fRPB2-7cr and *tef*1 gene region using EF1/EF2 [25]. The 25 µL PCR mixtures contained 12.5 µL of Go Taq G2 Green Master Mix (Promega, SAD, Madison, WI, USA), 1 µL of DNA template, and 1 µL of each forward and reverse primer at the concentrations 5 ng/µL and 9.5 µL ddH_2O. PCR reaction was conducted under protocols specific for each primer pair [25] in a PCRMax thermal cycler (Cole-Parmer, Cambridgeshire, UK). The sizes of PCR products were assessed by 1% agarose gel electrophoresis along with standard DNA markers. All successfully amplified PCR products were purified by ExoSAP-IT™ (Thermo Fisher Scientific, Waltham, MA, USA) cleanup reagent following the manufacturer's protocol prior to sending to Macrogen Europe (Amsterdam, The Netherlands) for bidirectional Sanger sequencing.

Sequence reads were assembled and edited using Geneious Prime 2025.0.2. software (https://www.geneious.com, accessed on 7 January 2025, Biomatters, Auckland, New Zealand). Assembled sequences were deposited at the National Center for Biotechnology Information (NCBI) GenBank database (https://www.ncbi.nlm.nih.gov/genbank/, accessed on 8 January 2025,) under the following accession numbers: *Sclerotinia sclerotiorum* strain SS (ITS = PQ849352), *Trichoderma koningiopsis* agg. strain STP8 (ITS = PQ849380, *rpb*2 = PQ867587, *tef*1 = PQ867588). The list of all sequences used in our phylogenetic analyses is available in the Supplementary File S1 [26–48].

The Basic Local Alignment Search Tool (BLAST, https://blast.ncbi.nlm.nih.gov, accessed on 1 March 2023) was used for searching similar sequences in GenBank. Sequence alignment of the dataset was achieved individually on ITS (*Sclerotinia*), rpb2 and tef1 (*Trichoderma*) using MAFFT vers. 7.490 [49,50] available as Geneious Prime plugin. After being aligned and trimmed, both *Sclerotinia* ITS rDNA alignment and *Trichoderma rpb*2 and *tef*1 alignments were accomplished using Geneious Prime 2025.0.2. *Monilinia johnsonii* was selected as the outgroup for phylogenetic analysis of *Sclerotinia* dataset, while *Protocrea farinosa* was used as the outgroup in the *Trichoderma* dataset. Phylogenetic analyses were conducted using Maximum Likelihood (ML) in IQTREE v1.6.12 [51,52] by applying the ultrafast bootstrap approximation with 10,000 replicates. The best model was selected by ModelFinder implemented in IQTREE (TIM2e model for *Sclerotinia* alignment and TNe + G4 for the *Trichoderma* dataset). Phylogenetic trees were visualized and annotated using FigTree 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/, accessed on 7 January 2025) and PowerPoint as part of Microsoft Office Professional Plus 2021.

2.3. Antagonism on Agar Culture Plates

The antagonistic effects of *T. koningiopsis* agg. (STP8) on the growth inhibition of *S. sclerotiorum* (SS) were investigated according to [53] using the dual-culture method. The fungal cultures were grown on PDA (Biolife, Italy) at 21 °C in 90 mm diameter Petri dishes. Mycelial discs with a diameter of 6 mm were removed from the edge of the seven-day-old cultures and transferred to 90 mm diameter Petri dishes containing PDA to form dual cultures. A mycelial disc of SS containing a well-developed sclerotium was placed on one side of a PDA plate, while a disc of STP8 was placed on the opposite side. For the control plates, a sterile agar disc was used instead of the STP8 mycelial disc. The testing for production of volatile metabolite was performed using the dual-culture method [54], in which the SS mycelial plug was placed on the agar in the center of the Petri dish lid and the STP8 was placed on the agar in the center of the Petri dish container. In both tests each treatment was carried out in five plates with four replicates (N = 40). The dishes were sealed with paraffin tape (Parafilm, Brand GMBH + CO KG, Germany) and incubated in the dark at 20 °C ± 1 °C for seven days. During the incubation period, the fourth and seventh day radial fungal colony growth of both species in the direction of the opposite colony was measured manually using a ruler. The maximum and minimum radial growth were measured and the average radial growth was calculated. The average radial growth was used to calculate the inhibition index (I = %) as follows: $I\ (\%) = (C - T/C) \times 100$, where I is the inhibition percentage; C is the radial growth of SS (mm) alone (control); and T is the radial growth of SS (mm) in the presence of STP8 [55]. An index value of 50% or more is considered as excellent performance. After measurement, the dishes containing the dual-cultures were incubated for 2 months to evaluate parasitism on sclerotia.

2.4. Biological Assay in Greenhouse Lettuce

The greenhouse experiment was set up according to a randomized complete block design in five replicates with five plants per replicate. Commercial lettuce seeds of the variety Sunny, which had been treated with carbendasim-thiram by the manufacturer (Nickerson-Zwaan Menaco B.V., Tuitjenhorn, The Netherlands), were sown in polystyrene boxes (containers for vegetable cultivation) in the potting mixture Klasmann-Delimann P 002 (Geeste, Germany). The potting substrate was previously autoclaved (2 h, 121 °C). Three-leaved seedlings (N = 100) were transferred to pots with a diameter of 9 cm and divided in four different treatments: (1) SS—seedlings infected with *S. sclerotiorum* by inserting a mycelial disc with a diameter of 6 mm and a sclerotium manually using a spatula near the root collar into the substrate; (2) STP8 + SS—seedlings inoculated with *S. sclerotiorum* additionally inoculated with STP8 spore suspension at a concentration of 4×10^6 spores mL^{-1} by pouring suspension into the substrate along the root collar; (3) STP8—seedlings inoculated with STP8 spore suspension at a concentration of 4×10^6 spores mL^{-1}; (4) control—untreated seedlings. The plants were harvested nine weeks after sowing and the following characteristics were evaluated: the number of leaves per plant, leaf length, leaf width, fresh weight, and dry weight of the plants.

Recovery of STP8 from the soil was performed after plants were harvested to evaluate its colonization. A soil sample was collected from each pot and each replicate (25 per treatment) in the STP8 and STP8 + SS treatments. Soil samples were brought to the laboratory and air-dried in paper bags at room temperature for seven days. The soil was subjected to the procedure for isolation of *Trichoderma* [24].

Obtained data were statistically analyzed: ANOVA, LSD for 5% and 1%, and Duncan's multiple-range test (MS Excel version 2306, MS Office 365).

3. Results

3.1. Taxonomic Identification of Fungal Strains

Phylogenetic analysis of SS ITS rDNA sequence and closely related species from the genus *Sclerotinia* showed strong affiliation of SS to a *S. sclerotiorum* (Figure 1). Sequence identity of SS was 100% with all analyzed *S. sclerotiorum* isolates [28–34]. The identity of *Sclerotinia* strain SS was further checked by available phenetic characters. Thus, all sclerotial characters fully complied with those of *Sclerotinia sclerotiorum* [56].

