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Trichoderma Spp. as Antagonist of *Rhizoctonia solani*

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Abstract

Trichoderma spp. are fungal species in a certain natural suppressive soil prevents the plant from infectious diseases caused by soil-borne pathogens. Among these soils borne pathogen, the fungus *Rhizoctonia solani* (*R. solani*) causes serious damages to economically significant crops and trees. The control strategies such as breeding for resistant cultivars, crop rotations, and application of fungicides are insufficient to manage diseases caused by *R. solani* because it persists in soil by producing sclerotia which is a hard-resistant structure. Moreover, fungicides are now unacceptable as they are not environment-friendly. The *Trichoderma* spp. are the potential biocontrol agents which inhibit *R. solani* by direct confrontation through mycoparasitic or antibiosis or competition as well as inducing plant defense responses. In this review paper, we provide first comprehensive report of a biological control activity (BCA) of *Trichoderma* spp. against various diseases caused by *R. solani*. We also report the cloning and functions of genes or proteins of *Trichoderma* spp. associated with suppression of diseases caused by a plant pathogen. Nevertheless, fast paced current research regarding *Trichoderma* spp. is required to fully exploit their actual potential against diseases caused by *R. solani* under field conditions.

Keywords: *Trichoderma* spp; Antagonism; *R. solani*; Interactions

Introduction

Pathogen

R. solani (R. *solani* JG Kuhn) [teleomorph *Thanatephorus cucumeris* (Frank) Donk] is an important soil born pathogen with a necrotrophic life style [1] that persist in the soil for extended periods by producing sclerotia a resistant survival structure. This fungus is a complex with more than 100 species that causes severe damage to many economically important agricultural and horticultural crops as well as trees worldwide [2]. It has a wide host range and distribution and causes sheath blight in some field crops, such as corn [3] and rice (*Oryza sativa* L.) [4]. To various extents, *R. solani* can cause seed and seedling diseases of eggplant, pepper, lettuce and zinnia [5]. It causes both stem canker and black scurf of potato (*Solanum tuberosum* L.) which lead to tuber yield reductions and losses in tuber quality [6]. The root rot disease of cotton is the most serious disease caused by *R. solani* [7]. In tomatoes, it causes root and crown rot under greenhouse conditions [8]. This is a vital fungus which causes seedling diseases of vegetables viz., seed root, root rot, pre-emergence damping off and post-emergence damping off [7]. Each year this fungus causes huge yield losses in more than hundred crops and horticultural species [9]. Recently in *R. solani* is considered an emerging problem in China. It is causing stem rot of sweet potato (*Ipomoea batatas*) [10]. This fungus is ubiquitous and cosmopolitan as saprophytes in soil and as plant pathogens. It is a species fourteen genetically distinct anastomosis groups (AG1 to AG13 and AGBI) with a unique degree of host specificity and reproductively incompatible to each other [9]. Collectively, the host-range of the *R. solani* species spans numerous plant species vital to the agriculture, forestry, and bioenergy industries, including but not limited to wheat, rice, barley, canola, soybean, corn, potato and sugar beet.

Trichoderma spp

The control of *R. solani* becomes difficult because of high survival rate of sclerotia, it's extremely broad host range and its ecological behaviour. Therefore, the strategies to control *R. solani* are limited because no cultivar is found to be complete resistance. Hence, agronomic controls such as crop rotation are heavily relied upon to fight this disease, though the polyphagous habit of some isolates can include commonly rotated crop species. Broad spectrum fungicides are also available but they have high toxicity and not eco-friendly. Moreover, chemical control methods may not be feasible nor economical for the control of many soil-borne pathogens. Hence biocontrol strategy offers an environmentally friendly alternative to protect plants from this soil born fungi. There are many studies reporting that biological control with genus *Trichoderma* (Teleomorph: Hypocrea) is found to be effective in control of *R. solani* [6] promoting plant growth as well as stimulating plant defense responses [11]. *Trichoderma* spp., are typically anaerobic, facultative, and cosmopolitan filamentous fungi that can be found in large numbers in agricultural soils and in other substrates such as decaying wood [2]. The genus *Trichoderma* (T) display a remarkable range of lifestyles and interactions with *R. solani* and can be used as biological control of plant diseases [12] (Figures 1-3).

For the control of plant pathogens, *Trichoderma* spp. [13] and/or their extracellular metabolites can be exploited as biocontrol agents or biological fungicides. These metabolites include volatile and water-soluble metabolites [14] and secondary metabolites of low molecular weight [15,16] collected 20 isolates of *Trichoderma* and found that out of all the isolates one of the *Trichoderma viride* isolate (T14) was identified as the highest producer of inorganic phosphate, IAA and siderophore exhibited high antagonistic against *R. solani*. *Trichoderma* spp. proteins association involved in the synthesis of deleterious secondary metabolites, completion, recognitions, signal transduction and genetic reprogramming of gene expression as well as in mycoparasitism of *R. solani*. Thirty-five strains of *T. viride* and *T. harzianum* were screened for their antagonistic ability against the rice sheath blight pathogen *R. solani* [17]. Biocontrol of *R. solani* in tomatoes cultivated under greenhouse and field conditions were analyzed by *T. harzianum* [18]. The rice sheath blight caused by *R. solani* was controlled by *T. asperellum* in tropical lowland rice [19]. The damping off greenhouse grown crops

