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RESEARCH

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Loop-mediated isothermal amplification (LAMP) assay proved the mechanism of biological control against root rot pathogens

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Abstract

Background The soil-borne fungi, *Rhizoctonia solani* and *Sclerotium rolfsii,* are major pathogens of Brassicae crops. This study was performed to clarify the relationship between the accumulation pattern of the genus *Trichoderma* and disease suppression in frequently inoculated soils with binucleate *Rhizoctonia* (BNR), *Rhizoctonia solani* and *Sclerotium rolfsii*.

Results As compared to the control group, five *Trichoderma virens* strains isolated from soil inoculated with *R. solani* or BNR significantly reduced the severity of *S. rolfsii* (85.6–100% covering percentage) and *R. solani* (95.7–100% covering percentage). Similarly, five *T. hamatum* strains obtained from soil inoculated with *R. solani* were shown to be highly suppressive against *S. rolfsii* (83.9–97.1% covering percentages) and *R. solani* (60.2–96.2% covering percentages). Four out of five *T. hamatum* strains obtained from soil infected with *S. rolfsii* exhibited considerable suppression against *S. rolfsii* (63.7–91.2% covering percentages), while the SM5 strain did not. The phylogenetic analysis of the TEF and ITS regions of *Trichoderma hamatum* revealed that most isolates were classified into the same cluster with homology of 99–100%. Five strains of each *T. virens* and *T. hamatum* were isolated from the suppressive soil with high antagonistic potentials against *R. solani* and *S. rolfsii.* Suppression and antagonistic activity of *T. hamatum* isolated from soil frequently inoculated with sterile barley grains were negligible, whereas *T. hamatum* isolated from frequently inoculated soil with BNR and *R. solani* demonstrated considerable suppression of the pathogens and antagonistic activity. Accumulation and quantification of *T. virens* and *T. hamatum* were confirmed using loop-mediated isothermal amplification (LAMP).

Conclusion In conclusion, disease suppression in frequently inoculated soil with BNR, *R. solani* and *S. rolfsii* was due to *Trichoderma* spp. accumulated selectively in each replicate of soil inoculation.

Keywords *Trichoderma virens*, *T. hamatum*, Soil environment, Binucleate *Rhizoctonia*, *Rhizoctonia solani*, *Sclerotium rolfsii*

Background

The necrotrophic devastating plant pathogen *Rhizoctonia solani* Khün has a practically widespread host range (Elsharkawy et al. 2022). Different symptoms, including damping-off in seedlings, crown, root, and stem rots, and foliar and sheath blights, are caused by this soil-borne fungus (Elsharkawy et al. 2022). *R. solani* and *Sclerotium rolfsii* Sacc. may remain dormant for years in the soil as sclerotia or mycelia inside infected plant material, even when circumstances are unfavorable. It is very rare for

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R. solani (Basidiomycota, Teleomorph; sexual stage) to develop sexual spores in nature, and the fungus never produces asexual spores.

It is difficult to reduce the incidence of soil-borne pathogens in cereal crops owing to their inherent persistence in soils and the ineffectiveness of chemical treatments (Elsharkawy et al. 2022). Consequently, biological control becomes an extremely promising approach for avoiding soil diseases. The type of soil which prevents diseases from spreading is called "disease decline soil". The addition of a new pathogen inoculum to the soil has no effect on disease development (Durán et al. 2017). Various studies have established that native soil microbial activity may play a crucial role in the suppression of *Gaeumannomyces graminis* var. *tritici* (Ggt) disease (Mendes et al. 2013). Naturally occurring "suppressive" soils restrict soil-borne pathogen establishment and reduce disease incidence (Jara et al. 2011). Suppressive soils for Ggt (and other soil-borne pathogens) have been identified and described globally (Bithell et al. 2012). Therefore, this process has the potential to be exploited as a biological control method. Ggt soil inoculation inhibited not only the disease caused by Ggt but also other wheat diseases caused by pathogenic fungi *Phialophora* sp. (Wildermuth 1982). Although *Trichoderma* is commonly regarded as a group of soil-borne fungi, there is certain evidence to show that some species may be opportunistic, avirulent plant symbionts and even parasites of other fungi (Samuels 2006). The identification methods of *Trichoderma* spp. by phylogenetic analysis using TEF and ITS regions were approved, especially with *T. hamatum* Bonord. (Samuels 2006). Although certain species of *Trichoderma*, such as *T. harzianum* Rifai, are found all over the world, others, such as *T. viride* Pers., are more strictly limited in their distribution.

Due to the lack of asexual spore formation by *S. rolfsii* and *R. solani*, the soil dilution plate method cannot be used for quantitative analysis. Consequently, the DNA fragment amplification technique distinct from polymerase chain reaction (PCR) loop-mediated isothermal amplification with PCR (LAMP) technique was developed to use in the detection of *S. rolfsii* and *R. solani*. Since its development and widespread usage in the early 2000s, the LAMP technique has been widely regarded as a simple, quick, and effective way to amplify DNA sequences with high sensitivity and specificity while requiring just a single temperature (Notomi et al. 2000). This method has been used to identify numerous plant-pathogenic fungi, such as *Didymella bryoniae* and *Colletotrichum truncatum*, since it is less sensitive to inhibitors than normal PCR (Tian et al. 2017). The LAMP test amplifies particular DNA sequences with great specificity using 4–6 oligonucleotide primers (Wastling et al. 2000). The LAMP reaction yields a significant amount of amplified product and by-product (magnesium pyrophosphate), which enables efficient identification of target DNA based on visual evaluation of turbidity or a color change that occurs after the administration of color-changing reagents (Ogura 2009). LAMP products may also be seen using agarose gel electrophoresis (Ma et al. 2010).

The species of *Trichoderma* that could be accumulated in the soil surface differs depending on the kind of filamentous fungus used for frequent inoculations. Disease suppression of *R. solani* and *S. rolfsii* and accumulation of *Trichoderma* spp. were confirmed with polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) analysis (Elsharkawy et al. 2022). The amount of *R. solani* and *S. rolfsii* may be decreased if *Trichoderma* spp. is responsible for preventing infection in the soil. Therefore, determining the frequency of soil pathogens and *Trichoderma* spp. is important. The accurate identification of *Trichoderma* isolates is necessary. Additionally, the antagonistic activity of the isolated *Trichoderma* should be investigated. Therefore, the aims of the present study are to explain the relationship between the accumulation pattern of the genus *Trichoderma* and the suppression of frequent inoculation of soil using LAMP technique. Additionally, the antagonistic effects of *Trichoderma* spp. isolated from the frequent inoculation of each soil fungus were evaluated against *R. solani* and *S. rolfsii.*

Methods

Source of the pathogens and *Trichoderma* isolates

Rhizoctonia solani AG1-IC RH28, *Sclerotium rolfsii* SR0205, and binucleate *Rhizoctonia* AG-A W1, which were isolated from the soil of sugar beet plants were kindly provided by Gifu University, Japan. The pathogen was isolated and identified following the methods of Villajuan-Abgona et al. (1993). *R. solani*, BNR, and *S. rolfsii* were frequently inoculated into the soil. *Trichoderma* species were isolated and identified as explained by Elad et al. (1981). Strains of *T. virens,* isolated from the frequent inoculation of soil with BNR (BV1, BV2, BV3, BV4 and BV5) and *R. solani* (RV1, RV2, RV3, RV4 and RV5), were utilized in the experiments. *T. hamatum* strains were isolated from soil frequently inoculated three times with BNR (BM1, BM2, BM3, BM4 and BM5), sterile barley grain (CM1, CM2, CM3, CM4 and CM5), *R. solani* (RM1, RM2, RM3, RM4 and RM5), and *S. rolfsii* (SM1, SM2, SM3, SM4, SM5).

Sterilized barley grains were used to grow the tested pathogens and *Trichoderma* spp. The test fungi were cultured for 3 days on PDA plates, and then, 10 disks (5 mm in diameter) from the cultures were transferred to 50 g

sterile barley grains in the 200-ml Erlenmeyer flask and incubated for 2 weeks at 25 °C. During the incubation period, the flasks were hand shaken every day in order to spread the mycelia evenly in barley grains. After air drying for one week at room temperature, barley grain inocula (BGI), stored at − 20 °C., BGI were ground just before using as a source of inoculum. The soil was subsequently mixed with the homogenized BGI at a concentration of 0.5% (w/w).