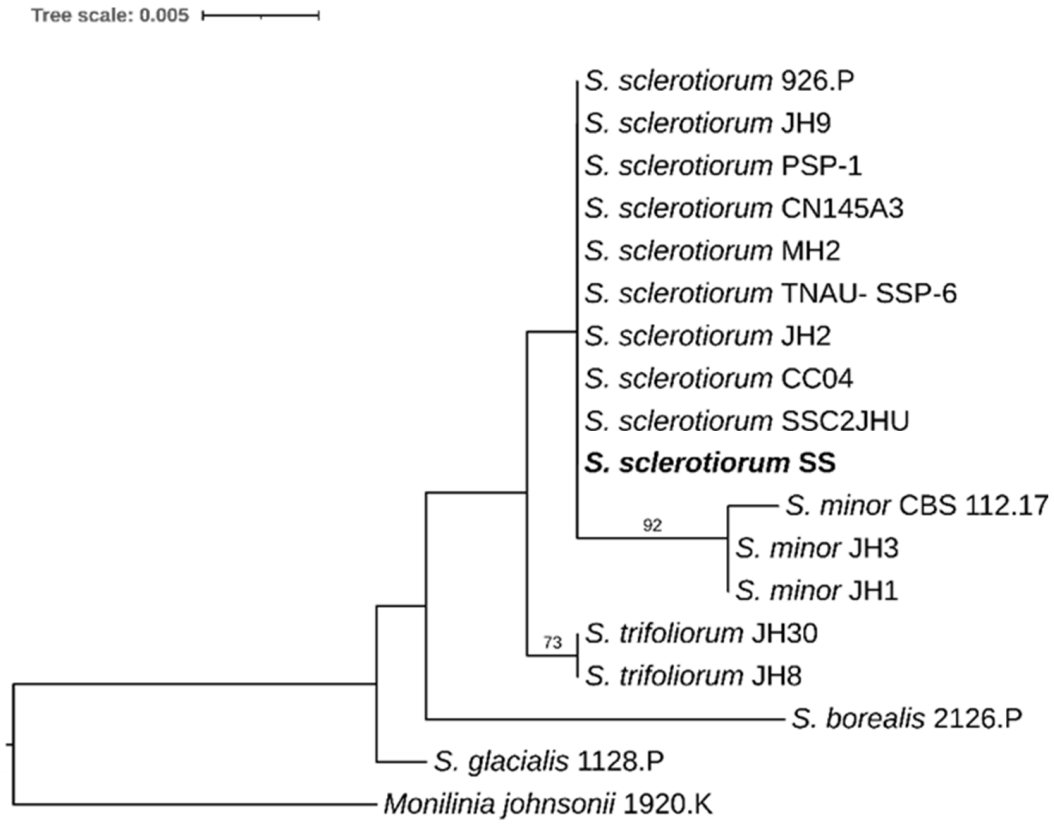

Figure 1. Phylogenetic tree based on Maximum Likelihood (ML) analysis of ITS sequence alignment of *Sclerotinia sclerotiorum* including the strain SS and closely related species. The species *Monilinia johnsonii* was used as an outgroup. Only ultrafast bootstrap support values ≥ 70% are presented at the branches.

To identify *Trichoderma* strain STP8 at the species rank, we followed the guidelines of Cai i Druzhinina [25] using three DNA barcode sequence data (ITS, *rpb*2, and *tef*1 gene regions). Megablast search of NCBIs GenBank nucleotide database using the *rpb*2 and *tef*1 sequences of *Trichoderma* strain STP8 showed that several closest hits belong to *T. koningiopsis* Samuels, Carm. Suárez & H.C. Evans. Phylogenetic analysis of STP8 and closely related taxa confirmed clear affiliation of STP8 to *T. koningiopsis* species group (Figure 2).

Comparison of STP8 sequence data to the reference (type) strain of *T. koningiopsis* (GJS 93-20, CBS 119075; *rpb*2 = EU241506, and *tef*1 = DQ284966) resulted in 97.91% of identity in *rpb*2, and 94.81% of identity in *tef*1, respectively. Since the STP8 sequences did not reach the similarity value to a reference strain as rpb2 ≥ 99% and tef1 ≥ 97% [25], we did not name STP8 isolate as *T. koningiopsis* sensu stricto but rather as a member of *T. koningiopsis* agg. until more taxonomic research is done.

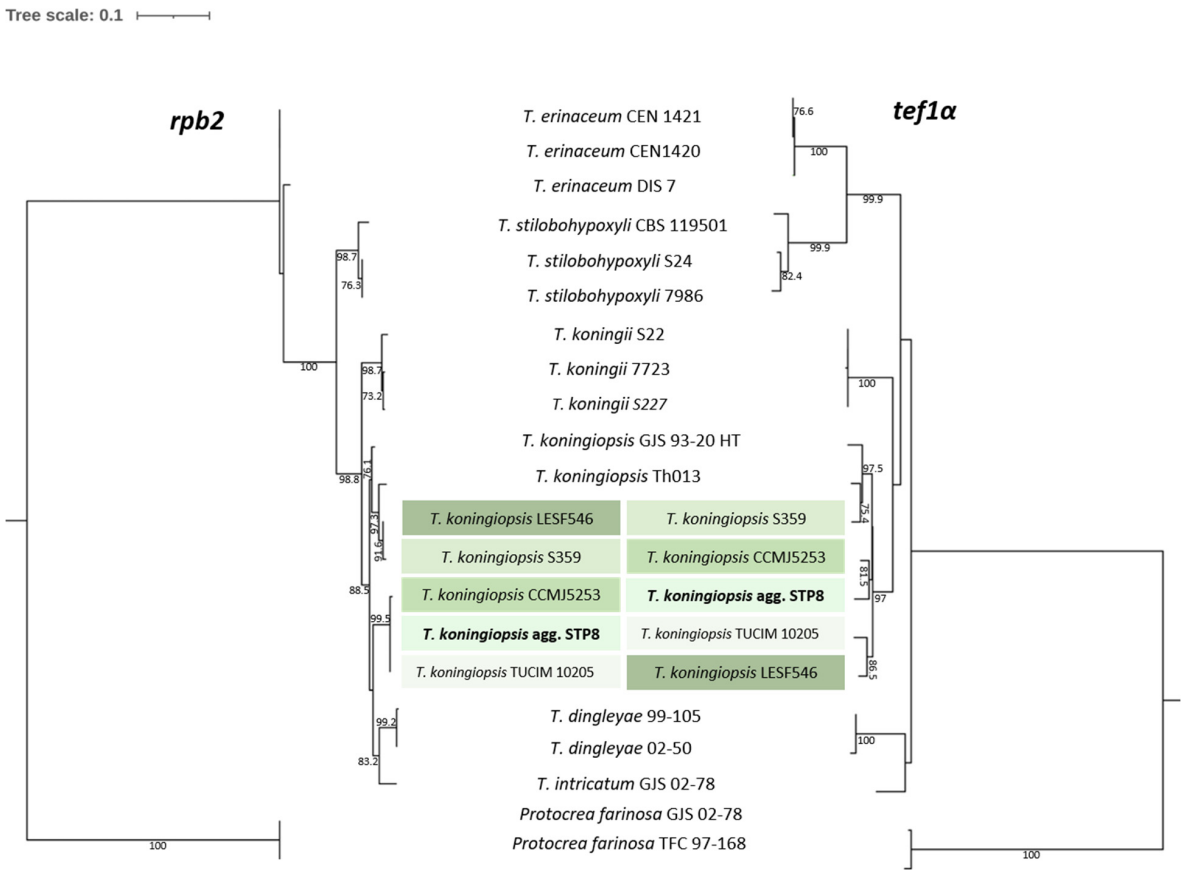

Figure 2. Phylogenetic trees based on Maximum Likelihood (ML) analysis of *rpb*2 and *tef*1 sequence alignments of *Trichoderma koningiopsis* agg. and closely related species of the genus *Trichoderma*. The species *Protocrea farinosa* was used as an outgroup. Only ultrafast bootstrap support values ≥ 70% are presented at the branches.