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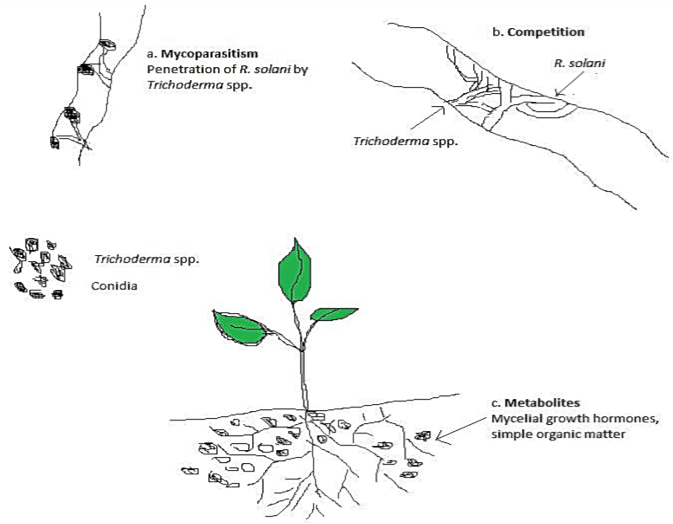

Figure 1: Activities of *Trichoderma* spp. in rhizosphere against *R. solani* (Sketch is drawn by author).

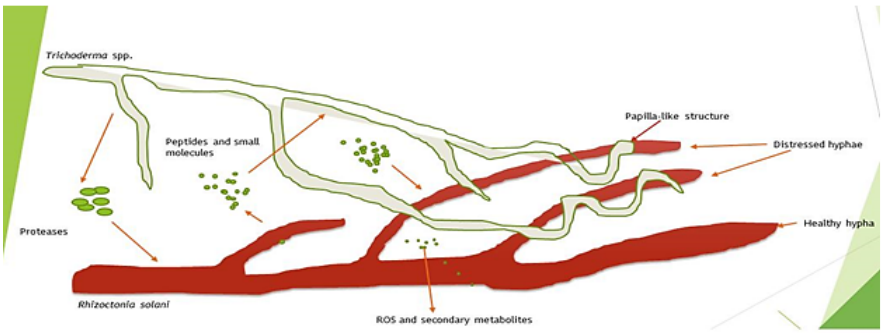

Figure 2: Schematic representation of the Mycoparasitism by which *Trichoderma spp*. antagonize *R. solani* within the soil community.

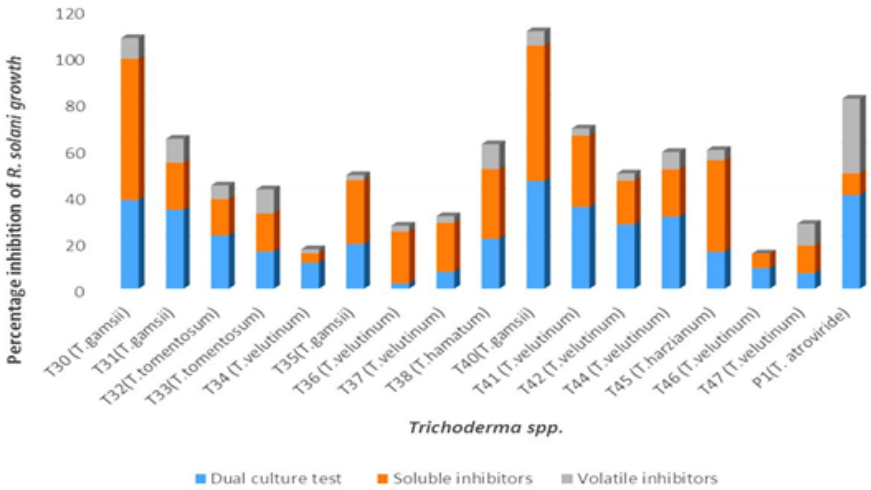

Figure 3: *In vitro* antagonism activity of *Trichoderma* spp. against *R. solani* [13].

caused by *R. solani* has been controlled with a formulation of various *Tricoderma* spp [5]. *T. virens*, demonstrated somewhat better control of stem canker of potato suggesting that this approach may provide improved biocontrol efficacy [6]. Sixteen isolates of *Trichoderma* spp. originating from the fields of sugar beet have been characterized where disease patches caused by *R. solani* were observed. Different antagonisitic mechanism was evident from their studies. However, the most antagonistic strain T30 was identified as *T. gamsii* [12].

Moreover, the biocontrol abilities of water soluble and volatile metabolites of *Trichoderma* spp (*T. asperellum*, *T. harzianum*) were evaluated against *R. solani* on bean plants under laboratory and greenhouse conditions [20]. First-time microsclerotia and submerged

Trichoderma spp.	Metabolites	Disease control
T. lignorum, T. virens, T. hamatum, T. harzianum and *T. pseudokoningii (Rifai)*	Unknown inhibitory substances; Extracellular metabolites or antibiotics, or lytic enzyme action	Damping-off of bean
T. virens isolates GL3 and *GL21; T.*	Antibiotics gliovirin and gliotoxin,	Damping-off of cucumber

Table 1: *Trichoderma* spp. antagonism against diseases caused by *R. solani.*

Trichoderma spp.	Trade name	Manufacturer	Country Registered
T. harzianum	Root Shield™, BioTrek 22G™, Supresivit™, T-22G™, T-22HB™	BioWorks, Wilbur-Ellis, Borregaard	USA, Europe
T. spp.	Promot™, Trichoderma 2000, Biofungus	J.H. Biotech, Mycontrol, Ltd., De Ceuster	USA, Belgium
T. viride	Trieco	Ecosense Labs	India
T. harzianum	Trichoderma 2000	Mycontrol (EfA1) Ltd.	Israel
T. harzianum	TUSAL®		Spain

