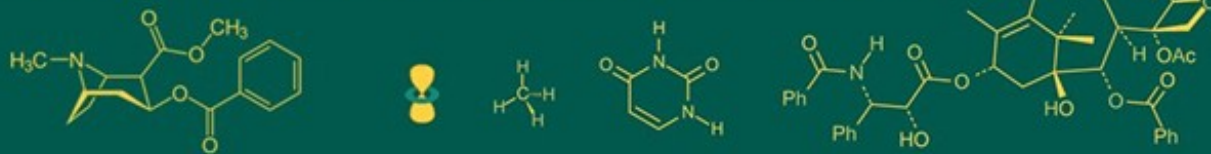


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Isolation and in vitro assessment of native *Trichoderma* spp. for biocontrol efficacy against major soil-borne plant pathogens

PD Pawar¹, KB Dharbale², SM Bhadke³, SS Hajgude⁴, AA Bharose⁵, CV Ambadkar⁶, RL Chavhan⁷, PK Jadhav⁸, YS Bhagat⁹

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ABSTRACT

The aim of the present study was to isolate and characterize native *Trichoderma* spp. The goal of this investigation was to identify and isolate native *Trichoderma* species, and assess the effectiveness of their in vitro biocontrol against the main soil-borne plant diseases. Samples of rhizosphere soil were taken from soybean, pigeonpea, and chickpea fields in Maharashtra, India's districts of Latur, Parbhani, and Dharashiv. The serial dilution plate method was used to obtain native *Trichoderma* isolates, which were then described using morphological and cultural traits. Initially, six isolates were recognized as *Trichoderma harzianum*, *T. virens*, *T. asperellum*, and *T. koningii*. Using the dual culture approach in a lab setting, the antagonism of the chosen native *T. harzianum* isolate was assessed against eight economically significant plant diseases. Among the pathogens studied, there was a notable variance in the prevention of pathogen growth. The chosen isolate of *Trichoderma* demonstrated broad-spectrum antagonistic activity, with mycelial growth inhibition ranging from 69.26 to 85.18%. *Fusarium oxysporum* f. sp. ciceri (80.18%), *Sclerotium rolfsii* associated with chili (83.15%), and *Mangifera pustulosis* (85.18%) showed the greatest inhibition. Additionally, *Pythium aphanidermatum* (78.52%), *F. oxysporum* f. sp. udum (74.07%), *Colletotrichum lindemuthianum* (73.15%), and *F. oxysporum* f. sp. pisi (71.48%) were all significantly inhibited. The *S. rolfsii* linked to rajma showed the least inhibition (69.26%). These results show that the chosen native *Trichoderma* isolate has promising broad-spectrum in vitro biocontrol potential against a variety of soil-borne plant pathogens. To confirm its effectiveness and assess its suitability for real-world disease management, more greenhouse and field research is needed.

Keywords: *Trichoderma*, Biological control, Soil-borne pathogens, Dual culture, Mycelial growth inhibition, Native isolates

Introduction

Soil borne plant pathogens are one of the major constraints limiting crop production and productivity as they can persist in soil and infect plants at different stages of their growth. Important pathogens from genera such as *Fusarium*, *Pythium*, *Sclerotium*, *Rhizoctonia* and other pathogenic fungi and oomycetes are associated with economically important diseases and may cause considerable yield losses under favourable environmental conditions. Their management is especially difficult since many soil-borne pathogens can survive for long periods in soil or infected crop debris, making eradication difficult.

Historically, chemical fungicides have been extensively used for the control of soil-borne diseases. Fungicides can be effective in suppressing disease, but repeated and indiscriminate application can lead to environmental contamination, the development of populations of resistant pathogens and disturbance of beneficial soil microorganisms. These limitations have increased the interest in environmentally safe and sustainable alternatives for plant disease management (Harman, 2006; Sharma *et al.*, 2014) [1].

Biological control with beneficial microorganisms has become an important part of sustainable disease management. *Trichoderma* spp. is one of the most extensively studied and widely exploited biological control agents among the microbial antagonists. Their rapid growth, ecological adaptability and ability to suppress a broad range of plant pathogens makes them a good candidate for biological control. *Trichoderma* spp. has been extensively studied in India against many soil-borne and foliar pathogens (Sharma *et al.*, 2014) [1]. *T. harzianum* and *T. viride* are the most widely used species.

