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Research Article

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Antagonistic Activity of *Trichoderma* Spp. to *Phytophthora* Infecting Plantation Crops and its Beneficial Effect on Germination and Plant Growth Promotion

Nidhina K, Sharadraj KM*, Prathibha VH, Hegde V and Gangaraj KP

Abstract

Trichoderma a genus under Ascomycotina, has gained immense importance since last few decades due to its biological control ability against several plant pathogens. In addition, the increased growth response induced by *Trichoderma* species has also been reported for much kind of crops. A total of sixteen *Trichoderma* strains were selected to evaluate their antagonistic behavior and plant growth promotion. PCR amplification of internal transcribed spacer regions was used to confirm the identities of *Trichoderma* isolates used. The in vitro tests against plant pathogens like *Phytophthora meadii, P. heveae, P. citrophthora, P. capsici* and *P. palmivora* in dual cultures showed all the *Trichoderma* isolates growing fast and inhibiting the growth of the pathogens by third day. Maximum growth inhibition of test pathogens was observed by antagonists CPCRI-TD-8 (72.7%) followed by CPCRI-TD-3 (71.7%) after 4 days of inoculation. The cellulase activity was also assayed by analyzing the glucose released in carboxymethyl cellulose solution with spectrophotometry. Highest cellulase activity (31.11 mg glucose liberated/mg protein/30minutes) was recorded by CPCRI-TD-8 which showed a high degree of antagonism to all tested pathogens each by more than 65%. This illustrated that the cellulolytic enzyme and metabolic products may have participated in growth suppression of test pathogens. Growth promoting activity of *Trichoderma* isolates on green gram seeds was tested under glasshouse conditions and seed germination percentage, root and shoot length, fresh weight, dry weight and vigour index were measured. All the tested *Trichoderma* isolates showed significant positive effects on vigour index in comparison with the control. Results from this study explored the plant growth promoting activity of *Trichoderma* sp. and suggests its use as a potential multifunctional bio-fertilizer.

Keywords

Trichoderma; Biocontrol; Cellulose; *Phytophthora;* Phylogeny

***Corresponding author:** Sharadraj KM, Division of Plant Pathology, Central Plantation Crops Research Institute, Kasaragod, 671124, India, Tel: 04994-232894; E-mail: sharatkskd@gmail.com

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Introduction

Trichoderma, a filamentous ascomycetes fungus frequently found in soil, growing on wood, other fungus and are able to utilize a variety of substrates demonstrating their adaptability to various ecological conditions [1]. Some strains establish long lasting colonization in root surfaces and induce localized and systemic resistance responses which explain their lack of pathogenicity to plants. They produce a wide range of antibiotics such as gliotoxin, viridin, antifungal secondary metabolites and cell wall hydrolytic enzymes [2].

Necrotropic hyper parasitism or mycoparasitism evolved by *Trichoderma* spp. is supported by many genes that are expressed before and during contact with the prey. Final death of the prey results from the sequential expression of cell wall degrading enzymes [3]. The cell wall account for approximately 30% of dry weight of the fungal cell and consists mainly of chitin, β-1,3-glucans, α-1,3-glucans and α-1,4-glucans [4]. The strains produce sensing enzymes that release cell wall fragment from the target pathogen which induce the expression of fungitoxic enzymes. The combined activities of these enzymes results in parasitism of target fungus and dissolution of cell wall.

Trichoderma spp. are well known for their ability to enhance plant growth. In rhizosphere they stimulate the plant growth by colonizing root surfaces and help to enhance root growth, plant productivity, resistance to abiotic stress and uptake of various nutrients that can be unavailable to plants in certain soils [1]. Crop productivity in fields can increase up to 30% after the addition of *Trichoderma koningii* [5]. Several studies have shown that root colonization by *Trichoderma* strains results in increased levels of defense related plant enzymes including chitinases, glucanases, peroxidases, polyphenol oxidases and lipoxygenases [6]. Moreover, these influences is sometimes crop specific or niche specific and can be altered by climatic variability and inconsistency of soil. Therefore, it is necessary to develop efficient strains in field conditions. Hence, the present study is to explore the efficacy of different *Trichoderma* strains on plant growth promotion and disease control.

Materials and Methods

Biological materials

Sixteen isolates of the *Trichoderma* spp. used in the present study were procured from Indian Institute of India Spices Research (IISR), Calicut. Phytopathogenic fungi viz., and *P. capsici* were collected from Plant Pathology Laboratory, Central Plantation Crops Research Institute (CPCRI), Kasaragod. All cultures were grew on potato dextrose agar at 28°C for 5 days and thereafter stored at 4°C.

Molecular identification of *Trichoderma* isolates

To confirm the identity of each *Trichoderma* species, amplification and further sequencing of the ITS region were performed. Extraction of total genomic DNA was done using Qiagen DNeasy plant minikit. The nuclear rDNA region of the internal transcribed spacers (ITS), including the 5.8S rDNA, was amplified with the universal primers ITS 1 (TCCGTAGGTGAACCTGCGG) and ITS 4 (TCCTCCGCTTATTGATATGC) as described by White et al. [7]. Each reaction consisted of 2 mM $MgCl_2$, 1× Buffer, 0.2

M dNTPs, 0.2 M of primers ITS1 (forward) and ITS4 (reverse), 1 U of Taq polymerase, and 1 µL of DNA (50 ng) in a 25 µl reaction volume. Amplifications were performed on a BioRad thermal cycler (BioRad, MJ Mini™), with an initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 45 sec, 55°C for 30 sec, 72°C for 45 sec, and a final extension at 72°C for 10 min. Samples were visualized on 1.5% agarose gel electrophoresis (Blue Marine) in 1X TBE buffer and sequenced. Resulting sequences were compared with BLASTn search tool against the GenBank database using default parameters. The nucleotide sequence data of each *Trichoderma* isolates has been deposited in the GenBank nucleotide sequence database.