Table 2: List of *Trichoderma* spp. based BCAs used against *R. solani.*

Trichoderma spp.	Reference
T. viride, T. harzianum	[18]
T. viride	[31]
T. harzianum, *T. virens* and *T. atroviride*	[33]
Trichoderma spp. isolates	[32]
T. asperellum	[20]

Table 3: Uses of various *Trichoderma* spp. for the control of sheath blight of rice.

conidia were formed by *T. harzianum* Rifai strain T-22 using liquid culture fermentation. These microsclerotia formulations reduced or eliminated damping off seedling caused by *R. solani* [21]. Root rot of cotton has been controlled by various *Tricoderma* spp (*T. harzianum, T. viridae, T. viresn, T. hamantum, T. konkoningii, T. pseudokoningii* and *Trichoderma* species) [7]. A new *Trichooderma* species *T. saturnisporum* has been recently discovered as a new biological control agent [22] and can be used against *R. solani* in future studies. In another report, *T. harzianum* induces the expression of plant defense related genes and produce high amount of ergosterol, indicating its ability to grow at a higher rate in soil which would explain its positive effects on bean plants growth and defence in presence of *R. solani* which causes root rot in beans [1]. *Trichoderma* spp. are also known to produce different antibiotic substance e.g. gliotoxin, gliovirin, viridin and trichoviridin [13]. *Trichoderma* spp. antagonism against various diseases caused by *R. solani* is shown in Table 1.

However, the commercial use of *Trichoderma* spp as biocontrol agents needs accurate identification, adequate formulation, and studies of the synergistic effects of their mechanisms of biocontrol [23]. *Trichoderma* spp. are the most successful bio-fungicides used in today's agriculture with more than 60% of the registered bio-fungicides worldwide being *Trichoderma*-based (Table 2).

The major limitations of microbe-based fungicides are their restricted efficacy and their inconsistency under field conditions. The origin of these difficulties is that microbes are slow to act, compared to chemicals, and are influenced by environmental factors [24-26]. Recently two new species of Trichoderma i.e. *Trichoderma shennongjianum* and *Trichoderma tibetense* have been isolated from soil samples from the Hubei and Tibet regions of China [27] and hopefully, these two-new species will be applied to manage *R. solani* in Future.

Biocontrol of damping off

R. solani causes several types of symptoms depending on host phenology at the time of infection, i.e. damping-off at initial stages or necrosis and sclerotium formation. Its spread in soil is sustained by organic matter (saprotrophic spread) or tissues of the infected host (pathogenic spread) through translocation processes. A biocontrol formulation system consisted of mixing vermiculite, powder wheat bran and biomass of isolates of Trichoderma and Gliocladium were used to control damping off in pepper and cucumber in green house [5]. An experiment was performed in pots to assess the *in vivo* disease-control efficiency of *T. harzianum* strain SQR-T37 and bio-organic fertilizer. The results indicate that the mycoparasitism was the main mechanism accounting for the antagonistic activity of SQR-T37. In one experiment, the population of *R. solani* was decreased from 10(6) internal transcribed spacer (ITS) copies per gram soil to 10(4) ITS copies per gram soil by the presence of the antagonist. In this experiment, 45% of the control efficiency was obtained when 8 g of SQR-T37 SQR-T37 hyphae per gram soil was applied. In a second experiment, as much as 81.82% of the control efficiency was obtained when bio-organic fertilizer (SQR-T37 fermented organic fertilizer, BIO) was applied compared to only 27.27% of the control efficiency when only 4 g of SQR-T37 hyphae per gram soil was applied. The results indicated that SQR-T37 was a potent antagonist against *R. solani* in a mycoparasitic way that decreased the population of the pathogen. Applying BIO was more efficient than SQR-T37 application alone because it stabilized the population of the antagonist [28]. Recently the first report emerges regarding microsclerotia and submerged conidia of *T. harzianum* formations through liquid culture fermentation. Then amending pots with dried microsclerotia of *T. harzianum* reduced or eliminated damping off melon seedlings caused by *R. solani* [21]. *Trichoderma harzianum* was used for controlling of tomato damping-off disease caused by *R. solani* in the greenhouse experiments. The percentage of infection using the bio-agent *T. harzianum* at concentrations of 5 g/kg and 10 g/kg seed were 3.33% to 16.67% compared with the untreated control which ranged 30.00% to 40.00% [29].

Biocontrol of rice sheath blight

Crop damage caused by sheath blight can decrease yield by unto 45%. Successful biological control of sheath blight by the bioagent *Trichoderma* spp. has been recorded [17,30-32] studied an experiment to evaluate the potential of indigenous *Trichoderma* spp. against *R. solani in vitro* as well as in the glass house. *In vitro* experiment showed that several strains belonging to *T. harzianum*, *T. virens* and *T. atroviride* revealed excelled biocontrol. These potential antagonist strains were further evaluated for their effectiveness in controlling sheath blight under glasshouse conditions. Among the 55 selected strains seven significantly controlled the disease [31] demonstrated that the application of isolates of *Trichoderma* isolates (T06, T09, T12, T52) to rice plants, grown under greenhouse conditions resulted in increased biomass, root length and plant size and reduced the severity of sheath blight. Among the known mechanisms involved in achieving these results was the production of phytohormones such as an indoleacetic acid (IAA), the production of biomolecules involved in metabolic pathways that cause walling off the *Trichoderma thallus*, phosphate solubilization and induced systemic resistance [19] conducted an experiment to control sheath blight by *T. asperellum* in tropical lowland rice and their results showed that moisture of four isolates of *T. asperellum* reduced disease severity by 19%, increased grain weight by 34% and increased yield by 41%.

