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Endophytic fungi associated with soybean plants and their antagonistic activity against *Rhizoctonia solani*

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Abstract

Background: Fungal endophytes produce many secondary metabolites that can reduce root rot diseases. Soybean is a particularly important crop worldwide. Endophytic fungi can be isolated, identified, and incorporated into sustainable agriculture for the biological control of many diseases.

Results: The aim of this study was to isolate some endophytic fungi for controlling the most important diseases of soybean plants and to study the mechanisms underlying this biocontrol regarding the suppression of pathogens. Ten endophytic fungi were isolated from soybean plants. Among them, the 3 fungi isolates that exhibited a high percentage of antagonistic activity against *Rhizoctonia solani*, the causal pathogen of root rot disease of soybean plants, were identified as *Trichoderma longibrachiatum* S12, *T. asperellum* S11, and *T. atroviride* PHYTAT7. The 3 fungi isolates had the ability to produce pectinase and chitinase and to solubilize phosphors. Moreover, they produced siderophores and indole acetic acid (IAA), which have a strong effect on the growth of the plants. The 3 isolates reduced disease severity by 64, 60, and 55%, respectively than the infected control.

Conclusion: The results suggest that certain endophytic fungi associated with soybean plants have potential for the management of root rot diseases in soybean. Moreover, these isolates can be considered as having a growth-promoting effect in soybean plants.

Keyword: Endophytic fungi, Root rot, Soybean, *Trichoderma* spp. biocontrol

Background

Endophytes are microorganisms that live in the intercellular space of healthy plant tissues without causing any disease in the plants (Abo-Elyousr et al. 2014a). They also supply nutrients to the host, stimulate plant resistance to pathogens, cold, and drought, and promote plant growth by secreting hormones or supplying nutrients to their host plant (Suryanarayanan et al. 2012). Nair and Padmavathy (2014) mentioned that the symbiosis between microorganisms and plants is well known. Plants protect and feed the endophytes, which in response produce bioactive substances that enhance the growth and resistance of plants in the environment.

Soybean (*Glycine max* L.) is a leguminous plant of the *Fabaceae* family that is commonly found in many locations worldwide (Chowdhury et al. 2016). Nutritionally, soybean comprises 45% of protein and 18% of oil; thus, it is also known as a miracle crop. Diseases such as root rot, brown spot, soybean rust, downy mildew, and stem blot have been a major problem and cause losses in soybean production in many countries. *Rhizoctonia solani* is well known and widely dispersed in roots, plant debris, and soil, causing major diseases in a diverse range of hosts, including root rot in soybean plants (Surbhi et al. 2020). Fungicide application is the main method used for the control of such diseases. However, the use of fungicides is currently forbidden in many countries and

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other methods for controlling of this disease are preferred, e.g., biological control and cultural practices (Arastehfar et al. 2019).

Traditional control methods have been suggested for root rot, such as the development of resistant cultivars and short rotation and culture practices; however, these methods are not always successful as *Rhizoctonia* spp. can remain for a long time in the soil via infested plant debris. Recently, several studies focused on alternative biological methods for the control of plant diseases that are characterized as being environmentally friendly, long lasting, and effective (Sallam et al. 2019). The use of beneficial microorganisms has been regarded as a hopeful method for the management of soil diseases (Fenta et al. 2019). Many beneficial microbes, such as *Pseudomonas putida*, Bacteriophages, *Trichoderma* spp., *Paenibacillus macerans*, *P. fluorescens*, *Streptomyces* spp., *Enterobacter* spp., *Acinetobacter* spp., and *Bacillus* spp., have been reported as efficient biocontrol agents against *Rhizoctonia* spp. (Sharma et al. 2017). Studies have focused on the mycoparasitic and antagonistic ability of isolates of *Trichoderma* to decrease the incidence of the diseases caused by plant pathogens (Abo-Elyousr et al. 2014b).

Therefore, the aim of this study was to isolate endophytic *Trichoderma* spp. from soybean plants and characterize their antagonistic activity against *the* causal pathogen of root rot disease of soybean. As well, their effect on disease reduction in pot experiments was studied.