Phylogenetic analysis

Phylogenetic analysis was performed with the ITS region sequence of *Trichodema* isolates and sequences available at NCBI database. Sequences were aligned with ClustalW followed by construction of phylogenetic tree using maximum likelihood method with the software MEGA 5.01 [8]. All positions containing gaps and missing data were eliminated. The bootstrap analysis was implemented using 1000 replicates of heuristic searches to determine the confidence levels of the inferred phylogenies.

Screening for the antagonistic activity *in vitro*

Dual culture technique was followed to assess the inhibitory effect of *Trichoderma* isolates on phytopathogens [9]. Mycelial disc (5 mm) of four days old *Trichoderma* isolates was confronted with phytopathogens in PDA plates at 60 mm distance in three replicates. After 4 days of incubation at 28°C radial growth inhibition was calculated according to Rodriguez et al. [10]. Mycoparasitism was observed in samples removed from the interaction zones according to Moussa [11].

Determination of cellulase activity

The cellulolytic enzyme production was made by growing all *Trichoderma* strains on liquid medium containing carboxy methyl cellulose as carbon source [12]. After incubating on a rotary shaker at 120 rpm for 7 days fermented culture broth was centrifuged at 9000 × g for 10 min at 4°C. Supernatant was used as crude enzyme solution for the estimation of extracellular protein content and total activity of cellulase. Protein content was estimated by Lowry's method using bovine serum albumin (BSA) as standard [13]. Cellulase production was quantitatively measured by DNS (3, 5-dinitrosalicylic acid) spectrophotometric assay as reported by Miller [14] and absorbance was taken at 540 nm using glucose as standard. The cellulase activity was estimated as the method described by Ghose [15] and calculated as amount of glucose liberated/mg protein/30minutes.

Detection of plant growth promoting traits

The growth promoting activity of the tested *Trichoderma* isolates on green gram (*Vigna radiate* (L) R. Wilczek) seeds was tested under glasshouse conditions. Surface sterilized seeds were coated with suspensions of each *Trichoderma* isolate according to a modified method proposed by Mukhtar et al. [16]. A seed coating was prepared from spore suspension supplemented with 2% of starch (w/v) as an adhesive. For untreated control seeds were dipped in sterilized distilled water. Spore suspension concentrations were adjusted to 1×10^6 conidia/ml by haemocytometer. Previously sterilized seeds were dipped in seed coating suspensions for 30 minutes and then air dried on filter paper in Petri plates for 24 hours. Dried *Trichoderma* coated seeds were sown in three replications (4 seeds per pot). Observations were recorded on 10th day and seed germination percentage; root and shoot length, fresh weight and dry weight were measured. Plant vigour index was determined as given below using the formula developed by Abdul-Baki and Anderson [17].

Plant vigour index=Germination percentage x (root length + shoot length)

All obtained data were statistically analysed using SPSS 16.0 software. The means were separated using the least significant difference at P=0.05.

Results and Discussions

The genus *Trichoderma* poses a major challenge for systematists because the phylogenetic relationships of many of its members are not clear till now. The nomenclature of these fungi is complicated because of their pleiomorphism that is, some of them can exist in two morphologically and physiologically different stages. Although several common species have lost their ability to reproduce sexually and have become clonal species or agamospecies; for example, *Trichoderma longibrachiatum, T. harzianum* and *T. parareesei* [18]. A total of 16 strains of *Trichoderma* used in these studies were identified at the species level by morphological characters using the existing taxonomic criteria (Table 1, Figure 1) analysis and by analysis of their ITS region gene sequences. Amplification of DNA sequences produced amplicons of 700 bp. The submitted nucleotide sequences of ITS regions were assigned with the GenBank accession numbers listed in Table 2. BLASTn analysis of the sequences resulted in hits with e-values of 0.0 and a maximum identity ranging from 97% to 100%. Thirteen isolates showed similarity ranging from 99 to 100% with the sequence results of *T. asperellum* (Table 2). Two strains (CPCRI-TD-1 and TD-4) showed 97% and 99% similarity with *T. harzianum*. So, these strains were identified as *T. harzianum.* One isolate (CPCRI-TD-15) was named *as T. longibrachiatum* because of its similarity with *T. longibrachiatum* was 99%. The information provided by [19] has been useful in evaluating the existing taxonomy of *Trichoderma* and the taxonomic significance of morphological characters. The dendrogram revealed that all the *T. asperellum* isolates segregated in one cluster and the strains of *T. harzianum* and *T. longibrachiatum* were not. From this tree, *T. asperellum* isolates constituted many sub clusters. *T. harzianum* was unique as it formed a separate branch and well supported by a bootstrap value of 100% (Figure 2). Many other molecular sequences data demonstrated that *T. harzianum* is a genetically variable complex, comprised by one morphological species and several phylogenic species [18,20].