3.2. Antagonism on Agar Culture Plates

Biocontrol of *S. sclerotiorum* (SS) was performed with the selected native strain T. koningiopsis agg. (STP8) under laboratory and greenhouse-controlled conditions. In vitro tests showed excellent antagonisms to *S. sclerotiorum* through competitiveness, antibiosis, and mycoparasitism. The mechanism of competition for space and resources was shown by the fact that isolate STP8 colonized the substrate faster, so that it occupied at least 54% of the Petri dish after only four days and the hyphae intermingled with those of *S. sclerotiorum*. In seven days, it had overgrown the mycelium of *S. sclerotiorum* with an inhibition of 77% (Table 1).

Table 1. Antagonism of *Trichoderma koningiopsis* agg. STP8 against *Sclerotinia sclerotiorum*.

Average Colony Radius of *Sclerotinia sclerotiorum* (cm)						
	Incubation Day: 4			Incubation Day: 7		
Rep.	Control [1]	Test [2]	I (%) [3]	Control [1]	Test [2]	I (%) [3]
1.	35	17	51.4	90	21	76.7
2.	39	17	56.4	90	21.8	75.8
3.	39	19	51.3	90	19.9	77.9
4.	37	16	56.8	90	21.16	76.5
$\bar{x}$	37.5	17.3	54	90	20.97	76.7

[1] Control = Culture SS + sterile mycelial plug instead of STP8. [2] Test = Dual cultures STP8 + SS. [3] I = Inhibition index.

The parasitism of isolate STP8 on the *S. sclerotiorum* mycelium appeared as browning in a 18.9 mm wide zone. No sclerotia were formed in the presence of *T. koningiopsis* agg. strain STP8, while the test of mycoparasitism of sclerotia resulted in complete decomposition of the sclerotia after two months. The parasitized sclerotia were smaller and had a completely macerated interior (medulla), while the rind (cortex) was softened and easily broken (Figure 3).

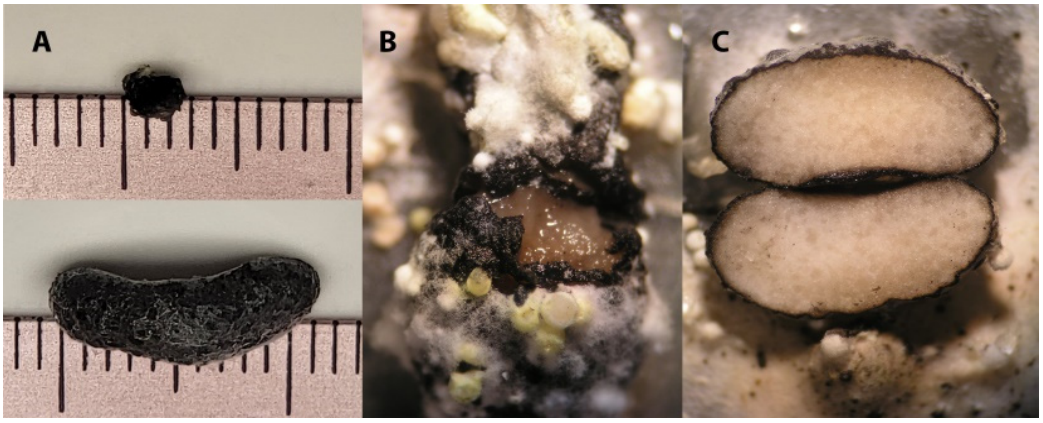

Figure 3. Sclerotia of *Sclerotinia sclerotiorum*: (**A**) parasitized by *Trichoderma koningiopsis* STP8 (above), healthy (below); (**B**) macerated (middle); (**C**) healthy, cross section (right).

When tested for antibiosis, the presence of volatile metabolites was confirmed by the growth inhibition of *S. sclerotiorum* at 75% after four days and 86% after seven days compared to the control (Table 2). Altogether, the strain STP8 showed the characteristics of a good biocontrol agent in vitro as it has the potential to significantly inhibit growth and development of *S. sclerotiorum* colonies and sclerotia.

Table 2. Antibiosis of *Trichoderma koningiopsis* agg. STP8 against *Sclerotinia sclerotiorum*.

Average Colony Radius of *Sclerotinia sclerotiorum* (cm)						
	Incubation Day: 4			**Incubation Day: 7**		
Rep.	**Control [1]**	**Test [2]**	**I (%) [3]**	**Control [1]**	**Test [2]**	**I (%) [3]**
1.	39	9	76.9	90	9.8	89
2.	39	12	69.2	90	19.5	78.3
3.	35	7.5	78.6	90	8.5	90.5
4.	37	9.5	74.3	90	12.6	86
$\bar{x}$	37.5	9.5	74.8	90	12.6	86

[1] Control = Culture SS + sterile mycelial plug instead of STP8. [2] Test = Dual cultures STP8 + SS. [3] I = Inhibition index.

3.3. Antagonism in Greenhouse Lettuce

The greenhouse experiments showed that STP8 reduce the onset of *S. sclerotiorum* infection and the infection rate, and could solubilize nutrients and stimulate plant growth. The differences were relevant in all treatments in which STP8 was added compared to the control. Statistically significant differences were observed between the *T. koningiopsis* agg. (STP8, STP + SS), the *S. sclerotiorum* (SS) treatment and the control in the morphological parameters evaluated (number of leaves per plant, leaf length and width, and fresh weight) (Table 3).

Table 3. Biocontrol of *Sclerotinia sclerotiorum* SS by *Trichoderma koningiopsis* agg. STP8 in greenhouse lettuce.

Treatment	Leaves/Plant	Leaf Length (cm)	Leaf Width (cm)	F.wt. (g) [1]	D.wt. (g) [2]
STP8	10.2 a [5]	17.5 a	8.6 a	11.3 a	0.1
STP8 + SS	9.0 b [5]	15.6 b	7.6 b	8.2 b	0.1
SS	5.8 c [5]	11.8 c	5.5 d	3.8 c	0.1
Control	8.7 b	14.7 b	6.7 c	8.1 b	0.1
LSD [3] 5%	0.4	1.0	0.1	1.3	n.s. [4]
LSD 1%	0.3	0.7	0.1	0.9	n.s.

[1] F.wt. = Fresh weight. [2] D.wt. = Dry weight. [3] LSD = Least significant difference. [4] n.s. = Non-significant difference. [5] a, b, c, d = Mean in each column, with same letters are not significantly different at $p < 0.05$, Duncan test.

The non-infected plants treated only with STP8 isolate (STP8) showed the best quality in terms of all morphological parameters. Plants infected with *S. sclerotiorum* (SS) resulted in the highest number of dead plants and the highest disease severity scores. *Sclerotinia sclerotiorum* infected the stem tissue and lower leaves and led to the death of the lettuce plants within seven to 10 days. There were no statistically significant differences in dry weight. Even the lettuce infected with *S. sclerotiorum* and treated with STP8 isolate (STP8 + SS) remained healthy and in better condition than the infected lettuce (SS) and the control lettuce plants. This indicates that STP8 also enhances plants' defense systems. Non-infected plants treated with STP8 were of the best quality based on the morphological parameters (number of leaves per plant, leaf length and width, and fresh weight) confirming the ability of STP8 to promote plant growth.