Methods

Source of the causal pathogen of soybean root rot

The highly pathogenic isolates of *Rhizoctonia solani* used in this study were previously isolated and tested for pathogenicity by (Sallam Nashwa et al. 2008) who identified the isolates using the morphological features of mycelia, as described by Barnett and Hunter (1986). Pure fungal isolates were then grown on potato dextrose agar (PDA) slants at 25±2 °C, then kept in a refrigerator at 4 °C for further studies.

Isolation of endophyte fungi from soybean plants

Healthy roots of soybean plants were collected, washed with tap water, and cut into small pieces, which were then surface sterilized in 2% sodium hypochlorite for 3 min and 70% ethanol for 3 min, washed with sterile distilled water 3 times, and left to dry in a laminar flow chamber. The pieces were transferred to a Petri dish containing PDA medium and incubated at 27±2 °C for 10 days. Pure cultures were transferred onto PDA slants.

Antagonistic capability of various endophytic fungi isolates against *Rhizoctonia solani*

The five tested endophytic fungi isolates were grown on PDA medium and incubated for 5 days at 28 °C for use as inocula. PDA Petri plates (9 cm in diameter) were inoculated at 3 cm from the edge with disks of *R. solani* (5 mm in diameter), followed by inoculation with the tested fungi on opposite sides of *R. solani*. Four replicates were used for each treatment. The control treatment consisted in plates with *R. solani* alone, without the inoculation of endophytic fungi. The plates were incubated for 7 days at 28 °C. When the growth of the pathogen was covered, the plate in control treatment in the linear growth of the tested pathogen was recorded in the treatments fungi (Zein El-Abdean et al. 2013). The percentage of mycelial growth inhibition was calculated using the following formula: percentage of mycelial growth inhibition = $[T–F/T] \times 100$, where T is the mycelia growth in the control and F is the mycelia growth in the tested isolate. The antagonistic fungi that afforded a high percentage of mycelial growth reduction were identified using a 28S rRNA molecular method.

Identification of *Trichoderma* spp. using polymerase chain reaction nucleotide sequencing (PCR-Seq)

Three isolates were used in this study. The nuclear rDNA region spanning the ITS1 ITS2 regions was used for the first amplification, which was performed in a total reaction volume of 50 μl including the following reagents: PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.01% gelatin), 200 μM of each deoxyribonucleotide triphosphate, 0.4 μM of each primer with 10 μl of the template DNA solution, and 1 U of Tth DNA polymerase (Toyobo). The PCR mixture was overlaid with 30 μl of mineral oil. The following thermal cycling was performed on a thermal cycler (PC-700; ASTEC): an initial denaturing step at 95 °C for 2 min; 30 cycles of 30 s at 72 °C for extension; and a final extension cycle of 7 min at 72 °C. One microliter of the first amplification mixture was used for the second amplification using the nested primer set ITS1 (White et al. 1990) and P3. The components of the reaction mixture and the thermal cycling conditions used for the second amplification were the same as those used for the first one. The PCR products from the second amplification were subjected to preparative electrophoresis in 1.5% agarose gels in Tris acetate EDTA (TAE) buffer. All amplifications yielded a single visible DNA product. The DNA product band was excised from the ethidium-bromide-stained gel and purified using a JETSORB kit according to the manufacturer's protocol. Direct sequencing of PCR products was performed on an Applied Biosystems 373A sequencer using a PRISM

Dye Terminator Cycle Sequencing kit (Applied Biosystems) according to the manufacturer's protocol and using the ITS1 and ITS2 primers (White et al. 1990). The nucleotide sequence data of the ITS2 and ITS2 regions were subjected to pairwise alignment via the method of Lipman and Pearson (1985) using the program GENETYX-MAC (Software Development).