The antagonistic potential of all the *Trichoderma* isolates were evaluated against *Phytophthora* sp. including *P. heveae, P. palmivora, P. citrophthora, P. capsici and P. meadii* by dual culture technique. Each of the tested *Trichoderma* isolates differentially limited the colony growth of the pathogens. The first apparent physical contact between antagonist and the pathogen occurred by 3rd day after inoculation, followed by growth inhibition. At four days of confrontation, *T. asperellum* CPCRI-TD-5 reduced mycelial growth of *P. palmivora* more than 90%, while *T. asperellum* CPCRI-TD-14 showed least inhibition (33.3%) against *P. palmivora*. This indicates that type and production of phytopathogen inhibitory compounds varies among *Trichoderma* species and among strains of the same species. Similar results were reported previously by Mpika et al. [21] whom tested thirty one *T. virens* strains that inhibited *P. palmivora* mycelia growth in a range of 33.2 to 97.8%, this inhibition was attributed to concentration of metabolites like glyotoxins, viridine,

Table 1: Cultural morphology of *Trichoderma* species grown in PDA.

Isolate number	Colony character	Conidiophore / Phialide character	Conidial shape
CPCRI-TD-1 & TD-4	Colonies growing rapidly, appear granular or powdery due to conidiation, rapidly turning yellowish green to dark green.	Phialides tend to be held at right angles with respect to other phialides.	Subglobose to obovoid
CPCRI-TD-2, TD-3, TD-5, TD-6, TD-8, TD-9, TD-10, TD-11, TD-13, TD-14, TD-16, TD-25 & TD-28	Colony appears to be a bit granular, with green conidia distributed throughout. An irregular yellow zone without conidia was present around the inoculums.	Inconspicous conidial warts, condiophores irregularly branched and branches typically paired. Phialides are straight.	Globose to subglobose
CPCRI-TD-15	Colonies growing rapidly, diffusing yellow pigment often forming on PDA and later turn dark green in color.	Conidiophores are elongate and lageniform to nearly cylindrical.	Globose to ellipsoidal

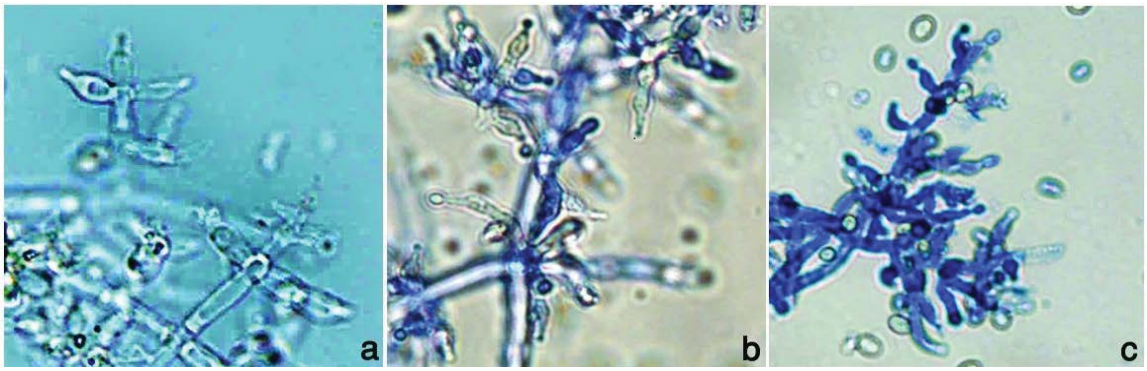

Figure 1: Morphology of *T. harzianum* (a), *T. asperellum* (b) and *T. longibrachiatum* (c) mycelia bearing conidiophores, after staining with lactophenol cotton blue.

Table 2: Identified *Trichoderma* spp. and comparison with referred GenBank.

Isolate number	Identified as	GeneBank accession number	Strains and reference	Identity (%)
CPCRI-TD-1	*T.harzianum*	LC076296 (DDBJ)	JN039045	97%
CPCRI-TD-2	*T.asperellum*	KT445766 (NCBI)	KC993073	100%
CPCRI-TD-3	*T.asperellum*	KT445767 (NCBI)	GU318216	100%
CPCRI-TD-4	*T.harzianum*	LC075589 (DDBJ)	KC819133	99%
CPCRI-TD-5	*T.asperellum*	KT445768 (NCBI)	GU318216	99%
CPCRI-TD-6	*T.asperellum*	LC075712 (DDBJ)	KC859434	99%
CPCRI-TD-8	*T.asperellum*	LC075713 (DDBJ)	KC479809	99%
CPCRI-TD-9	*T.asperellum*	LC075714 (DDBJ)	KC479809	99%
CPCRI-TD-10	*T.asperellum*	LC075715 (DDBJ)	KC859434	99%
CPCRI-TD-11	*T.asperellum*	KT445769 (NCBI)	KC479809	99%
CPCRI-TD-13	*T.asperellum*	LC075800 (DDBJ)	KC859434	99%
CPCRI-TD-14	*T.asperellum*	KT445770 (NCBI)	GU318216	99%
CPCRI-TD-15	*T.longibrachiatum*	LC075801 (DDBJ)	KP326575	99%
CPCRI-TD-25	*T.asperellum*	KT445771 (NCBI)	KC479809	100%
CPCRI-TD-16	*T.asperellum*	LC075802 (DDBJ)	KP763520	100%
CPCRI-TD-28	*T.asperellum*	LC075803 (DDBJ)	KP763517	100%