4. Discussion

The production and viability of sclerotia is essential for *S. sclerotiorum* survival, propagation and onset of disease. Therefore, reducing the production of sclerotia is critical in the control of this pathogen. Since sclerotia are overwintering structures that are subject to dormancy, they need a conditioning period of a one to three months in soil to overcome constitutive dormancy before germination, and the main onset of *Sclerotinia* disease mostly occurs two months after planting [11,57,58]. Isolated *T. koningiopsis* agg. STP8 inhibited in vitro growth of formed sclerotia after one week (more than 70% in the first week) and completely decomposed them within two months due to mycoparasitism. Also, STP8 prevented the formation of new sclerotia by simultaneously parasitizing on mycelia, which was in line with the research of [59]. According to [60], the high antagonism of *Trichoderma* spp. against *S. sclerotiorum* is due to the increasing productions of cell wall-degrading enzymes. The main extracellular cell wall-degrading enzymes responsible for mycoparasitism include chitinases and β-glucanases [61], which directly degrade host cell walls [19].

However, promising in vitro results do not often translate into successful in vivo (field) results [62]. Therefore, the *Trichoderma* isolate selected in vitro as suitable as bioagent, needs to be validated in research either in vivo under systems that resemble natural conditions or under field conditions [63]. Application of isolate STP8 in greenhouse trial showed that STP8 treatment when planting lettuce seedlings was beneficial, as it reduced the infection by *S. sclerotiorum* and promoted the growth of lettuce. Comparable results in lettuce were also obtained in a study by [64] with native Brasilian *Trichoderma* isolates, in which all 12 isolates of four different *Trichoderma* species promoted the growth of lettuce in the presence and absence of *S. sclerotiorum*. The positive effects on the lettuce growing in soil infested with *S. sclerotiorum* and eradication of sclerotia from soil using the native *Trichoderma* isolates were also observed in Poland [6,65]. Further, our results confirm previous findings that the most effective *Trichoderma* strains colonize the roots and are beneficial at least for the life span of annual plants, and that even if *Trichoderma* is only

present on the roots, the improvement in growth can be assessed on both the root and the foliage [3,7,17,65–68]. In similar trials on the S. *sclerotinia* control in onions by the native isolate of *T. atroviride* from New Zeland [58] the disease also occurred two months after planting, and authors concluded that the performance of *Trichoderma* bioagent can be much more influenced by the time it remains in contact with the pathogen than by the pressure of the disease itself. The results obtained here provide an idea for future research on the possible application of STP8 as a potential bioagent through preventive application to the soil.

Some studies of biological control targeting sclerotia of *S. sclerotiorum* have focused on the effectiveness of commercial products based on *Trichoderma* spp. [63,64,69–71]. Currently, there are nearly 400 species of *Trichoderma* [25], and more than 10 species have strains that are used commercially as a biological solution to control plant diseases [72] through 77 biopesticides on the world market [19] against more than 100 different phytopathogen fungal species [73]. Commercial *Trichoderma* products are based on an encapsulated fungal inoculum designed to maintain fungal propagules viable during transportation, storage, and application. During each phase, the loss of part of the propagules is possible, as they may die under inadequate conditions, or the lifespan of the product may be shortened [74]. Further, reintroduction into the soil, even of the most strongly rhizosphere competent *Trichoderma* bioagent, can be difficult [20]. When introduced into a new rhizosphere environment, *Trichoderma* must compete with a spectrum of native microbes, and is often affected by various natural abiotic factors. Thus, when it is applied in the field, the biological control effect is weakened [18,74]. However, even the ability to colonize the soil to a high degree is not a determining factor in evaluating the biocontrol potential of *Trichoderma,* as was noted by [75] where commercial *Trichoderma*-based biocontrol products were not effective in suppressing *S. sclerotiorum*, even though the treated soils were shown to be abundantly colonized with *Trichoderma*. The authors even emphasized the possibility that the allochthonous *Trichoderma* stimulated infection of lettuce by *S. sclerotiorum* as the number of healthy lettuce heads were in general lower than those in the control. A similar insignificant effect on disease control or sclerotial infection showed two *Trichoderma* strains imported in the UK, despite their high activity in original infection tests carried out in southern France, where they originated [69]. The authors speculated that this may have been due to the lower soil temperatures (18 °C) compared to the soil temperatures (28 °C) in the south of France, which makes these isolates poorly capable of infecting the sclerotia of native *S. sclerotiorum* isolate. Different behaviors in the control of *S. sclerotiorum* were shown by *Trichoderma* isolates from soils with different uses [63] indicating that under field conditions there are other biotic or abiotic factors that may influence the expression of the antagonism and that *Trichoderma* may have preference for the substrate. The type of crop is another factor influencing the *Trichoderma* species diversity in the soil [76]. Investigations of the rhizosphere in agricultural ecosystems around the world have established the dominance of *Trichoderma* species in the microbiota and a high level of their inter- and intraspecific genotypic diversity depending on crop type [77]. These findings confirm the need for careful selection of commercial *Trichoderma* antagonists for use in specific environments and under specific conditions.

Nowadays, as a stable taxonomic framework for the genus *Trichoderma* is available [25] different *Trichoderma* species have different biogeographic distribution patterns and, to some extent there is a preference for the substrate in natural [24,48,78,79] and agricultural environments [80–84]. The negative impact of *Trichoderma* species from bioproducts on the biodiversity of not only native *Trichoderma* species but also other organisms (e.g., plants, bacteria, fungi) is receiving more attention in research studies. The study of soil biodiversity on the island of Tenerife confirmed a low diversity of *Trichoderma*-specific communities

and the presence of ubiquitous, widespread species rather than endemic species [85]. All isolates showed high antagonistic and competitive ability, confirming extensive colonization by allochthones *Trichoderma* species and suppression of native strains. On the island of Sardinia similar results of reduction of native *Trichoderma* diversity because of replacement by extensive invasion of species from Eurasia, Africa, and the Pacific Basin were found, and possible introductions by humans or another biota were not excluded [86,87]. Allochthones *Trichoderma* strain can be introduced even with commercial potting substrate. According to [88], a higher level of diversity in *Trichoderma* species exists in potting media and as this material consists of different organic and inorganic ingredients but could therefore have been naturally occurring in these products or been introduced after composting [89,90]. In conclusion, since the efficacy of *Trichoderma* is related to the soil type, a suspension based on an autochthonous *Trichoderma* strain is more suitable than an allochthones strain introduced by commercial *Trichoderma* products.

5. Conclusions

Alongside the immense potential of *Trichoderma*, its effects as a plant partner go beyond the biocontrol of plant pathogens. The positive effect of *Trichoderma* on plant growth is recognized as an independent ability and is as remarkable and significant as its antifungal ability, as growth improvement was observed in the absence of any detectable disease and in sterile soil [91–95]. *Trichoderma* may cause significant biochemical changes in plant content of carbohydrates, amino acids, organic acids, and lipids [96]. *Trichoderma* researchers emphasize that considering biocontrol as the primary capability of *Trichoderma* may compromise the evolution of the biocontrol system, as it means that conditions are optimized for the wrong mechanism [18]. Therefore, it is proposed to expand the importance of *Trichoderma*-fungicide and extend its role in biocontrol to the promotion of plant growth (bio-stimulant) and disease resistance in addition to classical antibiosis and mycoparasitism, as there is overlap between biocontrol by *Trichoderma* and plant performance-enhancing activities. Bio-stimulants can benefit in greenhouse production and eco-farming of lettuce and can be applied in addition to standard fertilization treatments to improving nutrient use efficiency. In greenhouses water suspension of *Trichoderma* propagules are generally introduced into hydroponic systems infused directly into the nutrient solution. They can also be mixed into soil-less media, any inert growth media will suffice. Also, can be sprinkled directly onto plant roots like application in this study.