Evaluation of the antagonistic effect of fungi under greenhouse conditions

The inoculum was prepared in bottles containing barley grain medium using disks (5 mm) of *R. solani* isolates. The bottles containing barley medium were autoclaved at 121 °C and 1.5 kg/cm^2 for 20 min before inoculation with the pathogen. After inoculation, the bottles were incubated at 25±2 °C for 15 days. For soil infestation, *R. solani* barley grains were mixed with sterilized soil at a concentration of 3%. Four replicates were used for each tested isolate (5 seedlings/pot). Pot experiments were carried out to study the effects of the 3 selected antagonistic fungi, for controlling the root rot incidence in soybean plants. Seeds of the Giza 111cv. soybean cultivar were sown in *R. solani*-infested soils as described above (5 seeds/pot), and the antagonistic fungi were applied as a soil treatment by adding a 3% solution to pots 7 days after infestation with *R. Solani*. Untreated pots served as the control. At 30 days after sowing, root rot disease severity was recorded as described previously (Abo-Elyousr et al. 2014b). The experiments were arranged in a randomized complete block design with 4 replicates. Disease severity percentage (DS%) was estimated as follows: DS% = [ΣA/A (4T)] × 100

Where A is the disease rating on each plant, d max is the maximum disease rating possible, and 4T is the total number of plants (T) multiplied by the maximum discoloration grade 4. According to Dorrance et al. (2003), with slight modifications, the different degrees of disease in plants were classified into 4 categories: 0 = no root rot; 1 = 1 to 33% of roots with visible lesions or root rot; 2 = approximately 34 to 50% of the roots exhibited rot or damage; 3 = 51 to 80% of the roots exhibited rot; and 4 = pre-emergence damping-off and few if any roots.

Depiction of secondary metabolites and antifungal activity of endophytic fungi

Solubilization of minerals

Phosphate solubilization Pikovskaya medium was used to test the phosphate-solubilizing ability of the fungi isolates by dissolution of precipitated tricalcium phosphate Ca_3 $(PO_4)_2$. The fungi isolates were inoculated on the surface of PDA medium dried plates. The plates were incubated for 7 days at 28 °C. The solubilization index (SI) was calculated according to Edi-Premono et al. (1996).

Zinc solubilization As reported by Saravanan et al. (2003, b), the zinc-solubilizing ability of the tested fungi was detected based on the dissolution of precipitated zinc oxide on agar medium. On the surface of dried plates, a pinpoint inoculation of the fungal isolates was performed. Plates were incubated for 7 days at 28 °C. The SI was calculated as reported by Edi-Premono et al. (1996), as follows: ratio of the total diameter (colony diameter + halo zone diameter)/colony diameter.

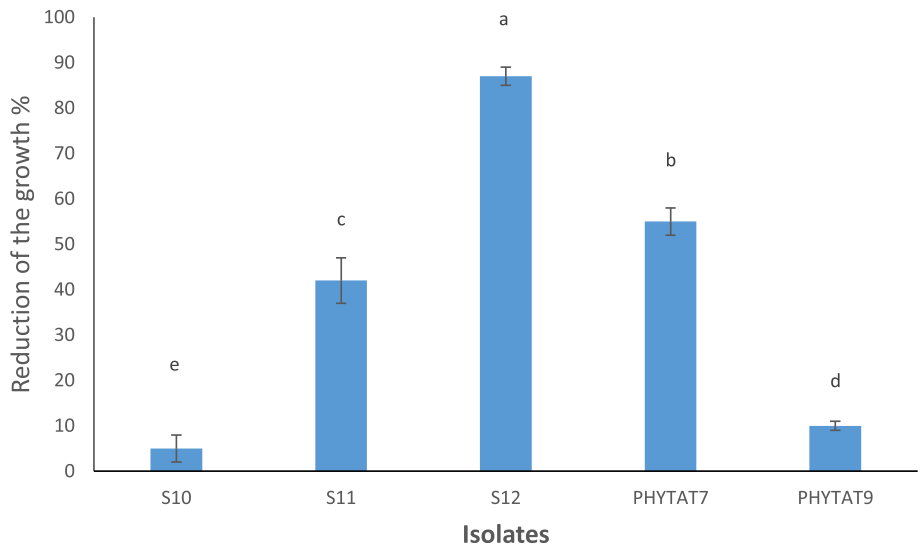

Fig. 1 In vitro study of the effect of five endophytic fungi isolates on the growth of *Rhizoctonia solani*. Values followed by the same letter are not significantly different, as determined by the LSD test ($P \leq 0.05$)

Fig. 2 Antagonistic activity of *Trichoderma* spp. isolates against *Rhizoctonia solani in vitro*

Production of specific enzymes by the bioagent

Using the disk plate method, the enzymatic activities of potent fungal bioagent were visualized on agar plates containing specific enzyme substrates (Acuna-Arguelles et al. 1995). These pathogens were screened for multiple enzymes, such as cellulases, pectinases, proteases, and amylase, on suitable substrates. The size of the clearing zone that developed around the colonies corresponded to the enzymatic activity.