Effect of *Trichoderma* virens and *T. hamatum* antagonists on *R. solani* and *S. rolfsii* in vitro

The dual culture method was used to evaluate the antagonistic activity of *Trichoderma* isolates against pathogens. Petri dishes (diameter 9 cm) containing SMS medium (SMS: KH_2PO_4 (680 mg), K_2HPO_4 (870 mg), KCl (200 mg), NH_4NO_3 (1 g), $CaCl_2$ (200 mg), $MgSO_4 \cdot 7H_2O$ (200 mg), $FeSO_4$ (2 mg), $MnSO_4$ (2 mg), $ZnSO_4$ (2 mg) and 10 g of sucrose) were inoculated with 5-mm disks of the pathogenic fungi (cultures that had been grown for one week), and disks of the isolated *T. virens* and *T. hamatum* strains were added on the other side (7 cm between both disks). The level of antagonism was assessed (El-kazzaz et al. 2022).

Suppression of radish root rot by *Trichoderma* hamatum

Suppression of *S. rolfsii* and *R. solani* by different strains of *T. hamatum* isolated from each replicate of soil inoculation was examined. The experiment was performed using a mixture of 1:1 (w/w) sandy loam soil with barley grain of each strain of *Trichoderma*, and the pathogens (0.5% w/w) were packed in plastic pots (diameter 6 cm, 8 cm depth). The soil was mixed with sterilized barley grains and the pathogenic fungi in the control group. Each pot was planted with ten radish (*Raphanus sativus* L. cultivar Akamaru hatsuka daikon) seeds. At the temperature range of 23–27 °C and 12-h photoperiod, pots were incubated in the greenhouse with fluorescent lights operating at 250–300 μE m^{-2} s^{-1}. After two weeks, the seedlings were assessed after being irrigated to maintain moisture. The experiment was performed three times, and the severity of the disease was evaluated using the following scale: 0: no symptoms (healthy seedlings); 1: < 50% of the hypocotyls covered with brown lesions; 2: > 50% of the hypocotyls covered with dark brown lesions; 3: seedlings dead after germination; 4: no germination.

Phylogenetic analysis of *Trichoderma hamatum* using the TEF and ITS regions

Trichoderma hamatum isolated strains from frequently inoculated soil were analyzed by nucleotide sequencing of the TEF and ITS regions. DNA extraction from fungal isolates and PCR were done, as explained by Elsharkawy et al. (2022). The primers used for ITS region were designed (ITS1: 5′-tccgtaggtgaacctgcgc-3′, ITS4: 5′-tcctccgcttattgatatgc-3′). Similarly, TEF region-specific primers were designed (TEF1-728: 5'-catcgagaagttcgagaagg-3′, TEF1 rev: 5'-gccatccttggagataccag-3'). The amplification product was purified using GeneEluteTM PCR Clean-UP Kit (SIGMA-ALDRICH Inc., Tokyo, Japan). The reaction sequence was carried out according to the recommended protocol by using BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Tokyo, Japan). Genetic Analyzer PRISM3100 and ClustulW were used to analyze the nucleotide sequence and draw the phylogenetic tree.

LAMP test specificity, validation, and sensitivity

DNA was extracted from the isolates *R. solani*, BNR, *S. rolfsii T. hamatum, T. virens* and *T. harzianum* (Elsharkawy et al. 2022). The DNA solution was adjusted to 10 ng/μl concentration using spectrophotometer U1800 (Hitachi High-Technologies Corporation, Tokyo, Japan) and was used in the LAMP reaction as a template.

LAMP primers were designed to detect *R. solani* species complex, *S. rolfsii* and *Trichoderma* spp. using PrimerExplolarV3 (http://primerexplorer.jp/lamp3.0.0/index.html). The designed LAMP primers are shown in Table (S1). The composition of the LAMP reaction was prepared as explained by Notomi et al. (2000); 0.2 μM from each of F3 and B3 primers, 1.6 μM from each of FIP and BIP primers, 0.8 μM from each of FLoop primer and BLoop primer, 1.4 mM dNTPs, 0.8 mM $MgSO_4$, 10 mM $(NH4)\ 2SO_4$, 0.1% (v/v) Tween20, 10 mM KCL, 20 mM Tris–HCL (pH8.8), 8 U Bst DNA polymerase, were used to obtain a total volume of 25 μl containing 10 ng template DNA. DNA as a control was used to verify the non-specific reaction (blank) without a reaction solution. The reaction temperature was 60 °C, and reaction time was 60 min. Turbidity of the reaction solution was detected using turbidity measuring instrument RT160C (Eiken Chemical Co., Ltd., Tokyo, Japan). The experiment was repeated in triplicate.

S. rolfsii and *R. solani* cultures grown on PDA at 25 °C for 30 days were utilized. Sclerotia formed on the surface of the culture were scraped with a sterile cotton swab, rapidly frozen in liquid nitrogen, and ground in a mortar and pestle. To prepare a fungal concentration of 100 mg/g, 5 g of ground sclerotia were mixed with 45 g soil (sand: loam, 1:1 w/w). Several dilutions (100 mg/g, 10 mg/g, 1 mg/g, 0.1 mg/g and 0.01 mg/g) from sclerotia with soil were prepared. DNA was extracted and purified from each sclerotia and soil mixture (El-Kazzaz et al. 2022). LAMP reaction was performed under the same conditions described previously. The calibration curve

was prepared in the amount of fungi by using the average time to reach 0.04 turbidity. DNA extracted from the sclerotia-free soil was used as a control to verify the specificity of the reaction.

Trichoderma virens and *T. hamatum* were grown on PDA medium and incubated for 30 days at 25 °C. The mycelia were collected by suspending them in sterile distilled water (SDW) with sterile cotton swabs, followed by double Kimwipe filtration (Sigma-Aldrich, Tokyo, Japan). Spore suspensions were prepared in different concentrations (10^8 spores/ml, 10^7 spores/ml, 10^6 spores/ml, 10^5 spores/ml and 10^4 spores / ml) using a hemocytometer. DNA was extracted using the Fast DNA Spin Kit (Qbiogene Inc., CA, USA) and 40 µl from each spore suspension according to the recommended protocol. Calibration curve and LAMP reaction were done as described previously. Control treatment was used to confirm specificity.

Quantities of *S. rolfsii, T. virens, T. hamatum* and *R. solani* in the soils frequently inoculated for 1, 2, 3, and 4 times were measured at 0, 7, 14, 21 days after inoculation (Elsharkawy et al. 2022). Five grams of soil was obtained, and DNA extraction and Lamp conditions were performed as explained previously. The experiment was repeated in triplicate.

Statistical analysis

The analysis of variance (ANOVA) was applied to the data. The experiments were repeated at least thrice, and treatment means were separated by Steel Dwass test using Excel "Tokei" for Windows (Social Survey Research Information Co., Ltd., Tokyo, Japan). The following formula was used to calculate disease severity (DS) (Elsharkawy et al. 2022):

$$\text{DS value} = [(0 \times N0) + (1 \times N1) + (2 \times N2) + (3 \times N3) + (4 \times N4)]/N$$

where N represents the total number of seeds planted and $N0$–$N4$ represents the average number of seedlings in groups 0–4, respectively.

The percentage of covering was determined as explained by Xue et al. (2021) using the following formula:

$$\text{PA}(\%) = [(C - T)/C] \times 100,$$

where C represents radial growth of control mycelial and T represents radial growth of the treatment mycelial.

The antagonistic activity was measured based on the scale 0–3 (El-Kazzaz et al. 2022), where 0 represents the pathogen completely overgrow *Trichoderma* and occupied the entire medium surface, on the other hand, 3 represents *Trichoderma* completely overgrow the pathogen and covered the entire medium surface.