The application of native *T. koningiopsis* agg. strain STP8 in this study increased yield parameters of lettuce and helped to inhibit *S. sclerotiorum*. Considering all, obtained results warrant further investigation toward field application of *T. koningiopsis* agg. STP8 as a bio-stimulant where its impact on yield parameters and chemical constituents in head lettuce would be evaluated. Since there is great interest in researching the molecular mechanisms by which plants perceive and respond to microbial signals [96] we plan to carry out further studies to understand the physiological and biochemical mechanisms of lettuce growth promotion by *T. koningiopsis* agg. STP8 and assess the possibility of commercializing the product for the market as a bio-stimulant.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microbiolres16020035/s1, Supplementary File S1: The list of sequences included in the phylogenetic studies.

Author Contributions: Conceptualisation, visualisation, validation, resources, S.T.-P.; methodology, formal analysis, investigation and data curation all authors; funding acquisition, S.T.-P. and A.M.; Writing—original draft, S.T.-P.; Writing—review & editing, all authors. All authors have read and agreed to the published version of the manuscript.

Funding: The authors would like to thank the Croatian Science Foundation for their support under the grants HRZZ-IP-2018-01-1736, HRZZ-DOK-2018-09-7081, and HRZZ-DOK-2021-02-4010. Topolovec-Pintarić S. acknowledges Institutional funding of research activities from University of Zagreb.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting this study's findings are available from the corresponding author upon reasonable request.

Acknowledgments: Topolovec-Pintarić S. acknowledges Institutional funding of research activities from University of Zagreb. We thank Ines Sviličić Petrić for obtaining the sequence of *Sclerotinia sclerotiorum*. All authors included in this section have consented to the acknowledgement.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Croatian Bureau of Statistics. Production of Vegetables, Fruits and Grapes in 2022—Provisional Data. Available online: https://podaci.dzs.hr/2022/en/29337 (accessed on 31 March 2023).
2. Subbarao, K.V. Lettuce drop. In *Compendium of Lettuce Diseases*, 1st ed.; Subbarao, K.V., Davis, R.M., Gilbertson, R.L., Raid, R.N., Eds.; American Phytopathological Society: St. Paul, MN, USA, 1997; pp. 19–21.
3. Elias, L.M.; Domingues, M.V.P.F.; Moura, K.E.; Salomão, D.; Harakava, R.; Patricio, F.R.A. Selection of *Trichoderma* isolates for biological control of *Sclerotinia minor* and *S. sclerotiorum* in lettuce. *Summa Phytopathol.* **2016**, *42*, 216–221. [CrossRef]
4. Mamo, B.E.; Eriksen, R.L.; Adhikari, N.D.; Hayes, R.J.; Mou, B.; Simko, I. Epidemiological characterization of Lettuce Drop (*Sclerotinia* spp.) and biophysical features of the host identify soft stem as a susceptibility factor. *PhytoFrontiers* **2021**, *11*, 182–204. [CrossRef]
5. Saharan, G.S.; Mehta, N. *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*, 1st ed.; Springer: Dordrecht, The Netherlands, 2008; pp. 301–376.
6. Smolińska, U.; Kowalska, B. Biological control of the soil-borne fungal pathogen *Sclerotinia sclerotiorum*—-A review. *J. Plant Pathol.* **2018**, *100*, 1–12. [CrossRef]
7. Vrandečić, K.; Ćosić, J.; Jurković, D.; Duvnjak, T. Weeds as inoculum source of *Sclerotinia sclerotiorum*. In Proceedings of the 6th Slovenian Conference of Plant Protection, Zreče, Slovenia, 4–6 March 2003; pp. 101–106.
8. Csüllög, K.; Tóth, B.; Lelesz, E.J.; Fehér, M.; Virág, C.I.; Kutasy, E.; Jász, B.; Tarcali, G.; Biró, G. First Report of *Sclerotinia sclerotiorum* on Watercress (*Nasturtium officinale*) in an Aquaponic System in Hungary. *Plant Dis.* **2022**, *106*, 767. [CrossRef] [PubMed]
9. Bolton, M.D.; Thomma, B.P.H.J.; Nelson, B.D. *Sclerotinia sclerotiorum* (Lib.) de Bary: Biology and molecular traits of a cosmopolitan pathogen. *Mol. Plant Pathol.* **2006**, *7*, 1–16. [CrossRef] [PubMed]
10. Zhou, F.; Zhang, X.L.; Li, J.L.; Zhu, F.X. Dimethachlon resistance in *Sclerotinia sclerotiorum* in China. *Plant Dis.* **2014**, *98*, 1221–1226. [CrossRef] [PubMed]
11. Panth, M.; Hassler, S.C.; Baysal-Gurel, F. Methods for Management of Soilborne Diseases in Crop Production. *Agriculture* **2020**, *10*, 16. [CrossRef]
12. Subbarao, K.V. Progress toward integrated management of lettuce drop. *Plant Dis.* **1998**, *82*, 1068–1078. [CrossRef] [PubMed]
13. Cook, R.J. Plant Health Management: Pathogen Suppressive Soils. In *Encyclopedia of Agriculture and Food Systems*, 1st ed.; Van Alfen, N.K., Ed.; Academic Press: Cambridge, MA, USA, 2014; pp. 441–455. [CrossRef]
14. Zhao, H.; Zhou, T.; Xie, J.; Cheng, J.; Chen, T.; Jiang, D.; Fu, Y. Mycoparasitism illuminated by genome and transcriptom e sequencing of *Coniothyrium minitans*, an important biocontrol fungus of the plant pathogen *Sclerotinia sclerotiorum*. *Microb. Genom.* **2020**, *6*, e000345. [CrossRef] [PubMed]
15. Li, G.Q.; Huang, H.C.; Acharya, S.N. Antagonism and biocontrol potential of *Ulocladium atrum* on *Sclerotinia sclerotiorum*. *Biol. Control* **2003**, *28*, 11–18. [CrossRef]
16. Villalta, O.N.; Wite, D.; Hunt, J.; Stewart, A.; Porter, I.J. Biological control of *Sclerotinia minor* on lettuce using *Trichoderma* and *Coniothyrium* species. *Acta Hortic.* **2012**, *944*, 51–58. [CrossRef]
17. Silva, L.G.; Camargo, R.C.; Mascarin, G.M.; Nunes, P.S.O.; Dunlap, C.; Bettiol, W. Dual functionality of *Trichoderma*: Biocontrol of *Sclerotinia sclerotiorum* and biostimulant of cotton plants. *Front. Plant Sci.* **2022**, *13*, 983127. [CrossRef]
18. Topolovec-Pintarić, S. *Trichoderma*: Invisible partner for visible impact in agriculture. In *Trichoderma: The Most Widely Used Fungicide*, 1st ed.; Shah, M.M., Sharif, U., Buhari, T.R., Eds.; IntechOpen: London, UK, 2019; pp. 15–30.
19. Tyśkiewicz, R.; Nowak, A.; Ozimek, E.; Jaroszuk-Sciseł, J. *Trichoderma*: The Current Status of Its Application in Agriculture for the Biocontrol of Fungal Phytopathogens and Stimulation of Plant Growth. *Int. J. Mol. Sci.* **2022**, *23*, 2329. [CrossRef] [PubMed]