Pectinase activity The tested fungal culture was inoculated on Czapek agar medium containing 2 $NaNO_3$; 1.0 K_2HPO_4; 2 peptone; 0.5 $MgSO_47H_2O$; 0.5 KCl; and 20 agar (g/l) in 1000 ml of distilled water at pH 6.8 and enhanced with 1% citrus pectin as a substrate for pectinase. The Petri dishes containing screening agar medium were incubated for 24 h at 30 °C. One percent cetyl trimethyl ammonium bromide was added to observe the zone of clearance, which revealed the pectinase activity.

Cellulase activity Medium containing carboxy methyl cellulose (CMC) (1% w/v) was used to detect the cellulase activity. The Petri plates were incubated for 24 h at 27 °C. After inoculation, the plates were screened for cellulase activity by flooding the plate with 1% Congo red solution for 15 min, followed by de-staining using a 1 M NaCl solution for 15 min. The clear zones around the growth of fungi indicated cellulase activity.

Amylase activity Medium containing starch (1% w/v) as a substrate was used to detect amylase activity. Plates were incubated at 27 °C for 24 h. The clear zones around the fungal growth were observed by staining the plate with 50 mM iodine.

Protease activity Medium containing casein (1% w/v) as a substrate was used to test protease activity. Enzymatic activity was determined after the inoculation of the plate with the tested fungi. The formation of a clear zone around colonies after precipitation with 1 M HCl solution indicated protease activity (Rodarte et al. 2011).

Production and assay of indole acetic acid

The fungi strains were inoculated individually in 250 ml Erlenmeyer conical flasks containing 50 ml of potato dextrose broth medium supplemented with 0.4% tryptophan, as a precursor. The inoculated flasks were then incubated in agitated conditions of 150 rpm for 7 days. After incubation, the fungal growth

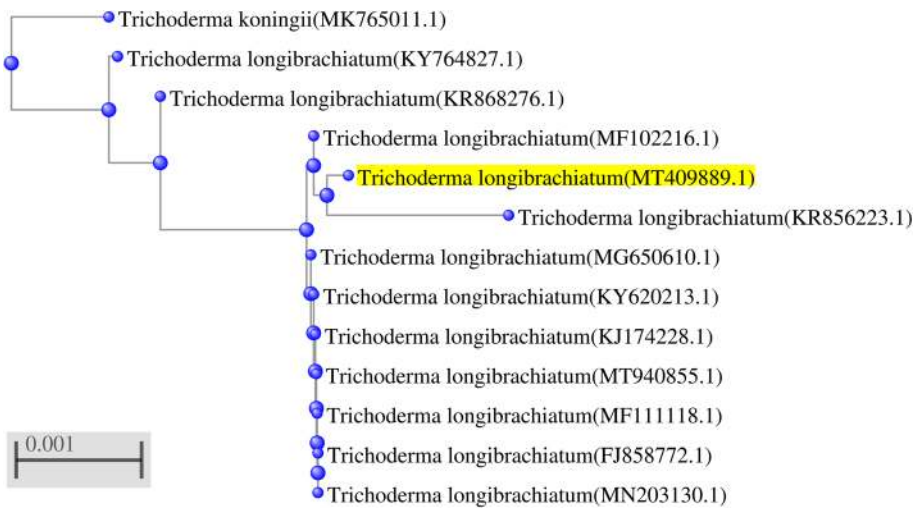

Fig. 3 Close homologs of *Trichoderma longibrachiatum* strain S12 (MT409889.1) shown in a phylogenic tree