Trichoderma spp. antagonistic activity involves a number of mechanisms, including competition for nutrients and space, mycoparasitism, antibiosis, production of secondary metabolites and cell-wall-degrading enzymes and induction of plant defense responses (Benítez *et al.*, 2004; Harman, 2006; Vinale *et al.*, 2008) [2].

Corresponding Author: YS Bhagat

Assistant Professor,
 Vasantrao Naik
 Marathwada Krishi
 Vidyapeeth (VNMKV)
 Parbhani, Maharashtra,
 India

3, 4]. These mechanisms can act individually or in combination and their efficacy is dependent on the *Trichoderma* strain, target pathogen and environmental conditions.

Several studies have reported antagonistic potential of *Trichoderma* against important soil-borne pathogens such as *Pythium aphanidermatum*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Fusarium oxysporum* (Hima *et al.*, 2016) [5]. The potential of *Trichoderma* as an environment friendly alternative to chemical disease management has also been highlighted in reviews (Verma *et al.*, 2007; Vinale *et al.*, 2008) [6, 4]. However, the biocontrol ability of *Trichoderma* differs with species and isolates due to differences in growth rate, competition, metabolite production, enzyme activity, mycoparasitism and environmental adaptation. Thus, screening of native isolates is important to identify effective strains, which are suitable to local conditions (Benítez *et al.*, 2004; Sharma *et al.*, 2014) [2, 1].

The dual culture method is extensively used for initial *in vitro* screening of *Trichoderma* isolates against plant pathogens. It gives a simple comparison based on the pathogen growth and percentage inhibition of mycelial growth and helps to identify promising isolates for further evaluation (Hima *et al.*, 2016) [5].

Hence the present study was planned to isolate and characterize native *Trichoderma* spp. and to test *in vitro* biocontrol potential of native *Trichoderma* spp. against major soil-borne plant pathogens. The selected isolate was further tested against several economically important pathogens to assess its wider potential in sustainable plant disease management (Mukherjee *et al.*, 2022; Sharma *et al.*, 2023) [7, 8].

2. Materials and Methods

2.1 Experimental site and materials

The present investigation was carried out during 2025-2026 in the Department of Plant Biotechnology, Vilasrao Deshmukh College of Agriculture Biotechnology, Latur, Maharashtra, India under laboratory conditions. The present study was carried out for the isolation and characterization of native *Trichoderma* spp. and there *in vitro* antagonistic activity against major soil borne and economically important plant pathogens.

Rhizosphere soil samples from agricultural and horticultural crops grown in Latur, Parbhani and Dharashiv districts of Maharashtra were collected for the isolation of native *Trichoderma* spp. The crops sampled were soybean (*Glycine max*), pigeonpea (*Cajanus cajan*) and chickpea (*Cicer arietinum*). The native *Trichoderma* isolates obtained from samples were purified and identified with the help of their cultural and morphological characters.

Eight plant pathogenic fungi cultures viz., *Fusarium oxysporum* f. sp. *ciceri*, *Pythium aphanidermatum*, *Sclerotium rolfsii* associated with chili, *F. oxysporum* f. sp. *pisi*, *S. rolfsii* associated with rajma, *Mangifera pustulosis*, *F. oxysporum* f. sp. *udum* and *Colletotrichum lindemuthianum* were obtained as existing pure cultures from the Department of Plant Biotechnology, Vilasrao Deshmukh College of Agriculture Biotechnology, Latur, Maharashtra. *In vitro* evaluation of antagonistic potential of native *Trichoderma* isolates was done using these cultures.

Potato Dextrose Agar (PDA) medium was used for isolation and purification of native *Trichoderma* spp., maintenance of fungal cultures and *in vitro* antagonism studies. Pure cultures of the test pathogens and *Trichoderma* isolates were stored on PDA slants at 5 ± 1 °C till further use.