trichodermine, furanone, and 6-pentyl-α-pyrone. Reduction of mycelial growth was correlated with *Trichoderma* isolate which significantly exhibited an antagonism effect against the pathogen (Figure 3). *T. asperellum* isolates *viz.,* CPCRI-TD-3, TD-8 and TD-25 were significantly superior to others since they were able to inhibit the growth of five pathogens each by more than 60% (Table 2). This antagonistic activity of *T. asperellum* agrees the report by Tondje et al. [22] which indicated a more inhibitory action on *P. megakarya*, virulent agent causing the black pod disease of cacao. Seven isolates provided inhibitory rate ranged from 52.38 to 92.86%, while nine isolates, exhibited less than 50% antagonism against the pathogen. Nevertheless, *T. harzianum* CPCRI-TD-1 revealed a lesser inhibitory activity on the mycelial growth of all pathogens tested. This activity contrast with the potent agents for the biocontrol of plant pathogens known as filamentous fungus *T. harzianum* [23].

According to Papavizas and Lumsden [24], the mechanisms involved in the control of pathogens by *Trichoderma* spp. are probably: antibiosis, lysis, competition and mycoparasitism. Several morphological changes were seen when inhibition zone were analysed under light microscope. *T. asperellum* strains viz., CPCRI-TD-25, TD-14, TD-9, TD-10 and TD-13 were capable of overgrowing and degrading *Phytophthora* mycelia, coiling around the hyphae with apressoria and hook-like structures. Lysis of hyphae of *P. capsici* with close contact of *T. longibrachiatum* (CPCRI-TD-15) hyphae was observed. According to Elad et al. [25], hyphal lysis is due to enzyme activity of *Trichoderma* isolates. Swelling of *P. citrophthora* hyphae with the mycelium of *T. harzianum* CPCRI-TD-4 was also observed under microscope. However, some antagonists (CPCRI-TD- 2, TD-3, TD-28 and TD-5) used different mechanism by just touching the hyphae without coiling (Figure 4a). While, *T. asperellum* (CPCRI-TD-1, TD-6, TD-8, TD-11) showed spore around pathogen not

attached to hyphae (Figure 4b). Similar interactions were reported by previous workers, who noticed inhibition of growth, lysis and parasitism of some species of *Phytophthora* by *Trichoderma* spp. [25,26]. However, Ayers and Adams [27] indicated that interactions observed in vitro do not necessarily confirm their operation for the decrease in pathogen populations and reduction in diseases observed in natural conditions. Further investigations are needed in order to characterize the interactions observed during these studies.

Lysis of hyphae is a frequently cited mechanism of antagonism used by fungi against soil borne plant pathogens [28]. Most of the biocontrol agents are known to produce different cell wall degrading enzymes which results in the lysis of hyphae of pathogen. Cellulose microfibrils in *Phytophthora* cell walls should make the fungus susceptible to enzymatic destruction by cellulases (β-1, 4-glucanases) present in organic litter layers. In vitro work on *Phytophthora* protoplast production for genetic studies has shown that cellulases will completely dissolve the cell wall [29]. In this comparative study, sixteen *Trichoderma* spp. produced and secreted cellulase when grown in the medium containing carboxy methyl cellulose as carbon source. Ahmad et al. [30] worked on *T. harzianum* for cellulase enzyme production by using different carbon sources and reported that CMC is the best for substantial amount of enzyme production. The ability of crude enzyme of *Trichoderma* species to hydrolyze cellulose revealed remarkable variation in enzyme activity among different strains of same species analyzed. The data recommended that *T. asperellum* strains CPCRI-TD-8 and TD-3 illustrated promising result by exhibiting 31.11 and 28.04 mg glucose liberated/mg protein/30minutes respectively. Lower activities were detected with *T. asperellum* strains CPCRI-TD-11 and TD-14 (16.78, 17.83 mg glucose liberated/mg protein/30 minutes). These findings are in line with the work conducted by Haq et al. [31]. Even though the enzyme activity varied over a wide range among the strains it positively correlates the growth inhibition by the dual culture test. It means that the production of cellulase enzyme would play a key role in the antagonism against the pathogens tested. Many reports suggested the involvement of signal transduction pathways components such as G proteins, cAMP and MAP kinase, in controlling extracellular enzyme and coiling around host hyphae [32,33].

Novel investigations emphasize the enhanced growth response of several plants following application of *Trichoderma* spp. [34,35]. In our present study strains of different *Trichoderma* species showed a various degree of increased plant growth response as reported by Chang et al. [36]. The results suggested that treated green gram plants were significantly superior to their untreated counterparts in terms of root length and shoot length measured (Figure 5). Particularly, the root length of green gram treated with *T. asperellum* strains CPCRI-TD-9 and TD-14 were as 2 fold length as the untreated seedlings for 10 days after sowing. Similarly the dry weights of seedlings treated with *Trichoderma* species were higher than that of untreated plants (Figure 6). No significant positive effects on germination were found in treatments with *T. asperellum* – CPCRI- TD-5, TD-10, TD-13 and *T. harzianum* TD-1. Vigour indexes of plants treated with those four *Trichoderma* isolates were significantly higher than the control. Maximum vigour index was recorded with *T. asperellum* strain CPCRI-TD-16 (Figure 7). *T. asperellum* strains CPCRI-TD-1, TD-2, TD-5, TD-11, TD-14, TD-28 and *T. longibrachiatum* TD-15 recorded significantly higher germination % than the other isolates (Table 3). But there were significant differences among these isolates in terms of other growth parameters measured. Similarly, earlier workers reported a positive influence of *Trichodema* spp. to a faster