Table 1 Molecular characterization of S12, S11, and PHYTAT7 by ITS analysis

Fungal isolates	Maximum score	Total score	Query cover	*E* value	Percent identity	Most similar organism	GenBank accession no.
S12	1138	1138	95%	0.0	98.46%	*Trichoderma longibrachiatum*	LT707585.1
S11	1059	1059	90%	0.0	98.20	*T. asperellum*	KR856220.1
PHYTAT7	994	994	96%	0.0	100%	*T. atroviride*	MT604177.1

was decanted by centrifugation at 10,000 rpm for 10 min and the cell-free supernatants were used as sources of indole acetic acid (IAA). The reaction mixture consisted of 1 ml of the cell-free supernatant and 2 ml of the Salkowski reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% perchloric acid). The mixture was then incubated at 28 °C for 30 min. Quantification was performed calorimetrically at 530 nm by comparison to an IAA standard curve (Gordon and Paleg 1957).

Qualitative estimation of siderophores

The production of siderophores by fungi isolates was assayed via the plate assay method, as described by Schwyn and Neilands (1987). After growth, fungal isolates (a 5-mm disk of each isolate) were potted on Chrome Azurol S blue agar plates and incubated for 48 h at 28±2 °C. The development of a yellow-orange zone around the colony was taken as a positive indication of siderophore production. The extent of siderophore biosynthesis was measured as the diameter of the colored zone (Alexander and Zeeberi 1991).

Statistical analysis

Data were subjected to statistical analysis using the MSTATC program of variance, and means were compared using the least significant difference (LSD) test at $P \leq 0.05$, as described by Gomes and Gomes (1984). The LSD at 5% probability was used for testing the significance of the differences among the mean values of the tested treatments.

Results

Antagonistic activity of the endophytic fungi isolates against *Rhizoctonia solani in vitro*

Five isolates of endophytic fungi were first tested for their antagonistic capability against *R. solani in vitro* before identification. The results presented in Fig. 1 showed that 3 fungal isolates exhibited inhibition percentages that were >80%. Isolate S12 afforded the highest percentage of inhibition (87%), followed by the isolate PHYTAT7 (55%) and isolate S11 (42%). Isolates PHYTAT9 and S10 yielded the lowest reduction of the pathogen. Based on these results, the three isolates were selected for subsequent experiments (Fig. 2a, b, and c).

Identification of the antagonistic fungi isolates using 16S rRNA gene sequencing

Pure cultures of the 3 fungal isolates were molecularly characterized, using 16S rRNA sequencing. Based on BLAST searches on the NCBI data libraries (Nucleotide Collection Database) for similarities of the 16S rRNA sequences, isolate S12 was the most similar to *Trichoderma longibrachiatum* (GenBank accession No. LT707585.1), with 98.46% identity and

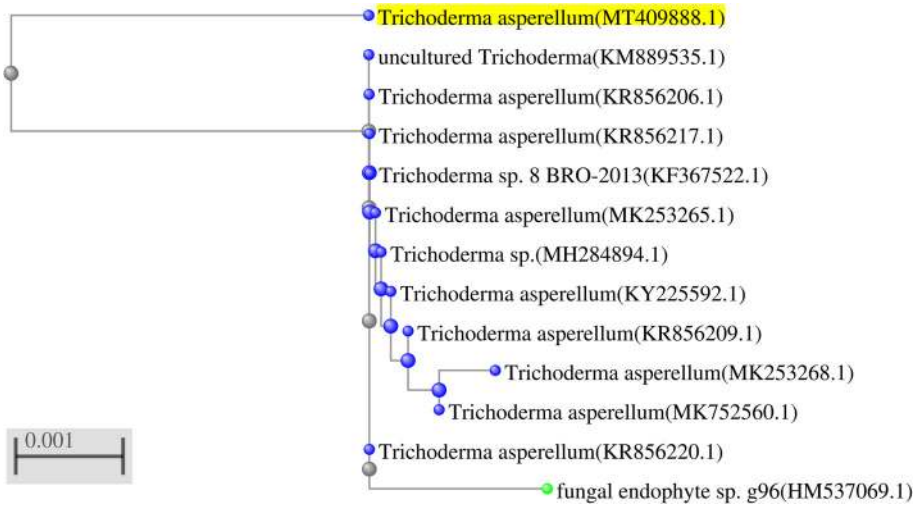

Fig. 4 Close homologs of *Trichoderma asperellum* strain S11 (MT409888.1) shown in a phylogenic tree