Biocontrol of black scurf of potato

Stem canker or black scurf caused by *R. solani* reduce tuber yield and quality. *Trichoderma* spp. demonstrated somewhat better control of these diseases suggesting that this approach of using *Trichoderma* spp may provide improved biocontrol efficacy. *Trichoderma harzianum*, nonpathogenic Rhizoctonia (np-R) and cattle manure compost amendment (CMC-H) applied in furrow could reduce black scurf incidence in organically grown potatoes. Incorporation of *T. harzianum* applied to the soil surface had a relatively small effect compared to the in-furrow treatment. Application of two isolates of nonpathogenic-binucleate Rhizoctonia (RS 521 and RU 56-8-AG-P) also significantly reduced the incidence of infected tubers in field experiments. Although treatments significantly reduced disease incidence and severity, total yield was unaffected. For the first time, the efficiency of *T. harzianum* and np-R in reducing the incidence of black scurf on daughter tubers was demonstrated using naturally infested soil and contaminated seed tubers [33]. *Trichoderma* spp. were regularly tested against black scurf disease of potatoes in a series of greenhouse experiments. Among these *Trichoderma* spp. such as *T. virense* and *T. harzianum* both spore suspension and commercial form of *T. virens* significantly reduce the severity of black scurf caused by *R. solani* as compared to *T. harzianum* [6] (Figure 4).

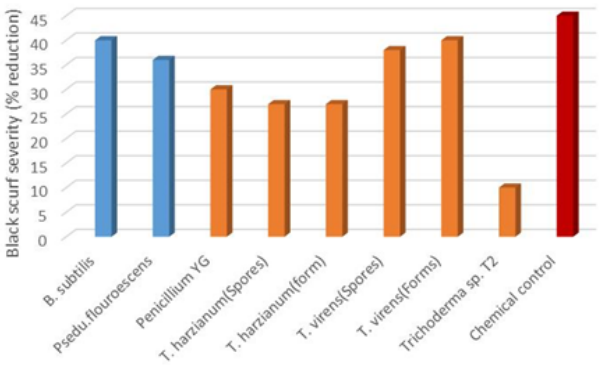

Figure 4: Black scurf severity of potato by selected biocontrol treatments and a chemical control.

Proteins	Encoding gene	Accession number (Gene Bank)	*Trichoderma* spp.
Peptaibol	N.A.	N.A.	*Trichoderma* spp.
Lipoxygenase	N.A.	N.A.	*T. atroviride*
Polyketide synthases (PKS)	*pks4*	N.A.	*T. atroviride*
	pks4	N.A.	*T. reesei*
	pks4	N.A.	*T. virens*
GliC Cytochrome P450	*gliC*	N.A.	*T. virens*
	gliF	N.A.	*T. virens*
γ-glutamyl cyclotransferase-like protein	*gliK*	N.A.	*T. virens*
Glutathione S-transferase	*gliG*	N.A.	*T. virens*
Methyltransferase	*gliN*	N.A.	*T. virens*
	gliN		
NRPS modules	*gliP*	N.A.	*T. virens*
O-methyltransferase	*gliM*	N.A.	*T. virens*
Cytochrome P450 monooxygenases	*tri4*	FN394496	*T. arundinaceum*
Major facilitator superfamily transporter	*Thmfs1*	JN689385	*T. harzianum*
L-amino acid oxidase	*Th-LAAO*	GU902953	*T. harzianum*
ABC transporters	*Taabc2*	AY911669	*T. atroviride*

Table 4: *Trichoderma* spp. proteins associated with antagonism and involved in the synthesis of secondary metabolites deleterious to *R. solani* [40,58].

Another *Trichoderma* strain was obtained via dilution methods from the rhizosphere soil of continuously cropped potato in Tiaoshan farm, Jingtai County, Gansu province. The strain was identified as *T. rossicum* based on morphological characteristics combined with ITS sequence analysis. This was the first record of *Trichoderma* species in China. The antagonism of this *Trichoderma* spp. was tested via dual culture and the inhibition index was found to above 0.70 shown that there was potential for this strain to be applied against *R. solani* [34,35]. Microbial preparations of *T. harzianum*, effective microbe (EM) culture and biological potassium fertilizer (BPF) were evaluated for the management of soil born inoculum of black scurf of potato caused by *R. solani*. These preparations were applied in three dosages to know their efficacy in reducing the disease. This application reduces the disease significantly, contributed to better crop stand and increased yield as compared to inoculated control and rest of the treatments [35]. In another study to control black scurf of potato caused by *R. solani* four commercial biocontrol formulations (*Bacillus subtilis* GB03, *Burkholderia ambifaria* type Wisconsin isolate J82, *T. virens* Gl-21, and *T. harzianum* strain T-22), a chemical seed treatment (thiophanate-methyl, mancozeb, and cymoxanil mixture, TMC), and a combination chemical/biological treatment, were compared with no-pathogen and pathogen-treated controls. All treatments reduced the incidence and severity of stem canker (37% to 75% reduction) relative to the pathogen control over both years, with the best control provided by the combination of chemical/biological treatment (TMC/Bamb). This research indicates that biocontrol treatments can assist in the control of Rhizoctonia disease of potato, persist in soil to some degree, and have significant effects on soil microbial communities long after application [36].