Results

Antagonistic activity of the isolated *T. virens* from frequently inoculated soil against *S. rolfsii* and *R. solani*

Five strains of *T. virens*, isolated from soil, which were frequently inoculated three times with *R. solani,* exhibited 95.7–100% covering percentage against *R. solani*, while the covering percentage against *S. rolfsii* was 85.6–100%. On the other hand, the five isolates of *T. virens,*

Table 1 The antagonistic activity of *Trichoderma virens* isolated from soil frequently inoculated three times with non-pathogenic binucleate *Rhizoctonia*, *Rhizoctonia solani*, and *Sclerotium rolfsii*

Strain name	Isolation source	Covering percentage (%) *Rhizoctonia solani*	Antagonistic activity	Covering percentage (%) *Sclerotium rolfsii*	Antagonistic activity
Control		0.0	0	0.0	0
RV1[1)]	*R. solani*	100	3	100	3
RV2	*R. solani*	100	3	100	3
RV3	*R. solani*	95.7	3	85.6	3
RV4	*R. solani*	100	3	100	3
RV5	*R. solani*	100	3	100	3
Control		0.0	0	0.0	0
BV1	BNR	100	3	100	3
BV2	BNR	100	3	100	3
BV3	BNR	100	3	100	3
BV4	BNR	100	3	100	3
BV5	BNR	100	3	100	3

[1)] *T. virens* isolated from soil inoculated repeatedly three times with *R. solani* and BNR was the RV1 ~ 5, BV1 ~ 5, respectively

which were isolated from soil frequently inoculated with BNR, exhibited a covering percentage of 100% against both pathogens (Table 1).

Antagonistic activity of the isolated *T. hamatum* from repeatedly inoculated soil against *S. rolfsii* and *R. solani*

Trichoderma hamatum, isolated from sterile barley grain, did not show antagonistic activity against both pathogens. While, *T. hamatum* strains (isolated from soil inoculated frequently three times with *R. solani*) exhibited 60.2–96.2% covering percentages of *R. solani*, and 83.9–97.1% covering percentages of *S. rolfsii*. The antagonistic values of RM1, RM3, RM4 and RM5 strains were 3 against *R. solani*, while it was 2 using the strain RM2. The antagonistic values of RM1-RM5 strains against *S. rolfsii* were 3 (Table 2). *T. hamatum* SM1-SM4 strains (isolated from *S. rolfsii* inoculated soil) resulted in covering percentages ranging from 63.7 to 91.2% against *S. rolfsii,* while it was 11.2%, using the strain SM5. On the other hand, SM1-SM5 strains resulted in covering percentages against *R. solani* ranged from 0.0 to 4.9%. SM1–SM5 strains did not exhibit antagonistic activity against *R. solani* (0), but they showed significant antagonistic against *S. rolfsii* (3), except for SM5 (0). The strains BM1–BM4, isolated from BNR inoculated soil, exhibited covering percentages of 100% against *R. solani* and *S. rolfsii,* while the covering percentages of the strain BM5 were 10.9 and 9.3% against *R. solani* and *S. rolfsii*, respectively (Table 2).

Suppression of radish root rot disease by *T. hamatum* and *T. virens* isolated from soil inoculated three times by *R. solani*, BNR and *S. rolfsii*

Five strains from *T. virens* isolated from *R. solani* inoculated soil showed significant suppression against *S. rolfsii* and *R. solani* compared to control (Table 3). The strain RV4 was the most effective, recording disease severity of 0.27 against *R. solani* compared with the control (3.5), while the strain RV2 was the most effective against *S. rolfsii*

Table 2 Antagonistic activity of *Trichoderma hamatum* isolated from soil frequently inoculated three times with non-pathogenic binucleate *Rhizoctonia*, *Rhizoctonia solani*, and *Sclerotium rolfsii*

Strain name	Isolation source	Covering ratio (%)	Antagonistic activity	Covering ratio (%)	Antagonistic activity
		Rhizoctonia solani		*Sclerotium rolfsii*	
Control		0.0	0	0.0	0
CM1[1)]	Sterilized barley	0.0	0	0.0	0
CM2	Sterilized barley	0.0	0	0.0	0
CM3	Sterilized barley	0.0	0	0.0	0
CM4	Sterilized barley	0.0	0	0.0	0
CM5	Sterilized barley	0.0	0	0.0	0
Control		0.0	0	0.0	0
RM1	*Rhizoctonia solani*	96.2	3	97.1	3
RM2	*R. solani*	60.2	2	93.9	3
RM3	*R. solani*	85.3	3	85.6	3
RM4	*R. solani*	86.2	3	83.9	3
RM5	*R. solani*	82.0	3	84.0	3
Control		0.0	0	0.0	0
SM1	*Sclerotium rolfsii*	4.9	0	91.2	3
SM2	*S. rolfsii*	4.2	0	63.7	3
SM3	*S. rolfsii*	0.0	0	84.7	3
SM4	*S. rolfsii*	0.0	0	81.5	3
SM5	*S. rolfsii*	0.0	0	11.2	0
Control		0.0	0	0.0	0
BM1	BNR	100	3	100	3
BM2	BNR	100	3	100	3
BM3	BNR	100	3	100	3
BM4	BNR	100	3	100	3
BM5	BNR	10.9	0	9.3	0

[1)] *T. hamatum* isolated from soil inoculated repeatedly three times with *R. solani, S. rolfsii*, BNR and sterile barley grain was the CM1 ~ 5, RM1 ~ 5, SM1 ~ 5, BM1 ~ 5 5, respectively

Table 3 Suppression of radish root rot disease by *Trichoderma virens* isolated from soil inoculated three times with the non-pathogenic binucleate *Rhizoctonia*, *Sclerotium rolfsii* and *Rhizoctonia solani*

Strain name	Isolation sources	Disease severity	
		Rhizoctonia solani	*Sclerotium rolfsii*
Control		3.50a*	3.67a
RV1[1)]	*Rhizoctonia solani*	0.68b	0.42c
RV2	*R. solani*	0.62b	0.33c
RV3	*R. solani*	0.64b	0.37c
RV4	*R. solani*	0.27c	0.50b
RV5	*R. solani*	0.57b	0.56b
Control		3.48a	3.65a
BV1	BNR	0.60b	0.30b
BV2	BNR	0.42c	0.34b
BV3	BNR	0.45c	0.18b
BV4	BNR	0.45c	0.21b
BV5	BNR	0.42c	0.27b

[1)] *T. virens* strains isolated from soil inoculated frequently three times with *R. solani* and BNR (RV1 ~ 5 and BV1 ~ 5, respectively)

* Same letters refer to insignificant differences and the different letters indicate significant differences at $P \leq 0.01$

recording 0.33 compared with the control (3.67). Similarly, five strains of *T. virens* isolated from BNR inoculated soil showed remarkable suppression against *S. rolfsii* and *R. solani* (Table 3). The strains BV2 and BV5 exhibited the best effect against *R. solani* recording 0.42 compared to the control (3.48), while the strain BV3 was the best against *S. rolfsii* recording 0.18 compared to the control (3.65).

Strains of *T. hamatum* isolated from frequent inoculation soil showed suppression against *S. rolfsii* and *R. solani*. Five strains of *T. hamatum* isolated from *R. solani* inoculated soil exhibited significant suppression against *S. rolfsii* and *R. solani* (Table 4). The strain RM5 showed the best effect recording the severity of 0.31 against *R. solani* compared to the control (3.74), while the strain RM2 resulted in 0.36 severity against *S. rolfsii* compared to the control (3.80). Among *T. hamatum* strains isolated from *S. rolfsii* inoculated soil, four strains showed significant suppression against *S. rolfsii*, but SM5 strain did not show suppression of the disease. On the other hand, all *T. hamatum* strains isolated from *S. rolfsii* inoculated soil did not show suppression against *R. solani* (Table 4). Among *T. hamatum* strains isolated from BNR inoculated soil, four strains showed significant suppression against *S. rolfsii* and *R. solani*. Non-significant reduction against both pathogens was found using BM5 strain (Table 4).