95% query coverage. The next closest homolog was the *T. longibrachiatum* isolate MIAE00828 (GenBank accession No. KM225906.1) (Fig. 3), whereas isolate S11 was identified as *Trichoderma asperellum* and was the most similar to the *T. asperellum* strain BHU-BOT-RYRL14 (GenBank accession No. KR856220.1) and *Trichoderma asperellum* strain BHU-BOT-RYRL11 (GenBank accession No. KR856217.1), respectively, (Table 1 and Fig. 4). In addition, the BLAST search showed that the highest match of isolate PHYTAT7 was the *Trichoderma atroviride* strain KABOFT6 (GenBank accession No. MT604177.1) with 100% sequence similarity (Table 1 and Fig. 5). The 16S rRNA sequences of isolates S12, S11, and PHYTAT7 were deposited within the GenBank sequence database under the accession nos. MT409889.1, MT409888.1, and MT476964.1, respectively.

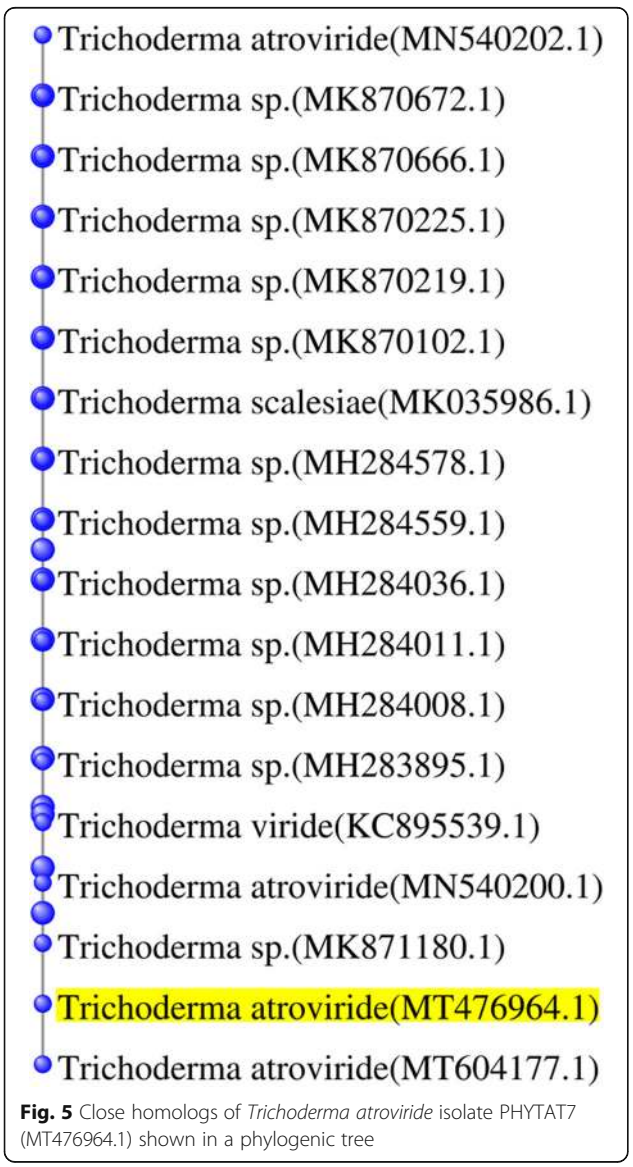

Fig. 5 Close homologs of *Trichoderma atroviride* isolate PHYTAT7 (MT476964.1) shown in a phylogenic tree

Table 2 In vivo study effect of three endophytic fungi isolates on growth of *Rhizoctonia solani* under greenhouse conditions

Fungal isolates	Disease severity (%)	Disease reduction (%)
Trichoderma longibrachiatum S12	35 [c]	57.3
T. asperellum S11	30 [d]	63.4
T. atroviride PHYTAT7	42 [b]	48.8
Infected control	82 [a]	0