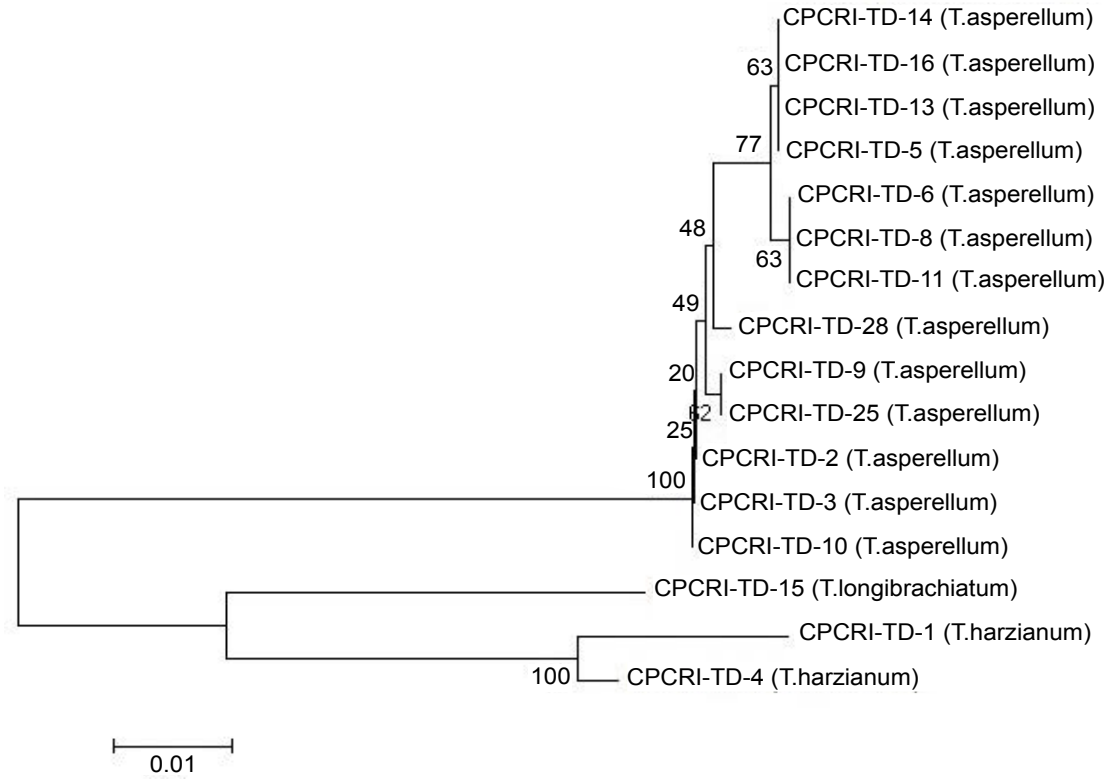

Figure 2: Phylogenetic tree depicting evolutionary relationships among species of *Trichoderma* based on ITS1-5.8S-ITS2 sequences.