20. Kubiak, A.; Wolna-Maruwka, A.; Pilarska, A.A.; Niewiadomska, A.; Piotrowska-Cyplik, A. Fungi of the *Trichoderma* Genus: Future Perspectives of Benefits in Sustainable Agriculture. *Appl. Sci.* **2023**, *13*, 6434. [CrossRef]
21. Rai, S.; Kashyap, P.L.; Kumar, S.; Srivastava, A.K.; Ramteke, P.W. Identification, characterization and phylogenetic analysis of antifungal *Trichoderma* from tomato rhizosphere. *SpringerPlus* **2016**, *5*, 1939. [CrossRef] [PubMed]
22. Haddad, P.E.; Leite, L.G.; Lucon, C.M.M.; Harakava, R. Selection of *Trichoderma* spp. strains for the control of *Sclerotinia sclerotiorum* in soybean. *Pesqui. Agropecuária Bras.* **2017**, *52*, 1140–1148. [CrossRef]
23. Sumida, C.H.; Daniel, J.F.S.; Araujod, A.P.C.S.; Peitl, D.C.; Abreu, L.M.; Dekker, R.F.H.; Canteri, M.G. *Trichoderma asperelloides* antagonism to nine *Sclerotinia sclerotiorum* strains and biological control of white mold disease in soybean plants. *Biocontrol Sci. Technol.* **2018**, *28*, 142–156. [CrossRef]
24. Samuels, G.J.; Hebbar, P.K. *Trichoderma: Identification and Agricultural Applications*, 1st ed.; The American Phytopathological Society: St. Paul, MN, USA, 2015; 190p.
25. Cai, F.; Druzhinina, I.S. In honor of John Bissett: Authoritative guidelines on molecular identification of *Trichoderma*. *Fungal Divers.* **2021**, *107*, 1–69. [CrossRef]
26. Holst-Jensen, A.; Kohn, L.; Jakobsen, K.; Schumacher, T. Molecular phylogeny and evolution of *Monilinia* (*Sclerotiniaceae*) based on coding and noncoding rDNA sequences. *Am. J. Bot.* **1997**, *84*, 686–701. [CrossRef]
27. Jaklitsch, W.M.; Põldmaa, K.; Samuels, G.J. Reconsideration of *Protocrea* (*Hypocreales, Hypocreaceae*). *Mycologia* **2008**, *10*, 962–984. [CrossRef]
28. Holst-Jensen, A.; Vaage, M.; Schumacher, T. An approximation to the phylogeny of *Sclerotinia* and related genera. *Nord. J. Bot.* **1998**, *18*, 705–719. [CrossRef]
29. Deng, D.; Sun, S.L.; Du, C.Z.; Xiang, C.; Long, J.C.; Chen, W.; Zhu, Z. Three *Sclerotinia* species as the cause of white mold on pea in Chongqing and Sichuan of China. *J. Integr. Agric.* **2021**, *20*, 2957–2965. [CrossRef]
30. Garfinkel, A.R.; Chastagner, G.A. Survey Reveals a Broad Range of Fungal Pathogens and an Oomycete on Peonies in the United States. *Plant Health Prog.* **2019**, *20*, 250–254. [CrossRef]
31. Islam, M.R.; Prova, A.; Akanda, A.M.; Hossain, M.M. First report of white mould caused by *Sclerotinia sclerotiorum* on pea in Bangladesh. *J. Plant Pathol.* **2020**, *102*, 941. [CrossRef]
32. Hu, J.; Handique, U.; Norton, E.R. First Report of *Sclerotinia* Boll Rot and Stem Blight of Cotton in Arizona. *Plant Dis.* **2018**, *102*, 1663. [CrossRef]
33. Venkatesan, R.M.; Muthusamy, K.; Iruthayasamy, J.; Prithiviraj, B.; Kumaresan, P.V.; Lakshmanan, P.; Perianadar, I.V. First Report of *Clonostachys rosea* as a Mycoparasite on *Sclerotinia sclerotiorum* Causing Head Rot of Cabbage in India. *Plants* **2023**, *12*, 199. [CrossRef]
34. Bezuidenhout, S. The Fungal Diversity and Mycotoxins Associated with South African Soybeans. Master's Thesis, University of Pretoria, Pretoria, South Africa, 2023. Available online: https://hdl.handle.net/2263/91324 (accessed on 8 January 2025).
35. Degenkolb, T.; Dieckmann, R.; Nielsen, K.; Gräfenhan, T.; Theis, C.; Zafari, D.; Chaverri, P.; Ismaiel, A.; Brueckner, H.; Döhren, H.; et al. The *Trichoderma brevicompactum* clade: A separate lineage with new species, new peptabiotics, and mycotoxins. *Mycol. Progress.* **2008**, *7*, 177–219. [CrossRef]
36. Samuels, G.J.; Dodd, S.L.; Lu, B.S.; Petrini, O.; Schroers, H.J.; Druzhinina, I.S. The *Trichoderma koningii* aggregate species. *Stud. Mycol.* **2006**, *56*, 67–133. [CrossRef]
37. Jaklitsch, W.; Voglmayr, H. Biodiversity of *Trichoderma* (*Hypocreaceae*) in Southern Europe and Macaronesia. *Stud. Mycol.* **2015**, *80*, 1–87. [CrossRef]
38. Inglis, P.W.; Mello, S.C.M.; Martins, I.; Silva, J.B.T.; Macêdo, K.; Sifuentes, D.N.; Valadares-Inglis, M.C. *Trichoderma* from Brazilian garlic and onion crop soils and description of two new species: *Trichoderma azevedoi* and *Trichoderma peberdyi*. *PLoS ONE* **2020**, *15*, e0228485. [CrossRef] [PubMed]
39. Hanada, R.E.; de Jorge, S.T.; Pomella, A.W.V.; Hebbar, K.P.; Pereira, J.O.; Ismaiel, A.; Samuels, G.J. *Trichoderma martiale* sp. nov., a new endophyte from sapwood of *Theobroma cacao* with a potential for biological control. *Mycol. Res.* **2008**, *112*, 1335–1343. [CrossRef]
40. Samuels, G.J.; Suarez, C.; Solis, K.; Holmes, K.A.; Thomas, S.E.; Ismaiel, A.; Evans, H.C. *Trichoderma theobromicola* and *T. paucisporum*: Two new species isolated from cacao in South America. *Mycol Res.* **2006**, *110 Pt 4*, 381–392. [CrossRef] [PubMed]
41. Jaklitsch, W.M.; Samuels, G.J.; Ismaiel, A.; Voglmayr, H. Disentangling the *Trichoderma viridescens* complex. *Persoonia.* **2013**, *31*, 112–146. [CrossRef] [PubMed]
42. Zhu, Z.X.; Zhuang, W.Y. Three new species of *Trichoderma* with hyaline ascospores from China. *Mycologia* **2015**, *107*, 328–345. [CrossRef]
43. Jaklitsch, W.M.; Stadler, M.; Voglmayr, H. Blue pigment in *Hypocrea caerulescens* sp. nov. and two additional new species in sect. *Trichoderma*. *Mycologia* **2012**, *104*, 925–941. [CrossRef] [PubMed]