Biocontrol of root and crown rot

Root and crown rots are severe diseases among various plants. Though many pathogens are responsible for causing root and crown rots. Among these pathogens, *R. solani* is more significant. *Trichoderma* spp. have proved to be effective biocontrol agents (BCAs) of *R. solani* that cause crown and root rot in certain host plants. *T. harzianum* mutants (Th650-NG7, Th11A80.1, Th12A40.1, Th12C40.1 and Th12A10.1 and ThF2-1) have been applied to manage root and crown rot disease caused by *R. solani* in tomatoes under green house and field conditions. Among the *Trichoderma* mutants (Th11A 80.1, Th12A10.1 and Th650-NG7) prevented tomato plants from 100% mortality. Canker level was also reduced as well as increase in other plant parameters (Development, fresh and dry weights) were observed) [18]. Root rots diseases caused *R. solani* pathogen is also the main disease in bean plants. Elevated level of incidence of root rots and reduction of yield were observed in bean plants. A study was conducted in Leon where root rot of bean is a common disease. This disease has been detected in almost 91% bean plants [37]. Infection caused by *R. solani* usually occurs through wounds or by a coating of an organ with mycelium. The mycelium then tears the cuticle and penetrates the epidermal cells. The pathogen is more aggressive in moist soil and at temperature 15°C and 18°C [38]. *T. harzianum* has been applied to manage root rot of beans in the Leon area. It increases the resistance of bean plants caused by root rot of *R. solani* and induces the expression of plant defense related genes. Further, the presence of high level of ergosterol was observed in beans plants which are a really positive effect on plant growth and defense against *R. solani* [1]. Root rot caused by *R. solani* is also a problem in cotton plants especially in areas

having a warm climate and irregular rainfed conditions. Both species of cotton viz. *hirsutum* and *arboreum* are affected with by root rot disease. Various *Trichoderma* spp (*T. harzianum*, *T. viride*, *T. virens*, *T. hamantum*, *T. koningii*, *T. pseudokoningii* and *Trichoderma* species) inhibited variably (15.32% to 88.12%) the *in vitro* growth of *R. solani*. The best biocontrol genes are recorded in *T. koningii* MTCC 796 for mycoparasitic activity to restrain the growth of test pathogen *R. solani* followed by *T. viride*. These mycoparasitic *Trichoderma* spp produce triterpenes during antagonism to inhibit the growth of *R. solani* [7].

Interaction of genes or proteins of *Trichoderma* spp. with *R. solani*

The mechanism by which *Trichoderma* strains displace phytopathogenic are essential of three types; direct competition for space or nutrients (Table 3), production of antibiotic metabolites whether volatile or non-volatile nature and direct parasitism of certain species of *Trichoderma* on plant pathogenic fungi [39]. Various genes have been cloned from *Trichoderma* spp. to use against *R. solani*. The first gene which was isolated from *T. virens* was *Tvsp 1*. This gene was cloned successfully and its function was analyzed and found that it encodes serine protease. This serine protease is used to control *R. solani* which affects the cotton seedlings. This gene was expressed in *Escherichia coli* and the pET-30 vector was used for their cloning. It was found degradation of the fungal cell wall by serine protease enzyme [40].

The other gene *trichodiene* gene *tri5* was isolated from *T. harzianum*. The characterization of this gene revealed that *tri5* gene was responsible for the synthesis of the enzyme trichothecene which inhibits the protein and DNA synthesis in the cells of the *R. solani* and inhibits their growth. The presence of *tri5* gene was confirmed by screening with other *Trichoderma* isolates [41]. The fungal cell wall degrading enzyme exo-β-1,3-glucanase was encoded by another gene and this enzyme showed strong parasitic activity against *R. solani*. This gene was isolated from *T. aspererllum* and characterized. The expression analysis of this gene was studied using real-time and reverse transcription-polymerase chain reaction (RT-PCR) [42]. Two various kinds of glucanases (β-1,3 and β-1,6 glucanase genes) isolated from *T. virens* found that these genes secrete cell wall degrading enzyme that helps in the biocontrol activity against *R. solani*. *T. virens* GV29.8 wild type and double over expression (DOE) transformant strains were used to detect the enzyme activity against pathogens like *R. solani* [43].

The gene *gluc 78* was isolated, cloned and sequence from *T. atroviride* and found that this gene has its significance in the cell wall degradation of the pathogens. The gene *gluc78* was cloned into pGEM-T-vector and the expression analysis was done against *R. solani* [44]. The g-protein α subunits genes, *TgaA* and *TgaB* were isolated and characterized from *T. virens* and found to exhibit strong antagonist activity against *R. solani* and *Sclerotium rolfsii* [45]. The other special enzyme endo-polygalacturonase which involves in cell wall degradation of *R. solani* as well as assists in the plant beneficial interactions was found to encode by the gene, ThPG1 which was isolated from *T. harzianum*. The expression study of this gene was studied by comparing the wild and mutant type strains. The full-length cDNA clone of ThPG1 gene was obtained by polymerase chain reaction and was cloned in the pSIL-pG1 vector. The phylogenetic relationship was obtained by Neighbor-joining (NJ) tree method [46]. Two clones-acetyl-xylane esterase AXE1 and endoglucanase Cel61b-showed significant upregulation during *in vivo* confrontation of a *T. harzianum* strain that successively demonstrated a very high antagonistic capability towards *R. solani*, while expression was progressively lower in a series of *T. harzianum* strains with intermediate to poor antagonistic activity. These clones are promising candidates for use as markers in the screening of improved T. *harzianum* biocontrol strains [47]. Recently a high throughput sequencing approach was utilized to conduct a comparative transcriptome analysis of *Trichoderma* spp interactions with *R. solani*. This approach was initially used to carry out comparative transcriptome analysis of *Trichoderma atroviride* IMI206040 during mycoparasitic interactions with the plant-pathogenic fungus *R. solani*. In this study, transcript fragments of 7,797 *Trichoderma* genes were sequenced, 175 of which were host responsive. According to the functional annotation of these genes by KOG (eukaryotic orthologous groups), the most abundant group during direct contact was "metabolism." Quantitative reverse transcription (RT)-PCR confirmed the differential transcription of 13 genes (including *swo1*, encoding an expansin-like protein; *axe1*, coding for an acetyl xylan esterase; and homologs of genes encoding the aspartyl protease papA and a trypsin-like protease, pra1) in the presence of *R. solani* [48].