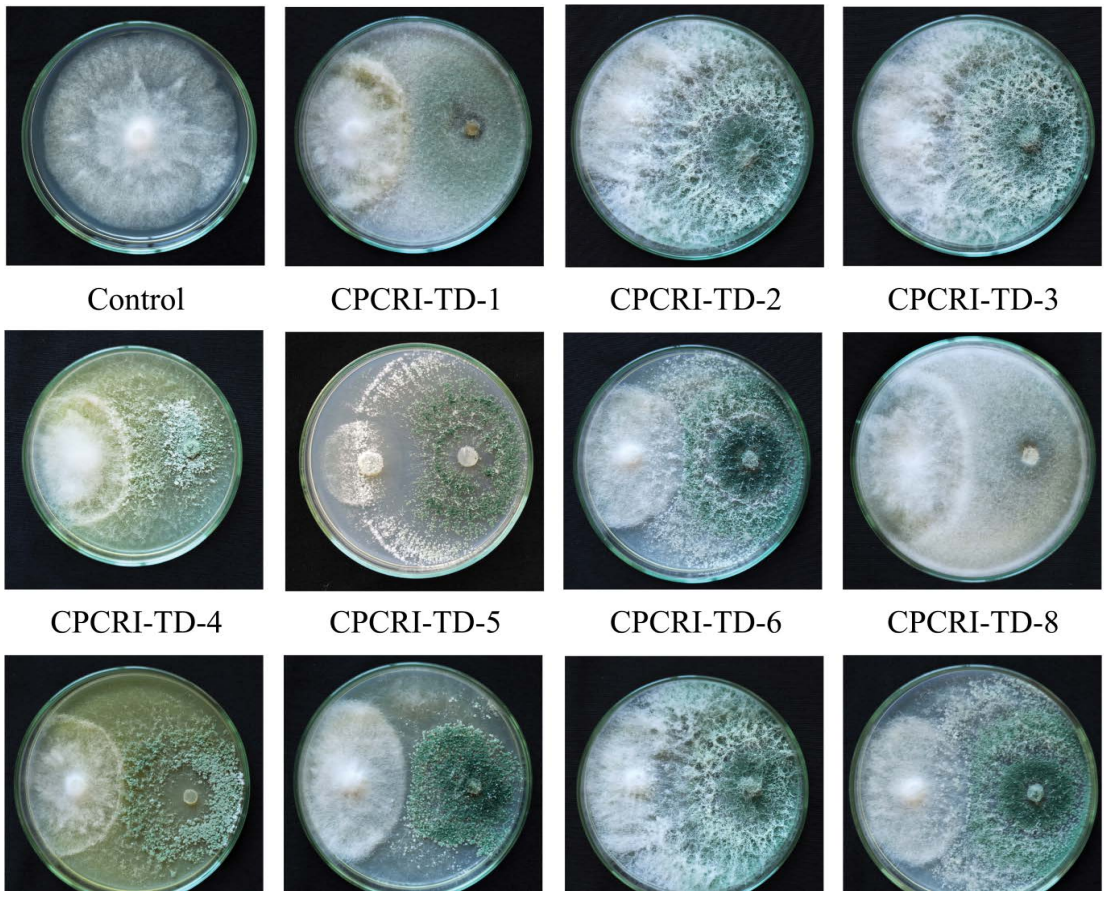

Figure 3: Antagonism between *Trichoderma* spp. and *Phytophthora meadii* at 7 DAI, (Antagonists petri dish have *Trichoderma* spp. at the right and pahtogen *P. meadii* at the left side).

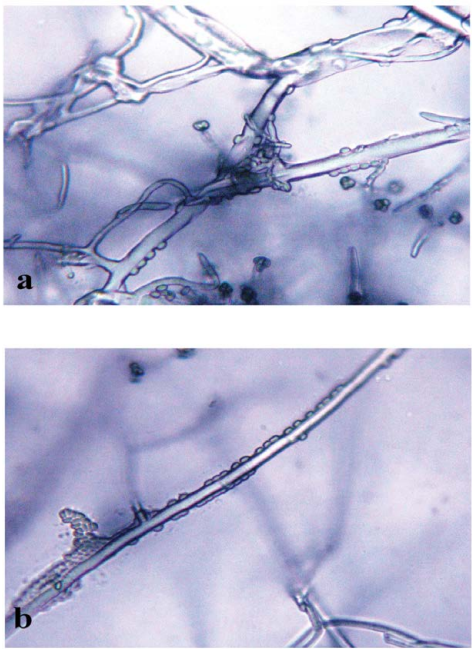

Figure 4a: *Trichoderma* mycelium coiled around *Phytophthora* mycelium. **4b.** *Trichoderma* spore around the pathogen.

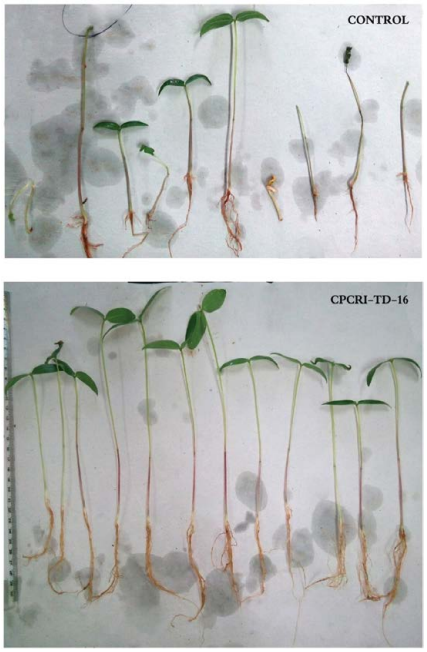

Figure 5: PGPR activity of *Trichoderma* sp. (CPCRI-TD-16) towards growth of green gram seedlings against control.