44. Smith, A.; Beltrán, C.; Kusunoki, M.; Cotes, A.M.; Kondo, T.; Deguchi, M. Diversity of soil-dwelling *Trichoderma* in Colombia and their potential as biocontrol agents against the phytopathogenic fungus *Sclerotinia sclerotiorum* (Lib.) de Bary. *J. Gen. Plant Pathol.* **2013**, *79*, 74–85. [CrossRef]
45. Montoya, Q.V.; Meirelles, L.A.; Chaverri, P.; Rodrigues, A. Unraveling *Trichoderma* species in the attine ant environment: Description of three new taxa. *Antonie Van Leeuwenhoek* **2016**, *109*, 633–651. [CrossRef] [PubMed]
46. An, X.Y.; Cheng, G.H.; Gao, H.X.; Li, X.F.; Yang, Y.; Li, D.; Li, Y. Phylogenetic Analysis of *Trichoderma* Species Associated with Green Mold Disease on Mushrooms and Two New Pathogens on *Ganoderma sichuanense*. *J. Fungi* **2022**, *8*, 704. [CrossRef]
47. Ding, M.Y.; Chen, W.; Ma, X.C.; Lv, B.W.; Jiang, S.Q.; Yu, Y.N.; Rahimi, M.J.; Gao, R.W.; Zhao, Z.; Cai, F.; et al. Emerging salt marshes as a source of *Trichoderma arenarium* sp. nov. and other fungal bioeffectors for biosaline agriculture. *J. Appl. Microbiol.* **2021**, *130*, 179–195. [CrossRef]
48. Jaklitsch, W. European species of *Hypocrea* Part 1. The green-spored species. *Stud. Mycol.* **2009**, *63*, 1–91. [CrossRef]
49. Katoh, K.; Misawa, K.; Kuma, K.; Miyata, T. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **2002**, *30*, 3059–3066. [CrossRef]
50. Katoh, K.; Standley, D.M. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780. [CrossRef]
51. Nguyen, L.-T.; Schmidt, H.A.; Von Haeseler, A.; Minh, B.Q. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274. [CrossRef] [PubMed]
52. Trifinopoulos, J.; Nguyen, L.-T.; von Haeseler, A.; Minh, B.Q. W-IQ-TREE: A fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Res.* **2016**, *44*, W232–W235. [CrossRef] [PubMed]
53. Porras, M.; Barrau, C.; Santos, B.; Arroyo, F.T.; Blanco, C.; Romero, F. Effects of temperature on in vitro response of *Trichoderma* strains against strawberry pathogen *Rhizoctonia solani* Kühn. *Plant Prot. Sciens.* **2002**, *38*, 620–622. [CrossRef]
54. Dennis, C.; Webster, J. Antagonistic properties of species groups of *Trichoderma* III, hyphae interaction. *Trans. Br. Mycol. Soc.* **1971**, *57*, 363–369. [CrossRef]
55. Royse, D.J.; Ries, S.M. The influence of fungi isolated from peach twigs on the pathogenicity of *Cytospora cincta*. *Phytopathology* **1978**, *68*, 603–607. [CrossRef]
56. Kohn, L.M. A monographic revision of the genus *Sclerotinia*. *Mycotaxon* **1979**, *9*, 365–444.
57. Clarkson, J.; Phelps, K.; Whipps, J.M.; Young, C.S.; Smith, J.A.; Watling, M. Forecasting *Sclerotinia* disease on lettuce: Toward developing a prediction model for carpogenic germination of sclerotia. *Phytopathology* **2004**, *94*, 268–279. [CrossRef] [PubMed]
58. McLean, K.; Braithwaite, M.; Swaminathan, J.; Stewart, A. Variability in control of onion white rot by *Trichoderma atroviride* under different disease pressures. *Australas. Plant Pathol.* **2012**, *41*, 341–346. [CrossRef]
59. Shaw, S.; Le Cocq, K.; Paszkiewicz, K.; Moore, K.; Winsbury, R.; Salmon, D.; Thornton, C.R.; Grant, M.; Studholme, D.J.; Zabala, T.; et al. Transcriptional reprogramming underpins enhanced plant growth promotion by the biocontrol fungus *Trichoderma hamatum* GD12 during antagonistic interactions with *Sclerotinia sclerotiorum* in soil. *Mol. Plant Pathol.* **2016**, *17*, 1425–1441. [CrossRef] [PubMed]
60. Troian, R.F.; Steindorff, A.S.; Ramada, M.H.S.; Arruda, W.; Ulhoa, C.J. Mycoparasitism studies of *Trichoderma harzianum* against *Sclerotinia* sclerotiorum: Evaluation of antagonism and expression of cell wall-degrading enzymes genes. *Biotechnol. Lett.* **2014**, *36*, 2095–2101. [CrossRef]
61. Lahlali, R.; Ezrari, S.; Radouane, N.; Kenfaoui, J.; Esmaeel, Q.; El Hamss, H.; Belabess, Z.; Barka, E.A. Biological Control of Plant Pathogens: A Global Perspective. *Microorganisms* **2022**, *10*, 596. [CrossRef]
62. Andreoli, Y.E.; Laich, F.S.; Navarro, C.A. Control in vitro de *Sclerotinia sclerotiorum* y *Gaeumannomyces graminis* por bacterias del grupo *Pseudomonas fluorescentes*. *Rev. Argent. Microbiol.* **1993**, *25*, 70–79. [PubMed]
63. Ibarra-Medina, V.A. Isolation and screening of *Trichoderma* strain antagonistic to *Sclerotinia*. *Rev. Mex. Micol.* **2010**, *31*, 53–63.
64. Da Silva, G.B.P.; Heckler, L.I.; Durigon, M.; dos Santos, R.F.; Lovato, M.; Finger, G.; Blume, E. Biological control of white mold (*Sclerotinia sclerotiorum*) in lettuce using Brazilian *Trichoderma* spp. strains. *Aust. J. Crop Sci.* **2019**, *13*, 803–809. [CrossRef]
65. Smolinska, U.; Kowalska, B.; Kowalczyk, W.; Szczech, M.; Murgrabia, A. Eradication of *Sclerotinia sclerotiorum* sclerotia from soil using organic waste materials as *Trichoderma* fungi carriers. *J. Hortic. Res.* **2016**, *24*, 101–110. [CrossRef]
66. Harman, G.E. Overview of mechanisms and uses of *Trichoderma* spp. *Phytopythology* **2006**, *96*, 190–194. [CrossRef] [PubMed]
67. Bader, A.N.; Salerno, G.L.; Covacevich, F.; Consolo, V.F. Native *Trichoderma harzianum* strains from Argentina produce indole-3 acetic acid and phosphorus solubilization, promote growth and control wilt disease on tomato (*Solanum lycopersicum* L.). *J. King Saud Univ. Sci.* **2020**, *32*, 867–873. [CrossRef]
68. Han, V.-C.; Michael, P.J.; Swift, B.; Bennett, S.J. Biological control of *Sclerotinia sclerotiorum*: Modes of action of biocontrol agents, soil organic amendments, and soil microbiome manipulation. *Biol. Control* **2023**, *186*, 105346. Available online: https://www.sciencedirect.com/science/article/pii/S1049964423001998 (accessed on 10 December 2024). [CrossRef]
69. Budge, S.P.; Whipps, J.M. Greenhouse trials of *Coniothyrium minitans* and *Trichoderma* species for the biological control of *Sclerotinia sclerotiorum* in celery and lettuce. *Plant Pathol.* **1991**, *40*, 59–66. [CrossRef]