2.2 Isolation and identification of *Trichoderma* spp

Rhizosphere soil samples were collected from different agricultural and horticultural crops of Latur, Parbhani and Dharashiv districts of Maharashtra. Native isolates of *Trichoderma* were isolated by serial dilution plate technique using Potato Dextrose Agar (PDA) media. The obtained colonies were purified by repeated hyphal-tip transfer and maintained on PDA slants at 5 ± 1 °C until further use.

The isolates were identified based on cultural and morphological characteristics such as colony growth, pigmentation, conidiophore branching pattern, phialide arrangement and conidial morphology according to the standard taxonomic descriptions of Nagamani *et al.*, 2015 [9] and Bissett (1991) [10].

2.3 Selection of *Trichoderma harzianum* for Broad-Spectrum Antagonistic Evaluation

The native *Trichoderma* isolates were initially evaluated for their antagonistic activity against *Pythium aphanidermatum*, the causal agent of ginger rhizome rot, by the dual culture technique as described by Dennis *et al.* (1971) [11]. The most effective isolate of *Trichoderma harzianum*, identified by previous screening, was selected for further evaluation against a broader spectrum of economically important plant pathogens.

The selected *T. harzianum* isolate was then tested *in vitro* against eight test pathogens viz., *F. oxysporum* f. sp. *ciceri*, *Pythium aphanidermatum*, *Sclerotium rolfsii* associated with chili, *F. oxysporum* f. sp. *pisi*, *S. rolfsii* associated with rajma, *Mangifera pustulosis*, *F. oxysporum* f. sp. *udum* and *Colletotrichum lindemuthianum*. The pure cultures of the pathogens were obtained from Department of Plant Biotechnology, Vilasrao Deshmukh College of Agriculture Biotechnology, Latur, Maharashtra and were used directly for the antagonistic studies.

2.4. *In vitro* antagonistic activity of selected *Trichoderma harzianum*

The antagonistic activity of the selected isolate of *T. harzianum* against the eight test pathogens was estimated by the dual culture technique (Dennis *et al.*, 1971) [11]. A 2-mm mycelial disk from the actively growing margin of a 7-day-old culture of *T. harzianum* and the respective pathogen were placed at opposite ends of a 90-mm Petri plate containing PDA medium. The untreated control plates were inoculated only with the respective pathogen.

The experiment was arranged in a Completely Randomized Design (CRD) with three replications. The plates were incubated at 28 ± 1 °C and the radial growth of each pathogen was recorded at the given observation intervals. The antagonism of the selected *T. harzianum* isolate was quantified through comparison of pathogen growth in dual culture with that of the untreated control.

2.5. Assessment of antagonistic activity

The antagonism efficiency of the selected *T. harzianum* isolate was assessed based on the reduction in radial growth of each test pathogen compared with the untreated control. Percentage inhibition of mycelial growth was calculated using the formula given by Vincent (1947) [12]:

$$I = [(C - T)/C] \times 100$$

where:

- **I** = Percent inhibition of mycelial growth
- **C** = Radial growth of the pathogen in the untreated control (mm)
- **T** = Radial growth of the pathogen in the dual culture treatment (mm)

2.6. Statistical analysis

The experiment was laid out in a Completely Randomized Design (CRD) with three replications. Analysis of variance (ANOVA) was used to analyze data on radial growth and percent inhibition of mycelial growth of test pathogens. The means of treatments were compared using the Critical Difference (CD) at 1% level of significance ($P \leq 0.01$). The standard error of the mean [SE_m] and the critical difference (CD) were determined using the standard procedures described by Gomez *et al.* (1984) [13]. Where necessary, percentage inhibition data were arcsine-transformed prior to statistical analysis.