Table 4 Suppression of radish root rot disease by *Trichoderma hamatum* isolated from soil inoculated three times with the non-pathogenic binucleate *Rhizoctonia*, *Sclerotium rolfsii* and *Rhizoctonia solani*

Strains	Isolation sources	Disease severity	
		Rhizoctonia solani	*Sclerotium rolfsii*
Control		3.69a*	3.78a
CM1[1)]	Sterilized barley grains	3.34a	3.48a
CM2	Sterilized barley grains	3.39a	3.40a
CM3	Sterilized barley grains	3.62a	3.55a
CM4	Sterilized barley grains	3.28a	3.60a
CM5	Sterilized barley grains	3.41a	3.49a
Control		3.74 a	3.80a
RM1	*Rhizoctonia solani*	0.49 b	0.57b
RM2	*R. solani*	0.47 b	0.36c
RM3	*R. solani*	0.56 b	0.51b
RM4	*R. solani*	0.48 b	0.60b
RM5	*R. solani*	0.31 c	0.68b
Control		3.49 a	3.66a
SM1	*Sclerotium rolfsii*	3.72 a	0.57b
SM2	*S. rolfsii*	3.76 a	0.54b
SM3	*S. rolfsii*	3.42 a	0.45b
SM4	*S. rolfsii*	3.38 a	0.60b
SM5	*S. rolfsii*	3.69 a	3.31a
Control		3.63a	3.75a
BM1	BNR	0.42b	0.21c
BM2	BNR	0.54b	0.36c
BM3	BNR	0.36b	0.26c
BM4	BNR	0.54b	0.64b
BM5	BNR	3.30a	3.54a

[1)] *Trichoderma hamatum* strains isolated from soil inoculated three times with sterile barley grain, *Rhizoctonia solani, Sclerotium rolfsii* and the non-pathogenic binucleate *Rhizoctonia* (CM1 ~ 5, RM1 ~ 5, SM1 ~ 5 and BM1 ~ 5, respectively)

* Same letters refer to insignificant differences and the different letters indicate significant differences at $P \leq 0.01$

Phylogenetic analysis using the TEF and ITS regions of *Trichoderma hamatum* isolated from frequently inoculated soil

The phylogenetic analysis using the ITS region revealed that among the 20 strains *T. hamatum*, which were isolated from the frequent inoculation of soil, 19 strains were classified into the same cluster and the percentage of homology was 99–100% (Fig. 1). However, only BM5 strain was different, and the percentage of homology with other strains was 89%. Among the 20 strains of *T. hamatum*, which were isolated from the frequent inoculation of soil in each fungus, 19 strains were classified into the same cluster using phylogenetic analysis of the TEF region and the percentage of homology was 99–100%. However, only SM3 strain was different recording 96% homology percentage with other strains (Fig. 2).

Evaluation of the sensitivity and specificity of designed primers for LAMP assay

The sensitivity of the detection primer of *R. solani* is shown in Fig. 3A. The primer was useful for the detection of both BNR and *R. solani*. Thus, the primer was not specifically detecting only *R. solani*, but in this experiment, the primer was very useful in the experimental system containing BNR. The primer was used as a specific detection primer for *R. solani*. The sensitivity of the detection primer of *S. rolfsii* was shown in Fig. 3B. The primer detected only *S. rolfsii*. Therefore, the primer was used for the specific detection of *S. rolfsii*.

The sensitivity of the detection primer *Trichoderma* spp. is shown in Fig. 3C. The primer detected in approximately equal reaction times the *T. hamatum*, *T. virens*, and *T. harzianum*. Therefore, the primer was used as a specific primer for the detection of fungus belonging to the genus *Trichoderma.*

Creating a calibration curve of the quantity of *Trichoderma* spores and the sclerotia of *Rhizoctonia solani* and *Sclerotium rolfsii* in mixed soil

The quantity of *R. solani* sclerotia in the soil is shown in Fig. 3A. The averages of time to reach the turbidity of 0.04 were 2898 s for the concentration of 0.1 mg/g, 2607 s for the concentration of 1 mg/g, 2334 s for the concentration of 10 mg/g and 2079s for the concentration of 100 mg/g. The calibration curve of the number of sclerotia was based on the formula $y=-0.0037x+11.662$ and R^2 value was 0.999. The log value used to calculate the quantity of sclerotia was $y=0.01e2.3026x$.

The quantity of *S. rolfsii* sclerotia in the soil is shown in Fig. 4A. The averages times to reach the turbidity of 0.04 were 2898 s for the concentration of 0.1 mg/g, 2514 s for the concentration of 1 mg/g, 2184 s for the concentration of 10 mg/g, and 1926s for the concentration of 100 mg/g. The calibration curve of the quantity of sclerotia was based on the formula $y=-0.0031x+9.7786$ and R^2 value was 0.992. The log value used to calculate the density of sclerotia was $y=0.01e2.3026x$.

The quantity of *S. rolfsii* sclerotia in the soil is shown in Fig. 4B. The averages of time to reach the turbidity of 0.04 were 2898 s. for concentration of 0.1 mg/g, 2514 s. for the concentration of 1 mg/g, 2184 s for the concentration of 10 mg/g, and 1926s for the concentration of 100 mg/g. The calibration curve of the density of sclerotia was based on the formula $y=-0.0031x+9.7786$ and R^2 value was 0.992. The log value used to calculate the density of sclerotia was $y=0.01e2.3026x$.

The concentrations of *T. virens* and *T. hamatum* in the soil are shown in Fig. 4C. The averages of time to reach the turbidity of 0.04 were 3358 s for the concentration of 1×10^4 spores/ml, 3123 s for the concentration of 1×10^5 spores/ml, 2875 s for the concentration of 1×10^6 spores/ml, and 2696 s for the concentration of 1×10^7 spores/ml. Calibration curve of the density of sclerotia was based on the formula $y=y=-0.0045x+15.968$ and R^2 value was 0.996. The log value used to calculate the number of sclerotia was $y=1000e2.3026x$.

Density of *Trichoderma* spp., *Rhizoctonia solani*, and *Sclerotium rolfsii* spores in soil inoculated frequently with *Rhizoctonia solani*

In *R. solani* frequently inoculated soil, DNA was extracted at 0, 7, 14 and 21 days. The densities of *R. solani* sclerotia after the first-time inoculation were 1.9, 11.8, 13.7, and 12.4 mg/g at 0, 7, 14 and 21 days after inoculation (DAI), respectively. For the second time inoculation, the densities were 16.0, 10.6, 7.1, and 5.5 mg/g at 0, 7, 14 and 21 DAI, respectively (Fig. 5). For the third time inoculation, *R. solani* densities were 9.1, 3.3, 3.8, and 3.6 mg/g at 0, 7, 14 and 21 DAI, respectively. For the fourth time inoculation, the densities were 3.1, 2.2, 1.6, and 1.3 mg/g at 0, 7, 14 and 21 DAI, respectively.

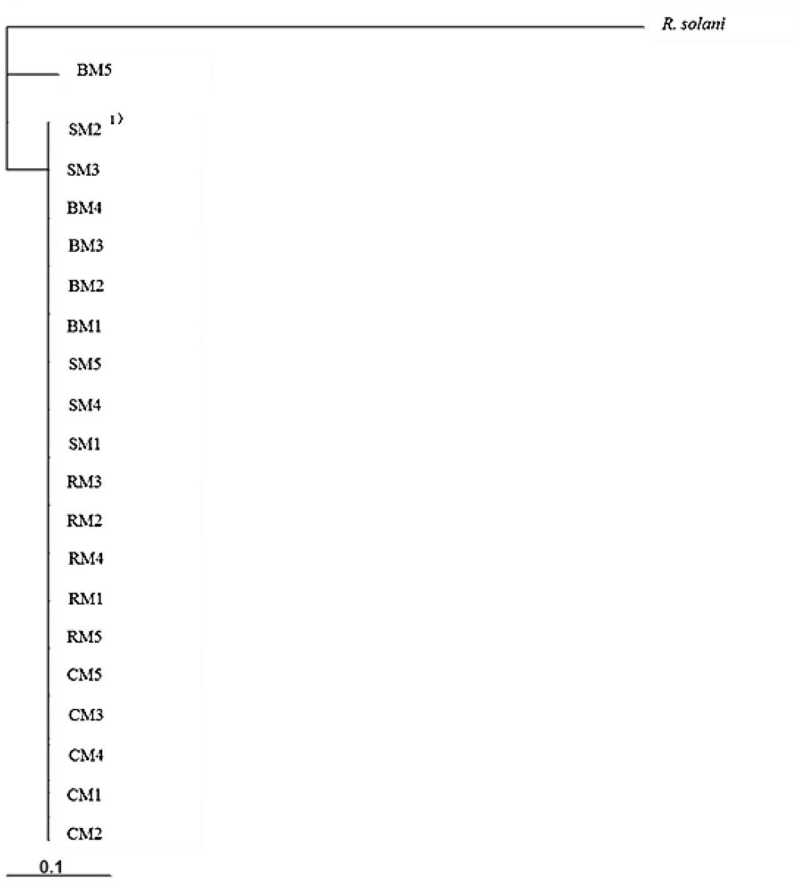
The densities of *Trichoderma* spp. spores in first-round inoculation were 0×10^4, 3.6×10^4, 8.5×10^4 and 22.9×10^4 spores/g soil at 0, 7, 14 and 21 DAI, respectively. For the second-round inoculation, the densities were 10.2×10^4, 27.6×10^4, 31.2×10^4 and 22.9×10^4 spores/g soil at 0, 7, 14 and 21 DAI, respectively. For the third round, the densities were 35.4×10^4, 19.0×10^4, 15.8×10^4 and 10.8×10^4 spores/g soil at 0, 7, 14 and 21 DAI, respectively. For the fourth round, the densities were 25.9×10^4, 7.96×10^4, 4.8×10^4 and 4.3×10^4 spores/g soil at 0, 7, 14 and 21 DAI, respectively.