70. Knudsen, G.R.; Eschen, D.J.; Dandurand, L.M.; Bin, L. Potential for biocontrol of *Sclerotinia sclerotiorum* through colonization of sclerotia by *Trichoderma harzianum*. *Plant Dis.* **1991**, *75*, 466–470. [CrossRef]
71. Escande, A.R.; Laich, F.S.; Pedraza, M.V. Field testing of honeybee-dispersed *Trichoderma* spp. to manage sunflower head rot (*Sclerotinia sclerotiorum*). *Plant Pathol.* **2022**, *51*, 346–351. [CrossRef]
72. Pedrero-Méndez, A.; Insuasti, H.C.; Neagu, T.; Illescas, M.; Rubio, M.B.; Monte, E.; Hermosa, R. Why Is the Correct Selection of *Trichoderma* Strains Important? The Case of Wheat Endophytic Strains of *T. harzianum* and *T. simmonsii*. *J. Fungi* **2021**, *7*, 1087. [CrossRef] [PubMed]
73. Thambugala, K.M.; Daranagama, D.A.; Phillips, A.J.L.; Kannangara, S.D.; Promputtha, I. Fungi vs. fungi in biocontrol: An overview of fungal antagonists applied against fungal plant pathogens. *Front. Cell. Infect. Microbiol.* **2020**, *10*, 604923. [CrossRef] [PubMed]
74. Yao, X.; Guo, H.; Zhang, K.; Zhao, M.; Ruan, J.; Chen, J. *Trichoderma* and its role in biological control of plant fungal and nematode disease. *Front. Microbiol.* **2023**, *14*, 1160551. [CrossRef]
75. Chitrampalam, P.; Figuli, P.J.; Matheron, M.E.; Subbarao, K.V.; Pryor, B.M. Biocontrol of lettuce drop caused by *Sclerotinia sclerotiorum* and *S. minor* in desert agroecosystems. *Plant Dis.* **2008**, *92*, 1625–1634. [CrossRef] [PubMed]
76. Jiang, Y.; Wang, J.L.; Chen, J.; Mao, L.J.; Feng, X.X.; Zhang, C.H.; Lin, F.C. *Trichoderma* biodiversity of agricultural fields in east China reveals a gradient distribution of species. *PLoS ONE* **2016**, *11*, e0160613. [CrossRef]
77. Kredics, L.; Hatvani, L.; Naeimi, S.; Körmöczi, P.; Manczinger, L.; Vágvölgyi, C.; Druzhinina, I. Biodiversity of the Genus *Hypocrea/Trichoderma* in Different Habitats. In *Biotechnology and Biology of Trichoderma*, 1st ed.; Gupta, V., Schmoll, M., Herrera-Estrella, A., Upadhyay, R.S., Druzhinina, I., Tuohy, M., Eds.; Elsevier: Amsterdam, The Netherlands, 2014; pp. 3–24.
78. Liu, B.; Glenn, D.; Buckley, K. *Trichoderma* communities in soils from organic, sustainable, and conventional farms, and their relation with Southern blight of tomato. *Soil Biol. Biochem.* **2008**, *40*, 1124–1136. [CrossRef]
79. Jaklitsch, W.M. European species of *Hypocrea* part II: Species with hyaline ascospores. *Fungal Divers.* **2011**, *48*, 1–250. [CrossRef] [PubMed]
80. Pięta, D.; Pastucha, A.; Patkowska, E. Bacteria and fungi communities occurring in the soil after the cultivation of cereal and potato. *Electronic J. Pol. Agric. Univ.* **2000**, *3*, 1. Available online: http://www.ejpau.media.pl/volume3/issue2/horticulture/art-01.html (accessed on 4 December 2024).
81. Hagn, A.; Pritsch, K.; Schloter, M.; Munch, J.C. Fungal diversity in agricultural soil under different farming management systems, with special reference to biocontrol strains of *Trichoderma* spp. *Biol. Fertil. Soils* **2003**, *38*, 236–244. [CrossRef]
82. Gherbawy, Y.; Druzhinina, I.; Shaban, G.M.; Wuczkowsky, M.; Yaser, M.; El-Naghy, M.A.; Prillinger, H.-J.; Kubicek, P.C. *Trichoderma* populations from alkaline agricultural soil in the Nile valley, Egypt, consist of only two species. *Mycol. Prog.* **2004**, *3*, 211–218. [CrossRef]
83. Kredics, L.; Láday, M.; Körmöczi, P.; Manczinger, L.; Rákhely, G.; Vágvölgyi1, C.; Szekeres, A. Genetic and biochemical diversity among *Trichoderma* isolates in soil samples from winter wheat fields of the Pannonian Plain. *Acta Biol. Szeged.* **2012**, *56*, 141–149.
84. Ma, J.; Tsegaye, E.; Li, M.; Wu, B.; Jiang, X. Biodiversity of *Trichoderma* from grassland and forest ecosystems in Northern Xinjiang, China. *3 Biotech* **2020**, *10*, 362. [CrossRef]
85. Zachow, C.; Berg, C.; Müller, H.; Meincke, R.; Komon-Zelazowska, M.; Druzhinina, I.S.; Kubicek, C.P.; Berg, G. Fungal diversity in the rhizosphere of endemic plant species of Tenerife (Canary Islands): Relationship to vegetation zones and environmental factors. *ISME J.* **2009**, *3*, 79–92. [CrossRef]
86. Kubicek, C.P.; Bissett, J.; Druzhinina, I.; Kullnig-Gradinger, C.; Szakacs, G. Genetic and metabolic diversity of *Trichoderma*: A case study on Southeast Asian isolates. *Fungal Genet. Biol.* **2003**, *38*, 310–319. [CrossRef] [PubMed]
87. Migheli, Q.; Balmas, V.; Komoñ-Zelazowska, M.; Scherm, B.; Fiori, S.; Kopchinskiy, A.G.; Kubicek, C.P.; Druzhinina, I.S. Soils of a Mediterranean hot spot of biodiversity and endemism (Sardinia, Tyrrhenian Islands) are inhabited by pan-European, invasive species of *Hypocrea/Trichoderma*. *Environ. Microbiol.* **2009**, *11*, 35–46. [CrossRef]
88. Al-Sadi, A.M.; Al-Oweisi, F.A.; Edwards, S.G.; Al-Nadabi, H.; Al-Fahdi, A.M. Genetic analysis reveals diversity and genetic relationship among *Trichoderma* isolates from potting media, cultivated soil and uncultivated soil. *BMC Microbiol.* **2015**, *15*, 147. [CrossRef] [PubMed]
89. Huang, K.; Li, F.; Wei, Y.; Chen, X.; Fu, X. Changes of bacterial and fungal community compositions during vermicomposting of vegetable wastes by *Eisenia foetida*. *Bioresour Technol.* **2013**, *150*, 235–241. [CrossRef]
90. Kausar, H.; Ismail, M.R.; Saud, H.M.; Habib, S.H.; Othman, R.; Bhuiyan, M.S.H. Changes of physical and chemical characteristics during microbial composting of rice straw at various pH levels. *Compost. Sci. Util.* **2014**, *22*, 153–163. [CrossRef]
91. Bal, U.; Altinatas, S. Effects of *Trichoderma harzianum* on lettuce in protected cultivation. *J. Cent. Eur. Agric.* **2008**, *9*, 63–70.
92. Harman, G.E. *Trichoderma*—Not just for biocontrol anymore. *Phytoparasitica* **2011**, *39*, 103–108. [CrossRef]
93. Topolovec-Pintarić, S.; Žutić, I.; Lončarić, I. Enhancing plant growth by *Trichoderma vi*ride based pellets. *Növénytermelés* **2011**, *60*, 177–180.

94. Topolovec-Pintarić, S.; Žutić, I.; Đemić, E. Enhanced growth of cabbage and red beet by *Trichoderma viride*. *Acta Agric. Slov.* **2013**, *101*, 87–92. [CrossRef]
95. Marra, R.; Lombardi, N.; Piccolo, A.; Bazghaleh, N.; Prashar, P.; Vandenberg, A.; Woo, S. Mineral Biofortification and Growth Stimulation of Lentil Plants Inoculated with *Trichoderma* Strains and Metabolites. *Microorganisms* **2022**, *10*, 87. [CrossRef]
96. Contreras-Cornejo, H.M.; Schmoll, M.; Esquivel-Ayala, B.A.; González-Esquivel, C.E.; Rocha-Ramírez, V.; Larsen, J. Mechanisms for plant growth promotion activated by *Trichoderma* in natural and managed terrestrial ecosystems. *Microbiol. Res.* **2024**, *281*, 127621. [CrossRef] [PubMed]