*Values in the same column, followed by the same letter are non-significantly different according to LSD test ($P = 0.05$)

Evaluation of the antagonistic effect of fungi under greenhouse conditions

The results presented in Table 2 showed that all the tested isolates of *Trichoderma* spp. reduced disease severity significantly. The addition of treatments comprising *T. asperellum* (S11) afforded the highest disease reduction than other treatments. It abated disease by (63.4%) compared to the infected control. *T. longibrachiatum* (S12) came second regarding the reduction of disease severity (57.3%), whereas *T. atroviride* (PHYT AT7) caused the lowest reduction in disease severity.

In vitro study *of* the mode of action of the endophytic fungal pathogens

Siderophore production

The results presented in Table 3 showed that all tested endophytic fungi isolates gave positive responses regarding the production of siderophores. Among the fungi isolates tested, S11 produced the maximum percentage of siderophores (2.3) compared to the control, followed by S12 and PHYT AT7, which afforded a siderophore production of 2.5.

Production of IAA

The results presented in Table 3 showed that all fungal isolates tested for the ability to produce IAA yielded positive results. The highest production rate was observed at S11, followed by S12 and PHYTAT7.

Table 3 Production of siderophore and IAA, by the endophytic fungal pathogens

Fungal isolates	Siderophore (Halo cm)	IAA µg/ml
Trichoderma longibrachiatum S12	2.5 [b]	3.4 [b]
T. asperellum S11	3.3 [a]	4.5 [a]
T. atroviride PHYTAT7	2.5 [b]	3.4 [b]

*Means followed by the same letter are not significantly different according to Duncan's multiple range tests at 5%

Table 4 Solubilization phosphate index measurement of S11 and S12 and PHYTAT7 after 7 days at 28 °C

Fungal isolates	Phosphate solubilization index (PSI)			Zinc solubilization index (PSI)		
	Colony diameter (cm)	(Zone of solubilization in cm	Phosphate solubilization index (PSI)	Colony diameter (cm)	(Zone of solubilization in cm)	Zinc solubilization index (PSI)
Trichoderma longibrachiatum S12	4.3	5.5	2.27	3.5	3.9	2.10
T. asperellum S11	4.7	4.9	2.04	3.5	3.8	2.01
T. atroviride PHYTAT7	2.8	2.5	1.89	2.6	2.2	1.80

Zinc and phosphate solubilization by fungal isolates

The zinc and phosphate solubilization ability of fungal isolates (S11, S12, and PHYTAT7) were tested in vitro. The data presented in Table 4 exhibited that S11 and S12 had positive effects on zinc solubilization and scored a strong solubilization index (SI) of (2.1), while PHYT AT7 scored a moderate SI of (1.8). Moreover, S11 and S12 had positive effects on phosphate solubilization, with an SI of (2.74), while the PHYTAT7 had an SI of (1.89).

Production of lytic enzymes

The data presented in Table 5 revealed that all tested isolates exhibited protease- and cellulase-producing ability and that they produced amylase and pectinase, with the exception of the isolate PHYTAT7, which did not produce the latter two enzymes.

Discussion

Rhizoctonia root rot of soybean is one of the most important diseases in the world. This disease is caused by *Rhizoctonia solani* (Kohn), which affects seed germination and seedling emergence, causing great losses in soybean yield (Abo-Elyousr et al. 2014b). Five endophytic fungi were isolated from soybean plants. The results revealed that all the tested *Trichoderma* spp. isolates were able to inhibit the mycelial growth of the pathogen with different degrees of antagonistic capability. Obtained data indicated that *T. asperellum* afforded the strongest antagonistic effect against the pathogen, followed by other isolates. The results are in accordance with the data of Singh and Chand (2006). *Trichoderma* spp. are known to have a strong antifungal effect partly because of their production of extracellular chitinase, amylase, and protease enzymes, which hydrolyze the main constituent of the fungal cell wall (Abo-Elyousr et al., 2014b). This can be explained in light of the results reported by Benhamou et al. (1998) who suggested that *T. harzianum* is affected through direct penetration of host hyphae. *Trichoderma harzianum* grows toward the hyphae of pathogenic fungi and degrades the cell walls of the pathogens via the activity of enzymes, which could be associated with physical penetration of the cell walls of the pathogens. Endophytes may stimulate plant growth and increase yield directly or indirectly (Khare et al. 2018).