3. Results and Discussion

3.1. Isolation and characterization of native *Trichoderma* isolates

Six indigenous *Trichoderma* isolates were effectively isolated and purified on Potato Dextrose Agar (PDA) medium. Considerable variation was noted among the isolates in colony color, texture, pigmentation, zonation and degree of conidial development. *T. virens* LTv-1 formed a more distinct green to dark-green colony with dense aerial growth, while *T. harzianum* LTh-1 produced a predominantly whitish to pale green, dense, cottony colony with progressive green pigmentation. *T. asperellum* LTAs-1 was a relatively uniform green colony with a compact, velvety to powdery appearance. *T. virens* LTv-2 showed prominent aerial mycelial growth with whitish growth associated with green pigmentation and marked radial development. *T. harzianum* LTh-2 showed a pale green to whitish growth with distinct peripheral aerial mycelium, while *T. koningii* LTK-1 developed green colony with pronounced white aerial mycelial growth and irregular radial distribution. These differences in cultural characteristics gave useful phenotypic differences (Bissett, 1991; Gams *et al.*, 1998; Samuels, 2006, Nagamani 2015) [10, 14, 15].

3.2. In vitro antagonistic activity of selected *Trichoderma* isolate against major soil borne pathogens

The antagonism activity of the selected native *Trichoderma* isolate was evaluated against eight economically important plant pathogens using the dual culture technique. Mycelial growth of each pathogen was recorded on the 7th day after inoculation (DAI), when the pathogen colony in the respective untreated control plates had attained complete growth. The extent of antagonism was assessed based on the average radial growth of the pathogen and percentage inhibition of mycelial growth. Considerable variation was observed in the response of the test pathogens to the selected *Trichoderma* isolate, with average mycelial growth ranging from 13.33 to 27.66 mm and inhibition of mycelial growth ranging from 69.26 to 85.18% (Table 2; Figure 2).

Among the pathogens tested, *Mangifera pustulosis* exhibited the highest susceptibility to the selected *Trichoderma* isolate, recording the lowest average mycelial growth of 13.33 mm and the highest inhibition of mycelial growth (85.18%). The second highest inhibition was recorded against *Sclerotium rolfsii* associated with chilli, where the pathogen showed an average growth of 15.16 mm, corresponding to 83.15% inhibition. *Fusarium oxysporum* f. sp. *ciceri* was also strongly inhibited, with an average mycelial growth of 17.83 mm and 80.18% inhibition.

Likewise, the average mycelial growth and inhibition percentage was 19.33 mm and 78.52% respectively against *Pythium aphanidermatum* with high level of antagonism. The average growth of *F. oxysporum* f. sp. *udum* was 23.33 mm with 74.07 % inhibition and *Colletotrichum lindemuthianum* was 24.16 mm with 73.15 % inhibition. The average mycelial growth was 25.66 mm with 71.48% inhibition of *F. oxysporum* f. sp. *pisi*.

The lowest antagonistic activity was comparatively observed against *Sclerotium rolfsii* associated with rajma, which showed the highest average mycelial growth of 27.66 mm and the lowest percentage of inhibition of mycelial growth (69.26%). Thus, the selected *Trichoderma* isolate inhibited all the eight test pathogens but the extent of inhibition differed significantly among pathogens indicating different susceptibility to the antagonism of *Trichoderma*.

The pathogens could be arranged in the order of susceptibility according to the percentage inhibition as follows: *M. pustulosis* (85.18%) > *S. rolfsii* (chilli) (83.15%) > *F. oxysporum* f. sp. *ciceri* (80.18%) > *P. aphanidermatum* (78.52%) > *F. oxysporum* f. sp. *udum* (74.07%) > *C.*

lindemuthianum (73.15%) > *F. oxysporum* f. sp. *pisi* (71.48%) > *S. rolfsii* (rajma) (69.26%). This variation shows a significant antagonism potential of the selected native *Trichoderma* isolate against diverse plant pathogens.