The novel *hmgR* gene of *T. koningii* strain MTCC 796 is liable to be express hmg-CoA reductase which is a key enzyme for regulation of terpene biosynthesis and mycoparasitic strains produced triterpenes during antagonism to inhibit the growth of *R. solani* [7]. The production of chitinase and antifungal metabolites in *T. atroviride* are because of *Tga1* gene which is a G protein α subunit. These proteins degrade the cells walls of *R. solani*. The sequences of this genes were cloned in Pgem-t vector and characterized. Moreover, the dual culture technique was used to determine the antifungal activity by planting wild-type and mutant Δtga1 strain of *T. atroviride* against *R. solani*. The antifungal activity between the wild and mutant type strains was analyzed by altering the *tga1* gene [49]. The other transcription factor Thctf1 isolated from *T. harzianum* which encodes 6-pentyl-2H-pyran-2-one (6-PP) and shows antifungal activity against *R. solani*. The sequences were analyzed using Laser gene package and cloned using pGEM-T vector [50]. Similarly, the transcription factor TmkA which is a mitogen-activated protein kinase isolated from the *T. virens* and found to have myco-parasitic activity against *R. solani* and *S. rolsfii* [51]. The gene *qid74* which is related to cell protection was isolated from *T. harzianum* CECT 2413 and was found to play an important role in cell protection and provide adherence to hydrophobic surfaces that help the fungus in mycoparasitic activity against *R. solani* pathogen. The function of this gene was studied by comparing the expression of genes in wild-type transformants and disruptants. The results showed that *qid74* gene was responsible for adhesion to the hydrophobic surfaces of the pathogenic fungi and helps in the antagonistic activity [52].

A gene *Taabc2* has a significant role in ATP binding cassette (ABC) transporter in cell membrane pumps was cloned from *T. atroviride* and characterized. This gene also has mycoparasitic activity. The expression of this gene was found to be more when they uptake the nutrients from the pathogenic fungi. The gene was cloned using a pGEM-T vector, expression of the genes was analyzed using RT-PCR. The antagonist activity against pathogens such as *R. solani* was done by dual culture plate assay with *T. atroviride* wild and mutant type strains [53].

Moreover, the genes encoding for proteinase prb1 and endochitinase ech42 were isolated and characterized from *T. harzianum* genes. These genes involved in the mycoparasitic activity against *R. solani*. For the production of these enzymes, the genes were induced by lectin-carbohydrate interaction a diffusible factor. This factor regulates the production of proteinase and endochitinase which helps in the mycoparasitism [54]. In *T. virens*, an adenalyte-cyclase encoding gene named *tac1* gene was isolated and cloned. This gene has its role in mycoparasitic activity against *R. solani* and *P. ultimum*

[55]. Recently a high throughput sequencing approach was utilized to investigate the transcriptome analysis of *Trichoderma* spp. during mycoprasitic interactions with the plant pathogenic fungus *R. solani*.

In a research conducted by [48] sequenced 7797 *Trichoderma* genes transcript fragments and found that 175 were host responsive. The functional annotation of these genes revealed the most abundant group during direct contact with *R. solani* was "metabolism. Moreover, the quantitative reverse transcription (RT)-PCR confirmed the differential transcription of thirteen genes in the presence of *R. solani*. In another study, the transcriptional responses of the most commonly found *Trichoderma* spp (*T. atroviride* and *T. virens*) were recently compared with *T. ressei* during confrontation with *R. solani*. Surprisingly the three *Trichoderma* spp exhibited variable transcriptomic response. Genes responsible to produce secondary metabolites, GH16 Beta glucanase, various proteases and cysteine-rich small proteins were expressed by *T. atroviride*. On the other hand, gliotoxin, precursors and glutathione were expressed by *T. virens*. The expression of differentially regulated genes by three different *Trichoderma* species indicates that these genes are orthologues present in all these three species. This information provides insights into mechanisms of interactions between *Trichoderma* spp and *R. solani* that may be exploited for the development of bio fungicides [56]. The transcription factors (TFs) from the *Trichoderma* spp. are still poorly investigated. Various TFs viz., AreA/Nit2, Msn2/Msn4, or AceI, are not involved in antifungal activity but involved in other activities like nitrogen repression, stress responses, and regulation of Plants. A transcription factor xylanase transcriptional regulator (Xyr 1) from *Trichoderma atroviride* is found to induce plant defense reactions. Surprisingly the deletion of xyr1 TFs caused in enhance completion with plant pathogens including *R. solani* [39].