The siderophores produced by fungi can repossess the limited iron, thus reducing its availability for the growth of phyto-pathogens. Thus, they promote plant growth indirectly (Alexander and Zeeberi 1991). Obtained results showed that the *isolate S11* produced the maximum percentage of siderophores, followed by *S12* and then *PHYTAT7.* These results agree with those reported by Rabbee et al. (2019), who found that *Trichoderma* spp. produce high concentrations of siderophores, which in turn inhibit fungal growth via deprivation of essential iron. Some endophytic fungi have the ability to synthesize IAA, which may explain the increase in the growth of some plants after their colonization with endophytic fungi (Shi et al. 2009). In the present study, the 3 isolates tested for the ability to produce IAA yielded positive results. These results agree with those of Palazzini et al. (2018), and Bereika et al. (2020). Chitinases are receiving increased attention because of their broad range of applications. Many of these chitinase-producing microorganisms have been used recently as an important biocontrol agent against fungal phytopathogens, by degrading the chitin component of the fungal cell wall (Nguyen et al. 2015). Obtained results agree with those reported by Myo et al. (2019).

In the present study, all isolates had ability to solubilize zinc and phosphate with different degrees.

Table 5 Production of amylase, protease, chitinase, and pectinase enzymes by the fungal pathogens

Fungal isolates	Lysis enzymes			
	Amylase	Protease	Cellulase	Pectinase
Trichoderma longibrachiatum S12	+	+	+	+
T. asperellum S11	+	+	+	+
T. atroviride PHYTAT7	–	+	+	–

(+) = positive effects (–) = negative effects

These agree with those reported by Wani et al. (2007). Altomare et al. (1999) found that a strain of *T. harzianum* produces many chemicals that solubilize rock phosphate, Zn, Mn^{4+}, Fe^{3+}, and Cu^{2+}, increases iron availability, and enhances iron uptake in vitro. Wakatsuki (1995) reported that microbes are potential alternatives that could cater plant zinc requirements by solubilizing the complex zinc in soil. Lytic enzyme production by pathogens has been postulated to play an important role in the biological control of pathogens (Myo et al. 2019). In the present study, the ability of fungal isolates to produce lytic enzymes was tested. All tested pathogens yielded production of lytic enzymes, which act as inducers of plant resistance. Lytic enzyme production by pathogens has been reported by Myo et al. (2019). Bhale and Rajkonda (2012) reported that the activities of extracellular hydrolytic enzymes exhibited different rates according to *Trichoderma* species, such as the cellulase and pectinase produced by a fungal biocontrol agent, and are responsible for the inhibition of plant pathogenic fungi.

Conclusion

The results suggest that *Trichoderma* spp. can be used for controlling rhizoctonia root rot in soybean plants caused by *R. solani* under greenhouse conditions. Enzymes successfully suppressed the fungal pathogen in vitro and in vivo. Further research is needed to identify an appropriate formulation and approve the application of a new strain for the control of the wide range of plant diseases.

Abbreviations

IAA: Indole acetic acid; S11: *T. asperellum*; S11: *T. longibrachiatum*; PHYTAT7: *T. atroviride*; SI: Solubilization index; LSD: Least significant difference; PDA: Potato dextrose agar; ZSB: Zinc solubilizing bacterial

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Authors' contributions

All authors contributed equally in the manuscript.
N.M. S. suggested the idea of the work and contributed to data curation and their validation as well as writing original draft. EFA contributed to the formal analysis of the data., M.H. and M.S. contributed to the reviewing and editing the manuscript. All authors reviewed and approved the final version of the manuscript.

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Availability of data and materials

Not applicable

Declarations

Ethics approval and consent to participate

This manuscript is in accordance with the guide for authors available on the journal's website. Also, this work has not been published previously and is approved by all authors and host authorities.

Consent for publication

Not applicable.

Competing interests

No potential conflict of interest was reported by the authors.

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