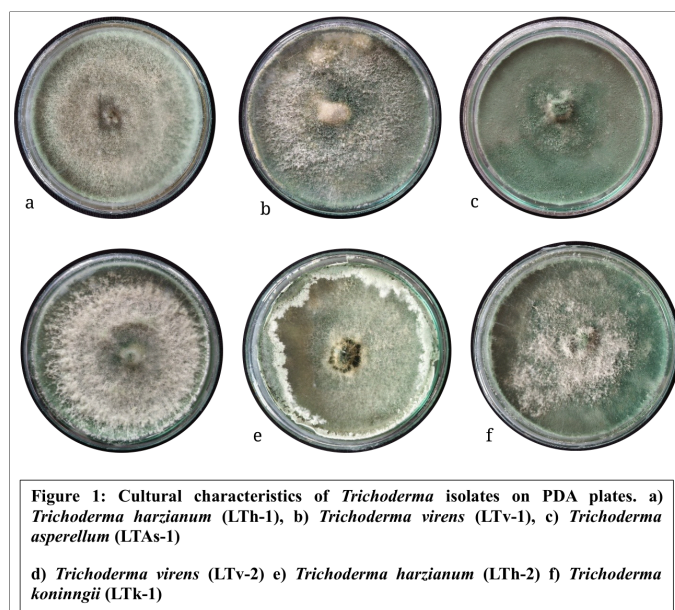


Figure 1: Cultural characteristics of *Trichoderma* isolates on PDA plates. a) *Trichoderma harzianum* (LTh-1), b) *Trichoderma virens* (LTv-1), c) *Trichoderma asperellum* (LTAs-1) d) *Trichoderma virens* (LTv-2) e) *Trichoderma harzianum* (LTh-2) f) *Trichoderma koningii* (LTK-1)

Figure 1. Cultural characteristics of *Trichoderma* isolates on PDA plates. a) *Trichoderma harzianum* (LTh-1), b) *Trichoderma virens* (LTv-1), c) *Trichoderma asperellum* (LTAs-1) d) *Trichoderma virens* (LTv-2) e) *Trichoderma harzianum* (LTh-2) f) *Trichoderma koningii* (LTK-1)

The observed inhibition could be due to several well-known antagonistic mechanisms of *Trichoderma* such as rapid colonization, competition for nutrients and space, myco-parasitism, antibiosis and production of hydrolytic enzymes and other secondary metabolites (Harman *et al.*, 2004; Howell, 2003; Vinale *et al.*, 2008) [3, 16, 4]. *Trichoderma* spp. is known to act against plant pathogens through several complementary mechanisms including direct parasitism of pathogenic fungi and production of biologically active compounds and cell-wall degrading enzymes (Harman *et al.*, 2004; Vinale *et al.*, 2008) [3, 4].

Previous studies have reported the antagonistic activity of *Trichoderma* against various groups of plant pathogens such as *Fusarium*, *Pythium*, *Sclerotium* and *Colletotrichum*, providing support for the potential of *Trichoderma* as a broad-spectrum biological control agent (Harman *et al.*, 2004; Vinale *et al.*, 2008) [3, 4]. The differential inhibition observed among the pathogens could be associated to their growth behaviour, biological characteristics and susceptibility to the antagonistic mechanisms of the selected *Trichoderma* isolate. The efficacy of *Trichoderma* varies according to the strain and target pathogen as the antagonistic interactions are influenced by the characteristics of the bio-control organism and pathogen (Vinale *et al.*, 2008) [4].

The native *Trichoderma* isolate selected showed broad-spectrum *in vitro* antagonistic activity, inhibiting the mycelial growth of all eight pathogens tested by > 69% overall. Maximum inhibition was recorded against *M. pustulosis* (85.18%) and minimum against *S. rolfsii* associated with rajma (69.26%) indicating the considerable potential of the selected isolate for further evaluation as biological control agent.

Table: 2 In vitro evaluation of *Trichoderma* isolate against major Soil born pathogen

Table 1. Cultural and morphological description used for identification of native isolate of *Trichoderma* spp.