The density of *R. solani* after first-time inoculation showed a significant increase with increasing the number

(See figure on next page.)

Fig. 1 Percentage of homology and phylogenetic tree were constructed using the sequence of ITS region of *Trichoderma hamatum* isolated from soil inoculated frequently three times with *Rhizoctonia* non-pathogenic binucleate, *Rhizoctonia solani*, and *Sclerotium rolfsii*. **A** The phylogenetic tree was constructed using the ITS region of *T. hamatum* isolated from soil inoculated frequently three times by *R. solani*, *S. rolfsii*, and BNR. **B** Percent homology of each strain using the sequence of ITS region of *T. hamatum* isolated from the frequently inoculated soils for three times with BNR, *R. solani* and *S. rolfsii. (*1) Five strains of *T. hamatum* isolated from soil inoculated three times with sterile barley grains, *R. solani*, *S. rolfsii*, and BNR were expressed as CM1 ~ 5, RM1 ~ 5, SM1 ~ 5, BM1 ~ 5

A)



B)

	CM1	CM2	CM3	CM4	CM5	RM1	RM2	RM3	RM4	RM5	SM1	SM2	SM3	SM4	SM5	BM1	BM2	BM3	BM4	BM5
CM1	-	100	100	100	100	100	100	100	100	100	100	99	100	100	100	100	100	100	100	89
CM2	-	-	100	100	100	100	100	100	100	100	100	99	100	100	100	100	100	100	100	89
CM3	-	-	-	100	100	100	100	100	100	100	100	99	100	100	100	100	100	100	100	89
CM4	-	-	-	-	100	100	100	100	100	100	100	99	100	100	100	100	100	100	100	89
CM5	-	-	-	-	-	100	100	100	100	100	100	99	100	100	100	100	100	100	100	89
RM1	-	-	-	-	-	-	100	100	100	100	100	99	100	100	100	100	100	100	100	89
RM2	-	-	-	-	-	-	-	100	100	100	100	99	100	100	100	100	100	100	100	89
RM3	-	-	-	-	-	-	-	-	100	100	100	99	100	100	100	100	100	100	100	89
RM4	-	-	-	-	-	-	-	-	-	100	100	99	100	100	100	100	100	100	100	89
RM5	-	-	-	-	-	-	-	-	-	-	100	99	100	100	100	100	100	100	100	89
SM1	-	-	-	-	-	-	-	-	-	-	-	99	100	100	100	100	100	100	100	89
SM2	-	-	-	-	-	-	-	-	-	-	-	-	99	99	99	99	99	99	99	88
SM3	-	-	-	-	-	-	-	-	-	-	-	-	-	100	100	100	100	100	100	89
SM4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	100	100	100	100	89
SM5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	100	100	100	89
BM1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	100	100	89
BM2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	100	89
BM3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	89
BM4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	89
BM5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Fig. 1 (See legend on previous page.)

of days after inoculation, but it tended to decrease in the second and subsequent inoculations. On the other hand, the density of *Trichoderma* spp. was initially below the detection limit of inoculation for the first time with *R. solani* and after 21 days the density of *Trichoderma* spp. increased to 3.12×10^5 spores/g soil (Fig. 5).

Density of *Trichoderma* spp., *Rhizoctonia solani*, and *Sclerotium rolfsii* spores in soil frequently inoculated with *Sclerotium rolfsii*

In *S. rolfsii* frequently inoculated soil, DNA was extracted at 0, 7, 14 and 21 days. The results showed that the densities of *S. rolfsii* after first-time inoculation were to 1.1, 13.8, 19.4 and 17.8 mg/g. For the second time of inoculation, the densities were 22.0, 11.1, 10.6 and 7.2 mg/g at 0, 7, 14 and 21 DAI, respectively. For the third time of inoculation, the densities were 15.0, 5.6, 4.3 and 4.9 mg/g at 0, 7, 14 and 21 DAI, respectively. For the fourth time inoculation, the densities were 4.7, 2.1, 0.7, and 0.7 mg/g at 0, 7, 14 and 21 DAI, respectively (Fig. 6).

The concentrations of *Trichoderma* spp. after the first round of inoculation were 0×10^4, 0.58×10^4, 4.3×10^4, and 15.6×10^4 spores/g soil at 0, 7, 14 and 21 DAI, respectively. For the second round of inoculation, the concentrations were 5.8×10^4, 24.3×10^4, 35.4×10^4, and 27.6×10^4 spores/g soil at 0, 7, 14 and 21 DAI, respectively. For the third round of inoculation, the concentrations were 42.6×10^4, 22.9×10^4, 15.8×10^4, and 6.61×10^4 spores/g soil at 0, 7, 14 and 21 DAI, respectively. For the fourth round of inoculation, the concentrations were 13.1×10^4, 7.96×104, 4.84×10^4, and 4.28×10^4 spores/g soil at 0, 7, 14 and 21 DAI, respectively.

The density of *S. rolfsii* after the first-time inoculation showed a significant increase with the increasing number of days after inoculation for the first time, but it tended to decrease in the second round of inoculation and subsequent. On the other hand, the density of *Trichoderma* spp. was initially below the detection limit in the first of *S. rolfsii* inoculation and after 21 days, it increased to 3.54×10^5 spores/g soil (Fig. 6).

Discussion

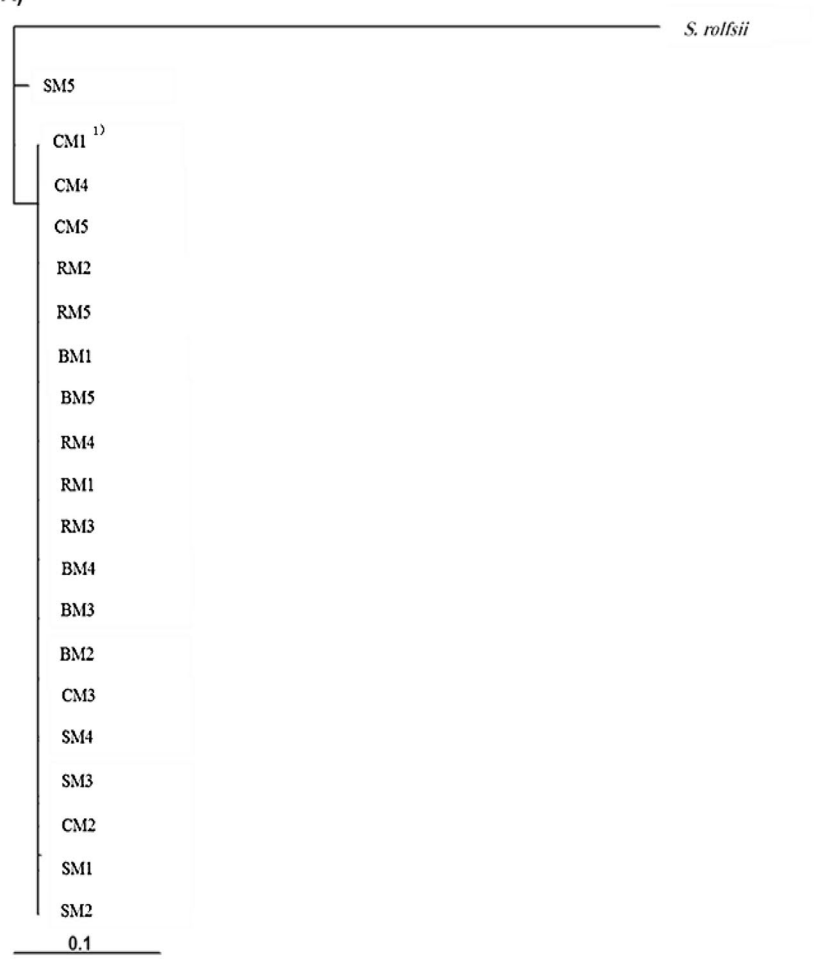
Trichoderma species are simply isolated from soil and root by traditional means, principally due to their quick growth, extensive conditions, and chlamydospore production, which promote rapid development on diverse materials (Elsharkawy et al. 2022). Host range of the genus *Trichoderma* is different among species, but *T. hamatum* was found within species lineage, and non-deterrent system in the same kind has been suggested. To prove the hypothesis that the intraspecific suppression system and non-suppression system are present in *T. hamatum* within the same species, the phylogenetic analysis using the ITS and TEF regions was performed. Most of *Trichoderma* spp. accumulated in the soil, which were repeatedly inoculated with BNR and *R. solani* are *T. hamatum* and *T. virens*, while most of the species composition of the genus *Trichoderma* in soil frequently inoculated with the *S. rolfsii* and sterile barley grain was *T. hamatum.* TEF and ITS regions are mainly used for species classification of *Trichoderma* spp. In recent years, the identification of species belonging to the genus *Trichoderma* by molecular biological analysis has become popular (Elsharkawy et al. 2022).