The proteins of *Trichoderma* spp. associated with antagonism of *R.*

Proteins	Encoding gene	Accession Number (Gene Bank)	*Trichoderma* spp.
High-affinity glucose transporter Gtt1	*Gtt1*	AJ269534	*T. harzianum*
Endopolygalacturonase Thpg1	*Thpg1*	AM421521	*T. harzianum*
Harzianic acid	N.A.	N.A.	*T. harzianum*
Proteases	*TaPapA*	AAT09023	*T. asperellum*
	TaPapB	AAU11329	*T. asperellum*
N.A.: Not available			

Table 5: *Trichoderma* spp. associated with antagonism with *R. solani* and involved in the competition [40,58].

Proteins	Encoding gene	Accession number (Gene Bank)	*Trichoderma* spp.
Seven-transmembrane receptor Gpr1	*gpr1*	N.A.	*Trichoderma atroviride*
G-protein one	N.A.	FD484960	*T. asperellum*
G-protein ypt3	N.A.	FD486508	*T. asperellum*
G-protein rab2	N.A.	FD485766	*T. asperellum*
α-subunit of G protein 1	*tga1*	AY036905	*T. atroviride*
α-subunit of G protein 3	*tga3*	AF452097	*T. atroviride*
Mitogen-activated protein kinases (MAPK)	*tmkA*	AY141978	*T. virens*
	tvk1	AY162318	*T. virens*
Adenylate cyclase Tac1	*tac1*	EF189190	*T. virens*
pH regulator PacC	*pacC*	N.A.	*T. virens*
pH regulator Pac1	*pac1*	EF094462	*T. harzianum*
Transcription factor ThCtf1	*ctf1*	EU551672	*T. harzianum*
VELVET Protein Vel1	*vel1*	N.A.	*T. virens*
Xylanase transcriptional regulator Xyr1	*xyr1*	N.A.	*T. atrovide*
N. A.: Not available			

Table 6: *Trichoderma* spp. proteins associated with antagonism and involved in *R. solani* recognitions, signal transduction and genetic reprogramming of gene expression [40,58].

Proteins	Encoding gene	accession number (Gene Bank)	*Trichoderma* spp.
41-KDa chitinase	*chit41*	N.A.	*T. flavus*
Chitinase 1	N.A.	FD484447	*Trichoderma asperellum*
33-KDa endochitinases	*chit33*	JK840912	*T. harzianum*
	(ech33)		
	Tv-cht1	AF395753	*T. virens*
	Tv-cht2	AF395754	*T. virens*
36-KDa endochitinases	*chit36Y*	AF406791	*T. asperellum*
42-KDa endochitinases	*chit42*	N.A.	*T. atroviride*
	echi42	FD485995	*T. asperellum*
	chit42	S78423	*T. harzianum*
	(ech42)		
	Tv-ech1	AF050098	*T. virens*
	Tv-ech2	AF395760	*T. virens*
46-KDa endochitinase	*chit46*	N.A.	*T. asperellum*
Endochitinases (GH 18)	*crchi1*	X80006	*T. harzianum*
N-acetyl-β-glucosaminidases	*exc1Y*	AJ314642	*T. asperellum*
	nag1	N.A.	*T. atroviride*
	eng18B	N.A.	*T. atroviride*
	nag1	N.A.	*T. harzianum*
	Tvnag1	AF395761	*T. virens*
	Tvnag2	AF395762	*T. virens*
N. A.: Not available			
b-1,3-glucanases	*tag83*	EU314718	*T. asperellum*
	lam1.3	AJ002397	*T. harzianum*
29-KDa b-1,3-glucanase	N.A.	N.A.	*T. harzianum*
36-KDa b-1,3-glucanase	N.A.	N.A.	*T. harzianum*
78-KDa b-1,3-glucanase	*bgn13.1*	X84085	*T. harzianum*
b-1,6-glucanase	*bgn16.2*	N.A.	*T. harzianum*
	Tvbgn3	AF395757	*T. virens*
b-1,3-glucanase	N.A.	N.A.	*T. koningii*
	Tvbgn1	AF395755	*T. virens*
	Tvbgn2	AF395756	*T. virens*
Endo-1,3(4)-b-glucanase	N.A.	FD486867	*T. asperellum*
Aspartic proteases	*TaAsp*	EU816200	*T. asperellum*
	TaPAPA	AAT09023	*T. asperellum*
	Sa76	EF063645	*T. harzianum*
	P6281	AJ967001	*T. harzianum*
	N.A.	FD485588	*T. asperellum*
Serine proteases	*Spm1*	FD486577	*T. asperellum*
	prb1	AAA34209	*T. harzianum*
	tvsp1	AY242844	*T. virens*
	prb1	AAA34209	*T. harzianum*

Table 7: Trichoderma proteins associated with mycoparasitism of *R. solani* [40,58].

solani are shown in Tables 4-6. *Trichoderma* spp. proteins associated with mycoparasistim of *R. solani* are also shown in Table 7.

Discussion

In the present review paper, the interaction of *Trichoderma* spp. with *R. solani* were described. The genus *Trichoderma* comprises of a large species complex having potential as biocontrol agents against *R. solani*. *Trichoderma* isolates can parasitize hyphae, sclerotia and other structures of *R. solani*. The metabolites of *Trichoderma* spp. induce competitiveness against the pathogen and induce resistant to host plant. To identify *Trichoderma* spp three different approaches are utilized viz., molecular, functional and morphological. The functional antagonistic activity reveals the widespread intraspecific diversity among the *Trichoderma* isolates. This functional antagonistic activity also shows some additional information regarding the interaction with *R. solani* as well as biocontrol potential by the *Trichoderma* spp. Morphological and molecular approaches are mere identification but the functional approach is the best approach for isolating better isolates or strains of *Trichoderma* against the pathogen. Morphological approaches to identify a certain strain of *Trichoderma* that could be used as biocontrol agent against *R. solani* sometimes may lead to mis identification. These *Trichoderma* spp could reduce the colonization and growth of pathogen both *in vivo* as well as i*n vitro*. More water-soluble metabolites of the *Trichoderma* spp are currently being evaluated to inhibit the proliferation and growth of *R. solani* [12].