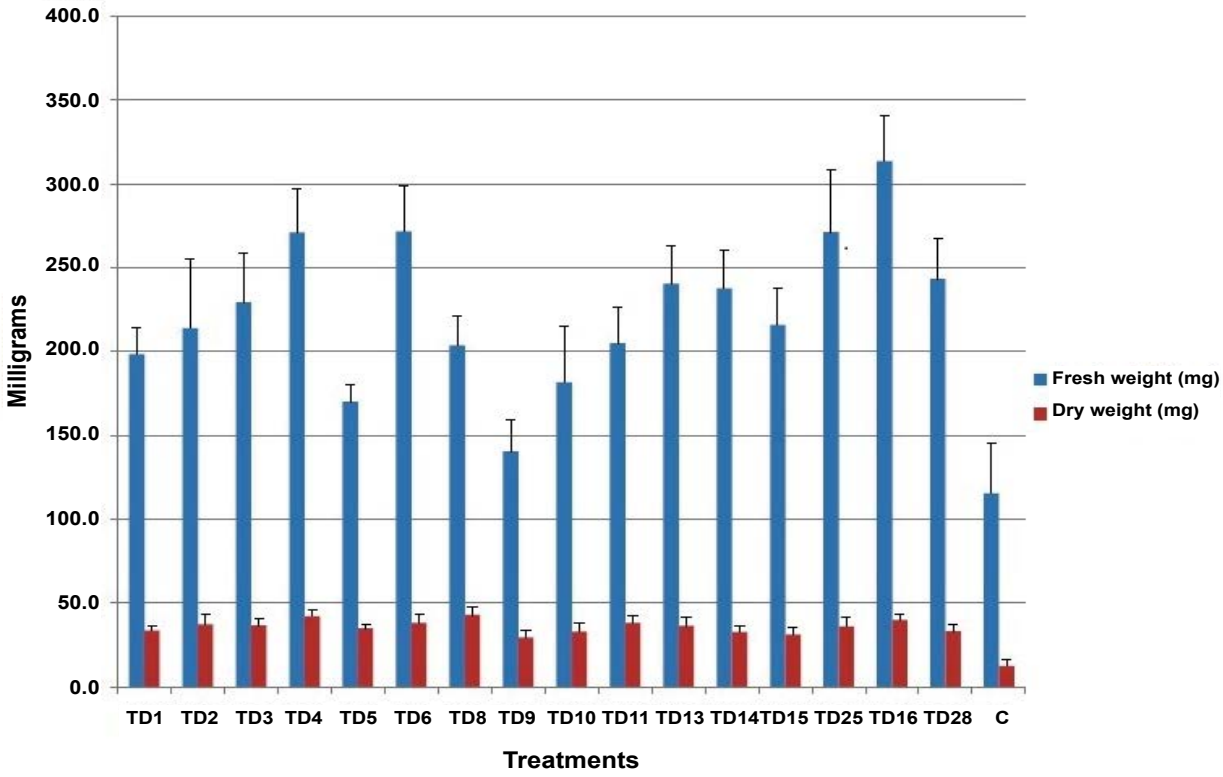

Figure 6: Growth response towards the *Trichoderma* treatment against green gram (*Vigna radiata*).

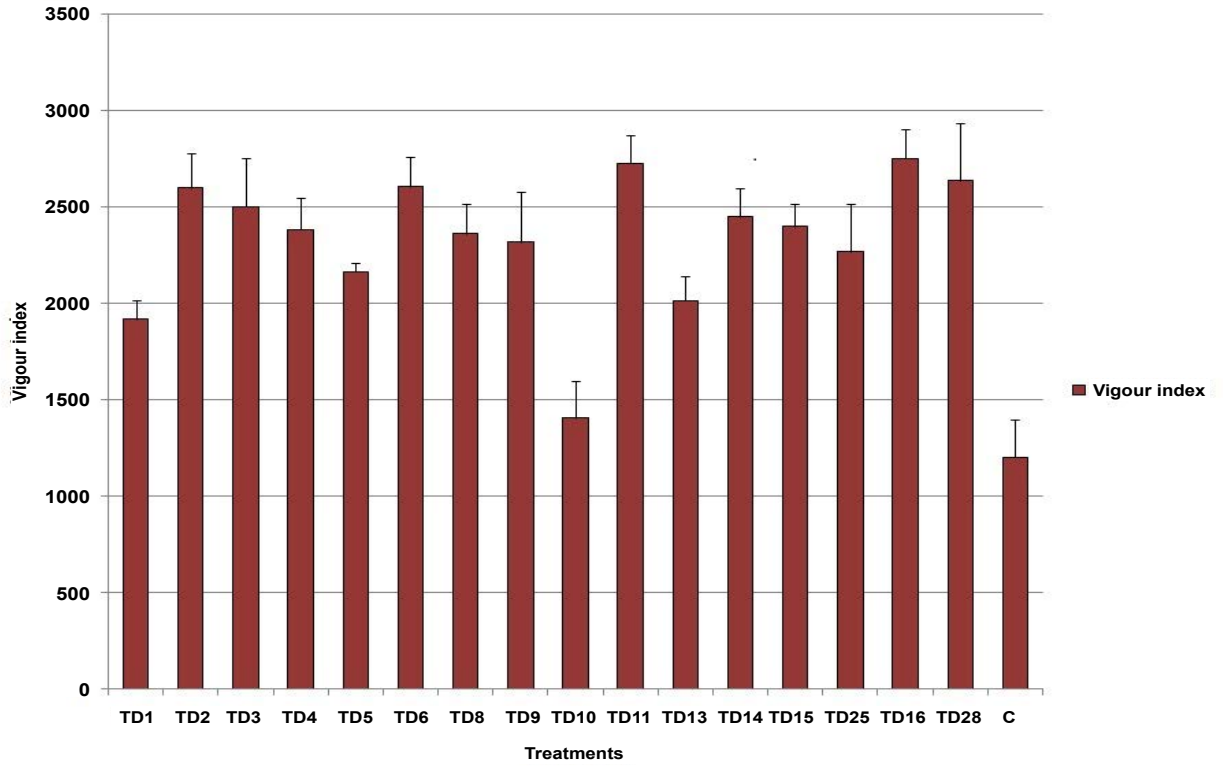

Figure 7: Vigour index, as influenced by *Trichoderma* strains.

Table 3: *In vitro* antagonism of *Trichoderma* spp. against *Phytophthora heveae*, *P. palmivora*, *P.citrophthora*, *P.capsici* and *P.meadii*.