Sr. No.	Name of isolate	Colony growth rate (mm)	Colony colour	Reverse colony colour	Colony edge	Conidial development	Conidiophore	Conidia: shape and size	Phialides: shape and arrangement
1	<i>Trichoderma harzianum</i> LTh-1	80-90	White, becoming light to green with age	Pale yellow to yellowish cream	Entire to slightly wavy	Moderate to abundant	Highly branched, predominantly pyramidal/tree-like	Globose, subglobose to broadly ovoid; approximately	Ampulliform to lageniform; solitary or in terminal whorls, commonly 2-5
2	<i>Trichoderma virens</i> LTV-1	80-90	White initially, developing green to dark green pigmentation	Cream to pale yellow	Entire	Moderate to abundant	Branched, often with a distinct main axis; repeatedly branched	Ovoid to broadly ellipsoidal; approximately	Ampulliform to lageniform; solitary or in small groups/whorls
3	<i>Trichoderma asperellum</i> LTAs-1	80-90	White initially, becoming green to dark green	Pale greyish cream to light olive-grey	Entire to slightly wavy	Moderate to abundant	Repeatedly branched, tree-like/pyramidal	Globose to oval/ellipsoidal; approximately	Flask-shaped, often enlarged in the middle; solitary or clustered
4	<i>Trichoderma virens</i> LTV-2	80-90	White to green, with green/dark-green sporulation	Cream to pale yellow	Entire	Moderate to abundant	Branched, repeatedly branched, with distinct main axis	Ovoid to broadly ellipsoidal; approximately	Ampulliform to lageniform; solitary or in small groups/whorls
5	<i>Trichoderma harzianum</i> LTh-2	70-80	White to pale green, becoming green with sporulation	Yellow to orange-yellow	Entire to slightly wavy	Moderate to abundant	Highly branched, pyramidal/tree-like	Globose to subglobose or broadly ovoid; approximately	Ampulliform to lageniform; clustered or in whorls
6	<i>Trichoderma koningii</i> LTK-1	80-90	Green to dark green	Pale cream to light yellow	Entire to slightly irregular	Abundant	Symmetrical, repeatedly branched with a recognizable main axis	Oblong to ellipsoidal/ovoid, smooth; approximately	Straight, lageniform to cylindrical; usually in whorls of 3-4

Treatment No.	Pathogen	mean mycelial growth (mm)	% inhibition
T1	<i>Fusarium oxysporum</i> f.sp <i>ciceri</i>	17.83	80.18 (63.56)
T2	<i>Pythium aphanidermatum</i>	19.33	78.52 (62.39)
T3	<i>Fusarium oxysporum</i> f.sp <i>pisi</i>	25.66	71.48 (57.72)
T4	<i>Colletotrichum lindamethium</i>	24.16	73.15 (58.79)
T5	<i>Sclerotium rolfsii</i> (Rajma)	27.66	69.26 (56.33)
T6	<i>Fusarium oxysporum</i> f.sp <i>udum</i>	23.33	74.07 (59.39)
T7	<i>Sclerotium rolfsii</i> (Chilli)	15.16	83.15 (65.76)
T8	<i>Mangifera pustulosis</i>	13.33	85.18 (67.36)
SE(m)		0.59	-
C.D. (1%)		2.43	-

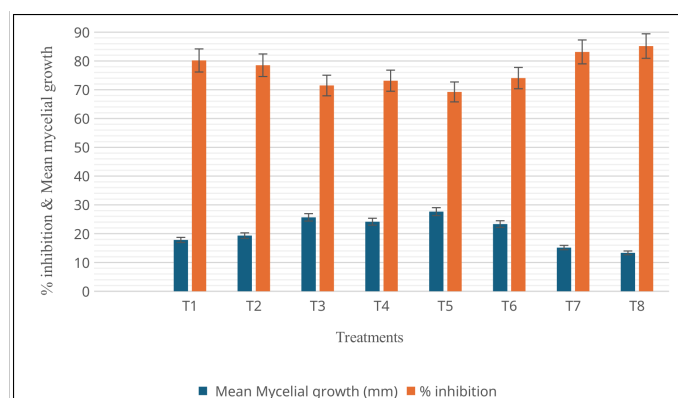


Figure 2. In vitro evaluation of *Trichoderma* isolate against major soil borne Pathogens