Trichoderma hamatum and *T. virens* were isolated from the frequently inoculated soil with antagonistic activity against both pathogens. *T. virens* showed significant antagonistic activity against both pathogens. The antagonistic activity of *T. hamatum* which is isolated from the frequently inoculated soil differed significantly depending on the type of fungi used in the repeated inoculation. *T. hamatum,* which is isolated from the frequent inoculation of soil with sterile barley grains, does not exhibit antagonistic activity for both pathogens. *T. hamatum*, which is isolated from repeated inoculation soil with sterile barley grains, has no antagonistic activity against both pathogens due to losing competition with the pathogen as a result from continuous cropping of the host plant. In contrast, *T. hamatum,* that is isolated from the frequent inoculation of soil with BNR and *R. solani* showed significant antagonistic activity against both pathogens. Also, *T. hamatum*, which was isolated from the frequent inoculation of soil with *S. rolfsii* showed significant antagonistic properties only against *S. rolfsii*. The growth of the pathogen was decreased by

(See figure on next page.)

Fig. 2 The percentage of homology and phylogenetic tree of TEF1 region of *Trichoderma hamatum* isolated from soil inoculated frequently three times by *Rhizoctonia* non-pathogenic binucleate, *Rhizoctonia solani*, and *Sclerotium rolfsii.* **A** The phylogenetic tree was constructed using the TEF1 region of *T. hamatum* isolated from soil inoculated frequently for three times with *R. solani, S. rolfsii,* and BNR. **B** Homologous percentages of the strains using the sequence of TEF1 region of *T. hamatum* isolated from the inoculated soils was repeated three times by BNR, *R. solani* and *S. rolfsii.* (1) Five strains of *T. hamatum* isolated from soil were inoculated three times by sterile barley grains, *R. solani, S. rolfsii*, and BNR were expressed as CM1 ~ 5, RM1 ~ 5, SM1 ~ 5, BM1 ~ 5

A)



B)

	CM1	CM2	CM3	CM4	CM5	RM1	RM2	RM3	RM4	RM5	SM1	SM2	SM3	SM4	SM5	BM1	BM2	BM3	BM4	BM5
CM1	-	100	100	99	100	100	100	100	100	100	99	99	96	99	100	100	99	100	100	100
CM2	-	-	100	99	100	100	100	100	100	100	99	99	96	99	100	100	99	100	100	100
CM3	-	-	-	99	100	100	100	100	100	100	99	99	96	99	100	100	99	100	100	100
CM4	-	-	-	-	99	99	99	99	99	99	99	99	96	99	99	99	99	99	99	99
CM5	-	-	-	-	-	100	100	100	100	100	99	99	96	99	100	100	99	100	100	100
RM1	-	-	-	-	-	-	100	100	100	100	99	99	96	99	100	100	99	100	100	100
RM2	-	-	-	-	-	-	-	100	100	100	99	99	96	99	100	100	99	100	100	100
RM3	-	-	-	-	-	-	-	-	100	100	99	99	96	99	100	100	99	100	100	100
RM4	-	-	-	-	-	-	-	-	-	100	99	99	96	99	100	100	99	100	100	100
RM5	-	-	-	-	-	-	-	-	-	-	99	99	96	99	100	100	99	100	100	100
SM1	-	-	-	-	-	-	-	-	-	-	-	99	96	100	99	99	99	99	99	99
SM2	-	-	-	-	-	-	-	-	-	-	-	-	96	99	99	99	99	99	99	99
SM3	-	-	-	-	-	-	-	-	-	-	-	-	-	96	96	96	96	96	96	96
SM4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	100	99	100	100	100
SM5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	99	100	100	100
BM1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	99	100	100	100
BM2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	99	99	99
BM3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	100
BM4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100
BM5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Fig. 2 (See legend on previous page.)

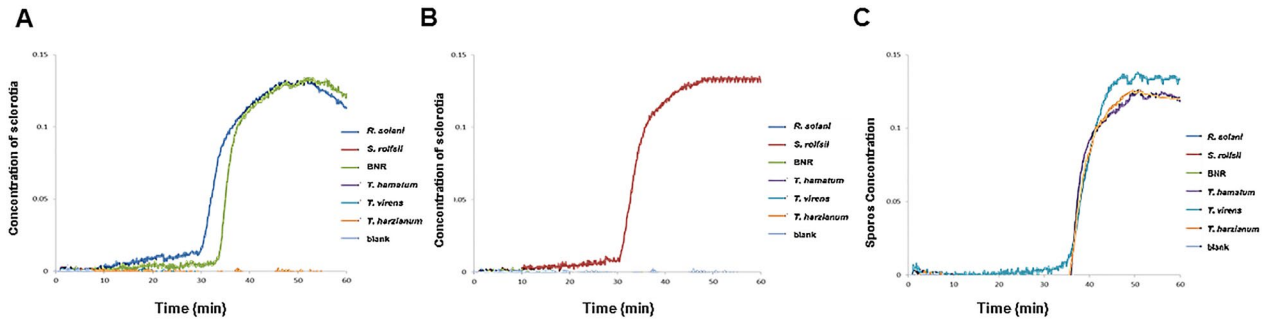


Fig. 3 Confirmation of the specificity of the detection primers of *Rhizoctonia solani* (**A**), *Sclerotium rolfsii* (**B**) and *Trichoderma* spp. (**C**)

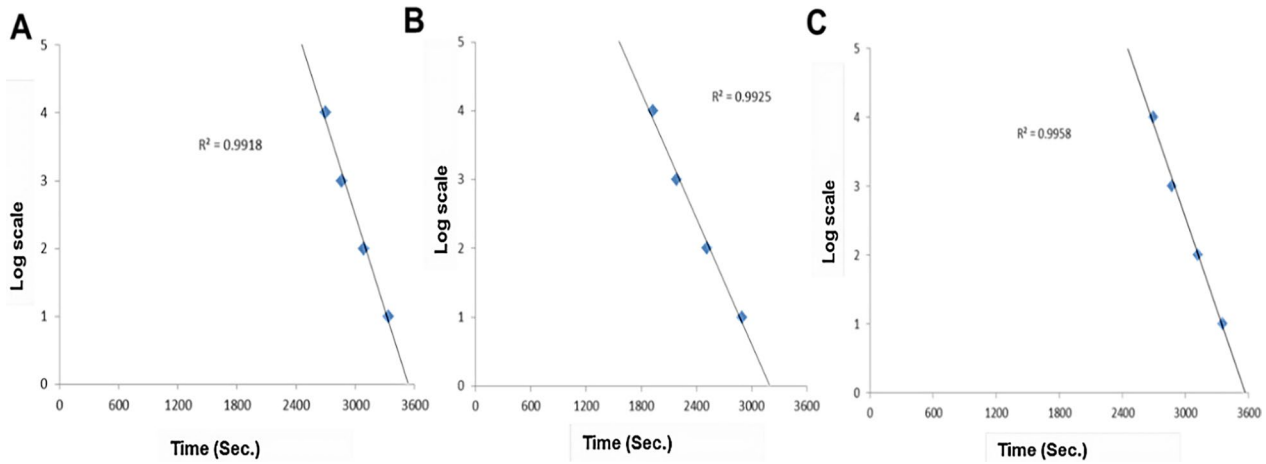


Fig. 4 Calibration curve of the sclerotia concentration of *Rhizoctonia solani* (**A**) and *Sclerotium rolfsii* (**B**) and spore concentration of *Trichoderma* spp. (**C**). **A** and **B** The vertical axis is a logarithmic scale, 0.1 mg/g = 1, 1 mg/g = 2, 10 mg/g = 3,100 mg/g = 4. **C** The vertical axis is a logarithmic scale, 10^4 spores/ml = 1, 10^5 spores/ml = 2, 10^6 spores/ml = 3, 10^7 spores/ml = 4