Isolate number	Mycelial growth inhibition (%)				
	P. heveae	***P. palmivora***	***P. citrophthora***	***P. capsici***	***P.meadii***
CPCRI-TD-1	57.75^{c}	52.38^{gh}	34.78^{c}	49.99^{ef}	54.29^{ef}
CPCRI-TD-2	67.14^{a}	64.29^{efg}	58.70^{ab}	82.81^{a}	63.81^{abcd}
CPCRI-TD-3	64.79^{ab}	83.33^{abc}	76.08^{a}	64.05^{bcd}	70.48^{ab}
CPCRI-TD-4	50.70^{de}	71.43^{cde}	43.47^{bc}	70.31^{bc}	72.38^{a}
CPCRI-TD-5	64.79^{ab}	92.86^{a}	43.48^{bc}	49.99^{ef}	63.81^{abcd}
CPCRI-TD-6	71.83^{a}	71.43^{cde}	34.78^{c}	73.43^{b}	72.38^{a}
CPCRI-TD-8	71.83^{a}	85.71^{ab}	73.91^{a}	65.62^{bcd}	66.67^{abc}
CPCRI-TD-9	64.79^{ab}	57.14^{fg}	56.52^{ab}	65.62^{bcd}	61.91^{bcde}
CPCRI-TD-10	64.79^{ab}	52.38^{gh}	67.39^{a}	70.31^{bc}	70.48^{ab}
CPCRI-TD-11	38.97^{f}	42.86^{hi}	34.78^{c}	49.99^{ef}	48.57^{f}
CPCRI-TD-13	34.27^{f}	66.67^{def}	71.74^{a}	68.74^{bcd}	73.33^{a}
CPCRI-TD-14	38.97^{f}	33.33^{i}	60.87^{ab}	35.92^{g}	63.81^{abcd}
CPCRI-TD-15	71.83^{a}	54.76^{fgh}	60.87^{ab}	70.31^{bc}	67.62^{abc}
CPCRI-TD-25	60.10^{bc}	85.71^{ab}	76.08^{a}	62.49^{cd}	64.76^{abc}
CPCRI-TD-16	55.40^{cd}	71.43^{cde}	56.52^{ab}	49.99^{ef}	55.24^{def}
CPCRI-TD-28	46.01^{e}	78.57^{bcd}	56.52^{ab}	59.37^{de}	55.24^{def}

*Values in the columns followed by the same letters are not significantly different (p<0.05) by Duncan's test; values are average of three replicates

Table 4: Effect of *Trichoderma* strains on green gram germination and growth parameters.

Isolate number	RGI (%)	Cellulase activity (v/mg protein)	Germination percentage*	Shoot length (cm)*	Root length (cm)*	Vigor Index*
CPCRI-TD-1	49.84	18.702	83.33^{ab}	14.07^{a}	8.92^{ab}	1916.66^{c}
CPCRI-TD-2	67.35	23.771	100.00^{a}	15.26^{a}	10.72^{ab}	2598.75^{ab}
CPCRI-TD-3	71.75	28.044	100.00^{a}	13.64^{a}	11.36^{a}	2500.00^{abc}
CPCRI-TD-4	61.66	20.086	91.67^{ab}	14.85^{a}	11.12^{ab}	2381.04^{abc}
CPCRI-TD-5	62.99	23.435	83.33^{ab}	15.52^{a}	10.42^{ab}	2162.41^{abc}
CPCRI-TD-6	64.77	23.572	100.00^{a}	14.86^{a}	11.18^{a}	2605.00^{ab}
CPCRI-TD-8	72.75	31.119	91.67^{ab}	14.75^{a}	11.00^{ab}	2360.24^{abc}
CPCRI-TD-9	61.20	21.733	91.67^{ab}	12.83^{ab}	12.45^{a}	2317.85^{abc}
CPCRI-TD-10	65.07	24.961	66.67^{b}	13.72^{a}	7.33^{bc}	1404.03^{d}
CPCRI-TD-11	43.03	16.785	100.00^{a}	16.05^{a}	11.22^{a}	2727.50^{ab}
CPCRI-TD-13	62.95	22.198	83.33^{ab}	14.91^{a}	9.23^{ab}	2012.50^{bc}
CPCRI-TD-14	46.58	17.834	91.67^{ab}	14.42^{a}	12.27^{a}	2447.50^{abc}
CPCRI-TD-15	65.08	26.856	91.67^{ab}	15.16^{a}	10.98^{ab}	2397.08^{abc}
CPCRI-TD-25	69.83	27.482	91.67^{ab}	14.68^{a}	10.03^{ab}	2266.46^{abc}
CPCRI-TD-16	57.72	19.074	100.00^{a}	16.37^{a}	11.12^{ab}	2750.00^{a}
CPCRI-TD-28	59.14	20.859	100.00^{a}	14.93^{a}	11.28^{a}	2636.25^{ab}
Control	-	-	91.67^{ab}	9.71^{b}	4.70^{c}	1201.04^{d}

*Values in the columns followed by the same letters are not significantly different (p<0.05) by Duncan's test; values are average of three replicates

germination and increase in percentage of emergence [37,38] (Table 4). In this work we found that increased root exploration in soils by *Trichoderma* sp. may be one of important factors for enhancing seedling growth of green gram. However, several mechanisms have been suggested to explain the role of *Trichoderma* in plant growth enhancement including production of growth stimulating factors, increased nutrients uptake through enhanced root growth, reduction of concentrations of plant growth inhibiting substances and control of deleterious root microorganisms [39]. Hence, more detailed studies in the various strains of different *Trichoderma* species are still needed in order to provide a better understanding of the mechanisms of promoting plant growth responses.

Conclusion

The *Trichoderma* species are well known as biocontrol agents for control of several crop diseases. It has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods. The key factor to the ecological success of this genus is the combinations of very active mycoparasitic mechanisms, promotion of plant development plus effective defense strategies induced in plants [40]. As opportunistic plant symbionts and effective mycoparasites, numerous species of this genus have the potential to become commercial biofungicides. The various strains of three *Trichoderma* sp. and their different mode of actions mentioned above, indicate their efficacy as a potent agent of biological control.

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Author Affiliation

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Division of Plant Pathology, Central Plantation Crops Research Institute, Kasaragod, 671124, India