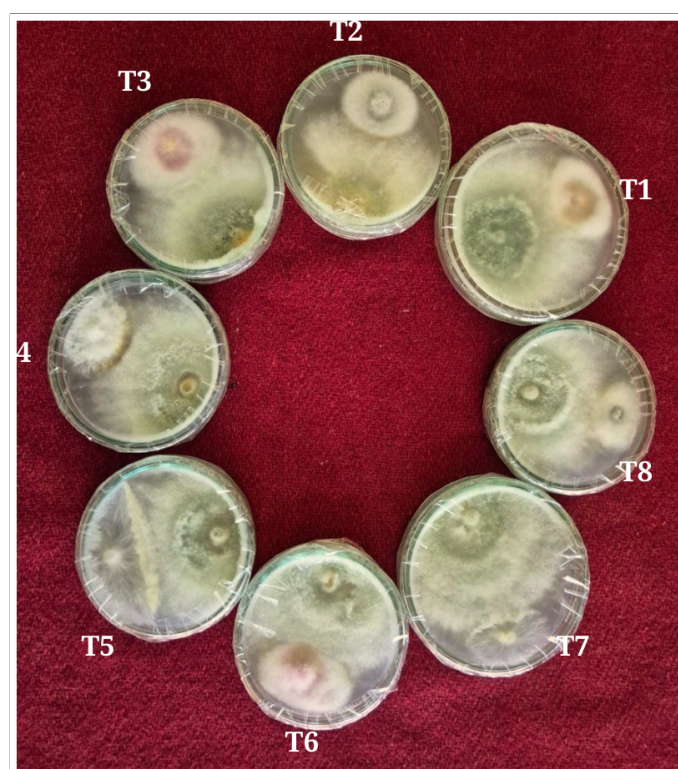


Figure 3. In vitro evaluation of *Trichoderma* isolate against major soil borne Pathogens

Treatment No.	Pathogen
T1	<i>Fusarium oxysporum</i> f.sp <i>ciceri</i>
T2	<i>Pythium aphanidermatum</i>
T3	<i>Fusarium oxysporum</i> f.sp <i>pisi</i>
T4	<i>Colletotrichum lindamethium</i>
T5	<i>Sclerotium rolfsii</i> (Rajma)
T6	<i>Fusarium oxysporum</i> f.sp <i>udum</i>
T7	<i>Sclerotium rolfsii</i> (Chilli)
T8	<i>Mangifera pustulosis</i>

Conclusion

The present investigation revealed the presence of diverse native *Trichoderma* isolates with different cultural and morphological characteristics supporting their preliminary identification as *T. harzianum*, *T. virens*, *T. asperellum* and *T. koningii*. The selected native *Trichoderma harzianum* isolate showed appreciable in vitro antagonistic activity against all the eight plant pathogens tested. But the magnitude of inhibition varied among the pathogens. Maximum mycelial growth inhibition was recorded against *Mangifera pustulosis* (85.18%) followed by *Sclerotium rolfsii* associated with chili (83.15%) and *Fusarium oxysporum* f. sp. *ciceri* (80.18%) while minimum inhibition was observed against *S. rolfsii* associated with rajma (69.26%).

The findings indicate that the selected native *Trichoderma* isolate possesses promising in vitro bio-control potential against diverse plant pathogens and may serve as a candidate for further biological control research. However, the present study was conducted under laboratory conditions; therefore, greenhouse and field evaluation, together with further investigation of the mechanisms responsible for pathogen suppression, are necessary before practical disease-management recommendations can be made.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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Authors

PD Pawar

PG Students, Vilasrao Deshmukh College of Agricultural Biotechnology, Latur, Maharashtra, India

KB Dharbale

PG Students, Vilasrao Deshmukh College of Agricultural Biotechnology, Latur, Maharashtra, India

SM Bhadke

PG Students, Vilasrao Deshmukh College of Agricultural Biotechnology, Latur, Maharashtra, India

SS Hajgude

PG Students, Vilasrao Deshmukh College of Agricultural Biotechnology, Latur, Maharashtra, India

AA Bharose

Dean, Vasantnao Naik Marathwada Krishi Vidyapeeth (VNMKV) Parbhani, Maharashtra, India

CV Ambadkar

Professor (CAS), Department of Plant Pathology, VNMKV, Parbhani, Maharashtra, India

RL Chavhan

Professor (CAS), Department of Plant Pathology, VNMKV, Parbhani, Maharashtra, India

PK Jadhav

SRF, Vasantnao Naik Marathwada Krishi Vidyapeeth (VNMKV) Parbhani, Maharashtra, India

YS Bhagat

Assistant Professor, Vasantnao Naik Marathwada Krishi Vidyapeeth (VNMKV) Parbhani, Maharashtra, India