Trichoderma sp. (Sahii and Khalid 2007). The *R. solani* mycelial growth and the incidence of damping-off and root rot disease were significantly reduced by *Trichoderma* sp. (Elsharkawy et al. 2022). The production of antibacterial metabolites and hydrolytic enzymes, MAPK and G-protein (mitogen-activated protein kinase), that has affected the various processes related to the biological control of the genus *Trichoderma*, such as the formation of infection structures, was reported (Zeilinger and Omann 2007). In addition, the functional properties and cloning of ABC transporter gene from the first biocontrol fungi isolated, *T. atroviride* P1, were elucidated (Ruocco et al. 2009). This ABC transporter gene can protect its own host from toxins and other harmful effects. Further study is needed to investigate the relationship between the action of the ABC transporter gene and the selective accumulation of *Trichoderma* spp. Comprehensive analysis of the entire gene using Amplified Fragment Length Polymorphism (AFLP) analysis and the next-generation sequencing analysis method should be done in the future.

The components of soil organics include fungi (mycelia), bacteria (microbial biomass), and spores (Nikitin et al. 2017). Previous studies showed that the introduction of live inocula led to a decrease in disease incidence and pathogen proliferation (Elsharkawy et al. 2022). The results revealed that the suppression of frequent inoculation of soil with *R. solani,* BNR and *S. rolfsii*, could be due to the selective accumulation of *Trichoderma* spp. The disease suppression mechanism for radish root rot disease by *Trichoderma* spp., either competing or parasitizing, acts directly against the pathogen and can be considered as a promising candidate, while the possibility of resistance induction on host plant is believed to be low. Dual culture of the root rot pathogens, and *Trichoderma* spp., has been reported and the vitality of the root rot pathogens in dual cultures was stated (Kumar et al. 2012).

The spore concentrations of *R. solani, S. rolfsii* and *Trichoderma* spp. were measured at 0, 7, 14 and 21 days after inoculation in *R. solani* and *S. rolfsii* repeated

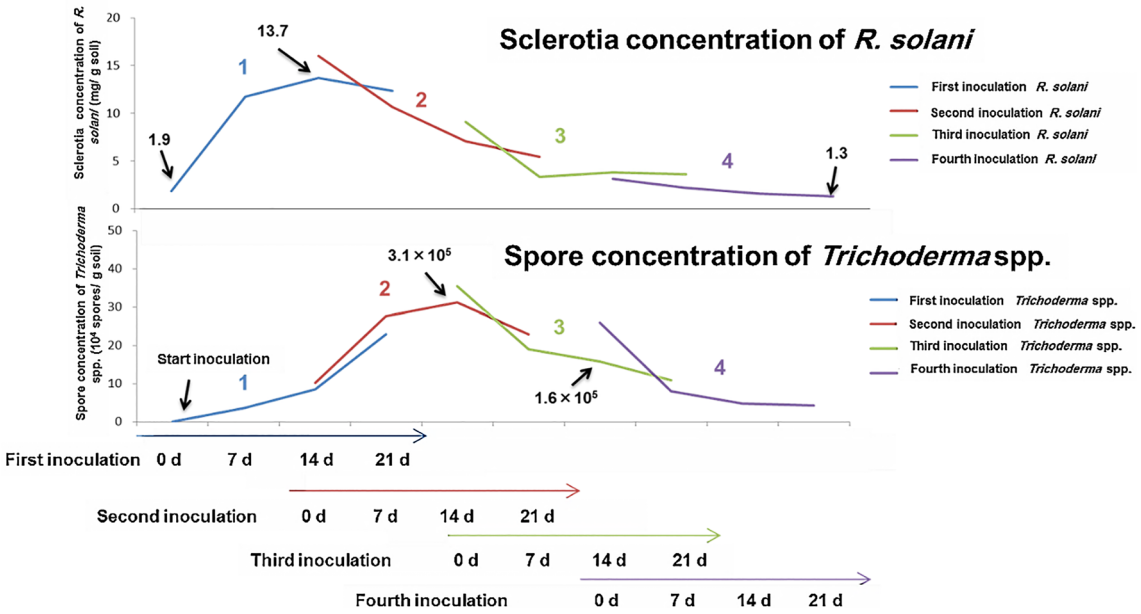


Fig. 5 Density of *Trichoderma* spp. and *Rhizoctonia solani* in soil inoculated frequently with *R. solani* at 0, 7, 14 and 21 days after inoculation

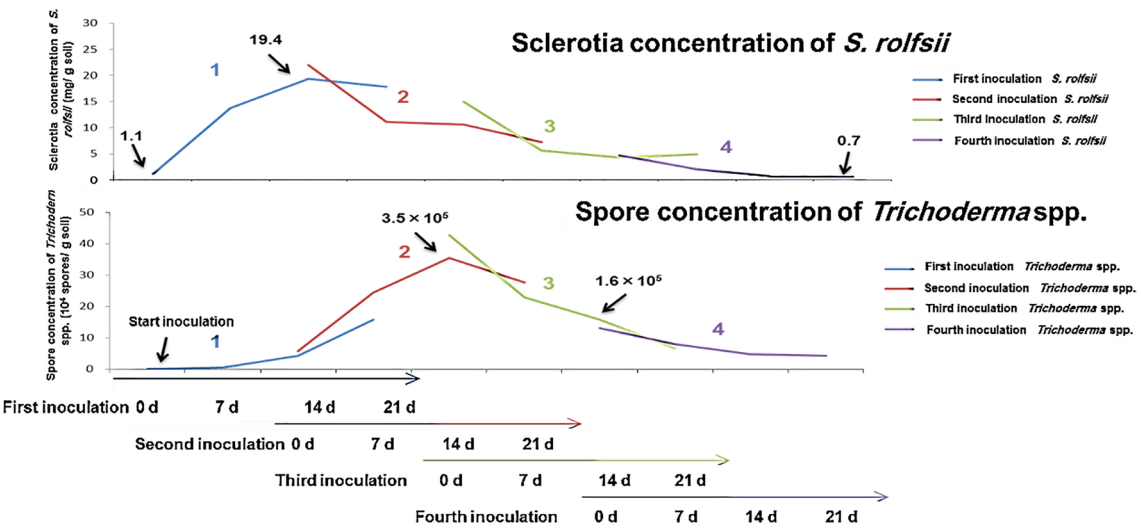


Fig. 6 Density of *Trichoderma* spp. and *Sclerotium rolfsii* in soil inoculated frequently with *S. rolfsii* at 0, 7, 14 and 21 days after inoculation

inoculation soil. Isothermal amplifications were used instead of PCR-based markers because they are more practical for usage in the fields (Choudhary et al. 2020). LAMP test based on the ITS gene was used for the detection of *R. solani* fungus, which causes blight in soybean seedlings (Lu et al. 2015). Furthermore, compared to conventional PCR, LAMP yields greater quantities of DNA. Even if the LAMP combination is mixed with untreated biological fluids, which frequently restrict PCR-based amplification, LAMP retains sensitive and specific amplification. After inoculation, the concentration of sclerotia of *S. rolfsii* and *R. solani* showed a rapid increase with increasing number of days, but it showed a sharp downward trend in the second round of inoculation and subsequent. On the other hand, *Trichoderma* spp. was initially below the detection limit on the first inoculation but increased rapidly in the second, third and fourth inoculations. The activity of the isolated *Trichoderma* spp. and species composition of the repeated inoculation of soil with BNR and

R. solani were almost similar. This was likely because BNR is very close to *R. solani*, and there is a possibility that the activity and recognition mechanisms for both fungi to the genus *Trichoderma* are very close. However, because there are differences in the concentration of *Trichoderma* spp. in the soil inoculated with BNR and *R. solani*, it was considered to have some differences in the activity and recognition mechanisms. The recognition in the antagonistic fungi and the parasites has been reported to be due to the attachment of the carbohydrate between the mycoparasite and lectin of the fungal host. Therefore, there is a need to find out the differences in lectin contents in the cell wall of *R. solani*, BNR and *S. rolfsii* (Zeilinger et al. 1999). In conclusion, Lamp results confirmed that *Trichoderma* species have selectively accumulated in each replication of soil inoculation, resulting in disease control in soil that has been repeatedly inoculated with BNR, *R. solani*, and *S. rolfsii*.