Antagonism is not a property of *Trichoderma* spp. because different strains or isolates of the same species can exhibit varying biocontrol potential against *R. solani*. The strains or isolates which genes are efficiently and rapidly expressed involved in antagonist activity against *R. solani* are infect better antagonists [57,58]. The varying mechanism of antagonistic activity of each strain highlighting the use of functional approaches to characterize and to identify good biocontrol strains of *Trichoderma* against *R. solani* [59]. Additionally, the strains of *Trichoderma* spp. recovered from the diseases fields were found to be better antagonist against *R. solani* than strains found in healthy fields [35]. Until now *Trichoderma* spp. have been successfully applied to diseases of *R. solani* mostly in Greenhouse studies with few studies conducted under field conditions [31].

The *Trichoderma* spp. are distributed worldwide and able to adjust to surrounding environmental conditions by regulating metabolism, growth, and sporulation. For the control of *R. solani* their extracellular metabolites have been continuously used as biological fungicides [14]. There metabolites were found to be B volatile as well as water soluble. Low molecular weight secondary metabolites were also evaluated against *R. solani* [15]. Moreover, *Trichoderma* spp avails more space and nutrients as compared to a pathogen which provides them a competitive advantage [60].

As far as interaction between *Trichoderma* spp. and *R. solani* is concerned, the mycoparistim is regarded as a major activity. *Trichoderma* spp. sense small molecules released by *R. solani* (Figure 3). Some of these molecules may be released by proteases enzymes. These molecules then bind to G-protein coupled receptors or nitrogen sensing receptors on the surface of *Trichoderma* spp. hyphae thereby eliciting a signaling cascade comprising G proteins and mitogen-activated protein kinases(MAPKs) which may then finally modulate unknown transcription factors(TFs). These factors encode enzymes for the biosynthesis of secondary metabolites. Lectins from *R. solani* and proteins protein harboring cellulose binding modules from hypha of *Trichoderma* spp. may collaborate in the attachment of *Trichoderma* spp. to the pathogen. This mycoparasites is expressed in different steps in a sequence viz., selection, attachment, direct penetration and secretion of fungi toxic enzymes. Moreover, the *Trichoderma* spp. are showing affinity of cell wall of *Trichoderma* spp. and *R. solani* which then lead to host cell wall penetration [15]. *Trichoderma* spp. are also suppressing *R. solani* by producing antifungal compounds. The antifungal compounds include antibiotics, mycotoxins and lower weight secondary compounds [15]. *Trichoderma* spp. are also well knowing plant growth regulators. They proliferate root and increase the yield by uptake of nutrients [19]. As compared to fungicides the effect of *Trichoderma* spp. against *R. solani* is higher because it persists in soil for a longer period after application [7].

Currently, functional genes and corresponding traits have been amplified by Scot Markers. These markers have become the maker of choice having high polymorphism and reproducibility [61]. The Scot polymorphism or Scot analysis is low cost and effective to use. Activation of biocontrol genes varied with various *Trichdorma* spp. are only triggered where there is contact with *R. solani* [62,60].

Moreover, the recent advent of genomic and transcriptomic data regarding the interaction *Trichoderma* spp. with *R. solani* has provided a wealth of information that allows a deeper understanding of this important fungal genus. Some of the molecular aspects such as the regulation and role of cell wall degrading enzymes and antagonistic secondary metabolites of *Trichoderma* spp. have been studied. The use of subtractive hybridization techniques, proteomics or expressed sequence tag (EST) approaches have been recently used with different *Trichoderma* spp. EST transcript of *Trichoderma* spp. have been used to understand and characterize its transcriptomes [63]. The full genomic analysis of *Trichoderma* spp. offered the opportunity to carry out as the systematic and comprehensive study of transcriptional response to the presence of *R. solani*. The availability of ESTs and annotated genomes have now raised the possibility to understand the interaction of *Trichoderma* spp. with *R. solani* in a better way. Recent transcriptomic analysis revealed that there is no common mechanism by which *Trichoderma* spp. attacks and kills *R. solani* but alternative strategies are used.

Conclusion

The pathogen *R. solani* is no doubt key determinant of most of our economically important crops by causing severe crop losses. *Trichoderma* spp. play a key role as biocontrol agents against diseases caused by *R. solani*. Information on mechanisms of antagonism as well as interaction with *R. solani* has been well documented. However, more research is needed for the wide-scale commercialization of the *Trichoderma* spp. against *R. solani*. To enhance the marketability of these fungi as BCAs, feasible commercial production processes are of utmost importance. The pursuit for isolating and cloning of *Trichoderma* genes which are interacting with *R. solani* is on and several encouraging results are being reported by researchers worldwide. With the increase in knowledge regarding the genes and proteins (Proteomics and Transcriptomics) of both *Trichoderma* spp. and *R. solani*, thus, it is expected that in near future, exploitation of this *Trichoderma* spp. would be maximized. Most of the molecular interactions between *Trichoderma* spp. and *R. solani* have been carried out in dual cultures. There is a need to considering soil microcosm having no of *Trichoderma* spp. It would be a good approach to understand the molecular interplay of a soil microbial community in response to *R. solani* and *Trichoderma* spp.

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Competing Interests Statement

The authors declare no competing commercial interests.

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