Conclusion

Trichoderma hamatum and *T. virens* constitute most of *Trichoderma* spp. accumulated in the soil, which were repeatedly inoculated with BNR and *R. solani.* On the other hand, *T. hamatum* constitutes most of the species' composition of the genus *Trichoderma* in soil frequently inoculated with the *S. rolfsii* and sterile barley grain. This could explain the vital role of *Trichoderma* spp. accumulation in root rot disease control.

Abbreviations

LAMP	Loop-mediated isothermal amplification
BNR	Binucleate *Rhizoctonia*
Ggt	*Gaeumannomyces graminis* Var. *tritici*
PCR-DGGE	Polymerase chain reaction denaturing gradient gel electrophoresis
BGI	Barley grain inocula
SDW	Sterile distilled water

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41938-024-00813-4.

Additional file 1.

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Author contributions

Conceptualization, M.M.E., S.K. and M.H.; methodology, M.M.E., S.K. and M.H.; software, M.M.E., S.K. and M.H.; validation, M.M.E., S.K., M.H., and N.M.A.; formal analysis, M.M.E., S.K. and M.H.; investigation, M.M.E., S.K. and M.H.; resources, M.M.E., S.K., M.H., and N.M.A; data curation, M.M.E., S.K., M.H. and N.M.A; writing—original draft preparation, M.M.E., S.K., and M.H.; writing—review and editing, M.M.E., S.K., and M.H.; visualization, M.M.E. and N.M.A.; supervision, M.M.E., S.K., and M.H.; project administration, M.H. and M.M.E.; funding acquisition N.M.A. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

All data and materials are available.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

Bithell SLS, McKay A, Butler R, New T, Bag P, Zealand N (2012) Predicting take-all severity in second-year wheat using soil DNA concentrations of *Gaeumannomyces graminis* var. *tritici* determined with qPCR. Plant Dis 96:443–451. https://doi.org/10.1094/PDIS-05-11-0445

Choudhary P, Rai P, Yadav J et al (2020) A rapid colorimetric LAMP assay for detection of *Rhizoctonia solani* AG-1 IA causing sheath blight of rice. Sci Rep 10:22022. https://doi.org/10.1038/s41598-020-79117-0

Durán P, Jorquera M, Viscardi S, Carrion VJ, Mora MD, Pozo MJ (2017) Screening and characterization of potentially suppressive soils against *Gaeumannomyces graminis* under extensive wheat cropping by Chilean indigenous communities. Front Microbiol 8:1552

Elad Y, Chet I, Henis Y (1981) A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. Phytoparasitica 9:59–67. https://doi.org/10.1007/BF03158330

El-kazzaz MK, Ghoneim KE, Agha MKM, Helmy A, Behiry SI, Abdelkhalek A, Saleem MH, Al-Askar AA, Arishi AA, Elsharkawy MM (2022) Suppression of pepper root rot and wilt diseases caused by *Rhizoctonia solani* and *Fusarium oxysporum*. Life 12:587. https://doi.org/10.3390/life12040587

Elsharkawy MM, Kuno S, Hyakumachi M, Mostafa YS, Alamri SA, Alrumman SA (2022) PCR-DGGE analysis proves the suppression of *Rhizoctonia* and *Sclerotium* root rot due to successive inoculations. J Fungi 8:133. https://doi.org/10.3390/jof8020133

Jara D, Herman A, Elizondo M, Ernesto A (2011) Root disease suppressive soils: "Take all decline (*Gaeumannomyces graminis* var. *tritici*) in wheat. A Agro Sur 39:67–78. https://doi.org/10.4206/agrosur.2011.v39n2-01

Kumar K, Amaresan N, Bhagat S, Madhuri K, Srivastava RC (2012) Isolation and characterization of *Trichoderma* spp. for antagonistic activity against root rot and foliar pathogens. Indian J Microbiol. 52(2):137–44

Lu C, Song B, Zhang H, Wang Y, Zheng X (2015) Rapid diagnosis of soybean seedling blight caused by *Rhizoctonia solani* and soybean charcoal rot caused by *Macrophomina phaseolina* using LAMP assays. Phytopathology 105:1612–1617. https://doi.org/10.1094/PHYTO-01-15-0023-R

Ma XJ, Shu Y, Nie K, Qin M, Wang D et al (2010) Visual detection of pandemic influenza A H1N1 Virus 2009 by reverse-transcription loop-mediated isothermal amplification with hydroxynaphthol blue dye. J Virol Methods 167:214–217. https://doi.org/10.1016/j.jviromet.2010.03.027

Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37:634–663. https://doi.org/10.1111/1574-6976.12028

Nikitin DA, Marfenina OE, Kudinova AG et al (2017) Microbial biomass and biological activity of soils and soil-like bodies in coastal oases of Antarctica. Eurasian Soil Sci 50:1086–1097. https://doi.org/10.1134/S1064229317070079

Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T (2000) Loop-mediated isothermal amplification of DNA. Nucleic Acids Res 28:E63. https://doi.org/10.1093/nar/28.12.e63

Ogura A (2009) Colorimetric detection of loop-mediated isothermal amplification reaction by using hydroxy naphthol blue. Biotechniques 46:167–172. https://doi.org/10.2144/000113072

Ruocco M, Lanzuise S, Vinale F, Marra R, Turra D, Woo SL, Lorito M (2009) Identification of a new biocontrol gene in *Trichoderma atroviride*: the role of an ABC transporter membrane pump in the interaction with different plant-pathogenic fungi. Am Phytopathol Soc 22:291–301

Sahii IY, Khalid AN (2007) In vitro biological control of *Fusarium oxysporum* causing wilt in *Capsicum annum*. Mycopath 5:85–88

Samuels GJ (2006) *Trichoderma*: systematics, the sexual state, and ecology. Phytopathology 96(2):195–206. https://doi.org/10.1094/PHYTO-96-0195. (**PMID: 18943925**)

Tian Q, Lu C, Wang S, Xiong Q, Zhang H, Wang Y, Zeng X (2017) Rapid diagnosis of soybean anthracnose caused by *Colletotrichum truncatum* using a loop-mediated isothermal amplification (LAMP) assay. Eur J Plant Pathol 148:785–793

Villajuan-Abgona R, Kageyama K, Hyakumachi M (1993) Biological control of *Rhizoctonia* damping-off of cucumber by binucleate *Rhizoctonia*: Prevention of hyphal penetration. Ann Phytopath Soc Jpn 60(6):780

Wastling SL, Picozzi K, Kakembo ASL, Welburn SC (2000) LAMP for human African trypanosomiasis: a comparative study of detection formats. Plos Neglec Trop D 4:e865. https://doi.org/10.1371/journal.pntd.0000865

Wildermuth GB (1982) Soils suppressive to *Gaeumannomyces graminis* var. *tritici*: effect on other fungi. Soil Biol Biochem 14:569–573

Xue M, Wang R, Zhang C, Wang W, Zhang F, Chen D, Ren S, Manman Z, Hou J, Liu T (2021) Screening and identification of *Trichoderma* strains isolated from natural habitats in China with potential agricultural applications. Biomed Res Int 2021:7913950. https://doi.org/10.1155/2021/7913950. PMID:34970627;PMCID:PMC8714372

Zeilinger S, Omann M (2007) *Trichoderma* biocontrol: Signal transduction pathways involved in host sensing and mycoparasitism. Gene Regul Syst Biol 1:227–234

Zeilinger S, Galhaup C, Payer K, Woo SL, Mach RL, Fekete C, Lorito M, Kubicek CP (1999) Chitinase gene expression during mycoparasitic interaction of *Trichoderma harzianum* with its host. Fungal Genet Biol 26:131–140

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