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RESEARCH ARTICLE OPEN ACCESS

Effect of Various pH Levels on the Growth and Sporulation of *Trichoderma viride* Isolates and Assessing their Antagonistic Activity against Soil-borne Pathogens

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Abstract

***Trichoderma viride* strains, which are filamentous fungi commonly found in soil, possess the ability to parasitize various fungi harmful to plants. In this study, ten strains of *T. viride* were isolated from different locations in Uttar Pradesh, India, and examined for their cultural, physiological, morphological, and antagonistic characteristics against soil-borne pathogens. The *T. viride* isolates were assessed at different pH levels for their growth, sporulation, and antagonistic efficacy under *in vitro* conditions using the dual culture technique against five major soil-borne pathogens that cause significant diseases in cereal and pulse crops. Among the ten isolates tested, the 49CP isolate from Sultanpur exhibited the maximum growth and sporulation at a pH of 6.5 as well as the highest inhibition percentages of mycelial growth in the pathogens: 63.23% against *Fusarium oxysporum* f. sp. *ciceri*, 65.85% against *Sclerotium rolfsii*, 53.33% against *Sclerotinia sclerotiorum*, 53.84% against *Pythium* sp., and 48.00% against *Rhizoctonia bataticola*. *Trichoderma viride* is also recognized for its effectiveness as a biocontrol agent against soil-borne pathogens, which are responsible for significant crop losses.**

Keywords: Antagonistic, pH, *Trichoderma*, Pathogens, Soil Borne

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INTRODUCTION

Trichoderma species are filamentous fungi that are commonly found in soil and root ecosystems, where they actively interact with root, soil, and leaf environments. These fungi produce secondary metabolites that can trigger localized or systemic plant defense responses. When *Trichoderma* strains colonize roots, they often promote root growth, enhance crop yield, increase resilience to environmental stress, and improve nutrient absorption and utilization. The highest protein content, measured 0.41% was observed in chickpea seedlings, bioprimed with a *Trichoderma viride* formulation (*T. viride* @ 5 g/kg seeds), and increased germination, root and shoot length of chickpea crops.[1] *Trichoderma* species are also recognized for their effectiveness as antagonists against various soil-borne diseases, which have led to their exploration as bio-control agents for plant diseases. The motivation for this study stems from the benefits of using bio-control agents, which are considered safer and have a lower environmental impact than synthetic pesticides, as highlighted.[2,3] Among the commonly used species, *Trichoderma harzianum* and *Trichoderma viride* have been developed into several commercial biological control products to inhibit the growth and development of various soil-borne pathogenic fungi. *T. viride* is marketed in Europe, while *Trichoderma harzianum* is marketed in India, both targeting different soil-borne pathogens in crop fields, greenhouses, and vegetable crops.[4]

Many *Trichoderma* species exhibit characteristics of opportunistic, nonpathogenic plant symbionts, forming mutualistic endophytic associations with numerous plant species. These species act as beneficial partners to plants, serving as natural bio control agents against various harmful phytopathogenic fungi. *Trichoderma* is one of the most effective mycoparasites, making it a promising biocontrol agent against a broad range of soil-borne phytopathogens. The efficacy of *T. viride* in inhibiting pathogens such as *Rhizoctonia solani*, *S. rolfsii*, and *Fusarium* spp. across different crops.[5] The alternative methods such as protoplast fusion have been developed to enhance its antagonistic properties for bio control potential of *Trichoderma* species as noted.[6] Its widespread use as a fungal biocontrol agent is largely due to its notable antagonistic activity against soil-borne plant pathogens.

MATERIALS AND METHODS

The present study investigated the effects of various pH levels on the growth, sporulation, and antagonistic effectiveness of *T. viride* isolates, a fungus known for its ability to combat plant pathogens. The experiments were conducted at the Biocontrol Laboratory in the Department of Plant Pathology at C.S. Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India.

Collection of soil samples and isolation and purification of *T. viride*

Fifty soil samples were collected from the rhizosphere of chickpea fields across various districts of Uttar Pradesh, each taken at a depth of approximately 5 to 6 cm. The samples were carefully placed in polythene bags, with each bag clearly labeled to indicate the location and date of collection. The samples were then transported to the laboratory for further analysis. *Trichoderma* species were isolated using the *Trichoderma* selective medium (TSM), which consisted of the following components: $MgSO_4.7H_2O$ (0.2 g), K_2HPO_4 (0.9 g), KCl (0.15 g), NH_4NO_3 (1.0 g), glucose (3.0 g), chloramphenicol (0.20 g), Apron 35SD (0.01 g), Captan (0.2 g), Rose Bengal (0.15 g), agaragar (20 g), and distilled water (1 liter).[7] The soil samples were processed using the serial dilution technique,[8] followed by incubation of the plates at 28°C ± 1°C for 5 days. The appearance of colonies was noted and recorded between the third and fifth days. Individual colonies were selected and maintained in pure culture for further examination. *T. viride* isolates were purified using the single spore isolation method, with subculturing performed from the growing tip of each new colony. A small quantity of spores was aseptically taken and streaked onto potato dextrose agar (PDA) plates using a sterilized inoculating needle.

Morphological characterization of *Trichoderma* sp.

The *Trichoderma* genus can be identified when grown on TSM by observing specific colony

characteristics, such as growth pattern, growth rate, odor, and color.[9]

Microscopic features

Identifying *Trichoderma* species typically involves examining these characteristics under a light microscope, as described.[10-12] Key morphological features used for identification include the branching pattern of conidiophores, the elongation and shape of conidiophore tips (whether coiled, straight, or undulating), the structure, size, and shape of phialides, and the morphology of conidia.

Coding of *T. viride* isolates

Chickpea rhizospheric soil samples were collected from various districts of Uttar Pradesh, including Etawah, Kaushambi, Faizabad, Sultanpur, Mirzapur, and Bhadohi. These samples were coded as 02CP, 10CP, 13CP, 21CP, 25CP, 45CP, 49CP, 64CP, 70CP, and 117CP, respectively.

Reconfirmation of cultures by ITCC, New Delhi

The *Trichoderma* pure culture was obtained using the single spore technique. Microscopic observation of mounted slides of the cultures was performed, and the cultures were sent to the ITCC in New Delhi, India, for accurate identification and to obtain accession numbers for the pure culture.

Collection of disease samples, isolation, and purification of pathogen

PDA medium was prepared for isolating the pathogen, following the method outlined.[13] The medium consisted of 200 g of peeled potato, 20 g of dextrose, 20 g of agar, and 1000 mL of distilled water. Pathogen identification involved comparing the cultural and morphological characteristics of the fungus with those described[14] for *Fusarium oxysporum* f. sp. *ciceri*. The color nomenclature was based on the standards provided.[15] The growth habit, cultural traits, and morphological features of the fungus were examined on the PDA medium.

Effect of various pH levels on growth and sporulation of *T. viride* isolates

The potato dextrose broth (PDB) medium was prepared with different pH levels (4.5, 5.5, 6.5, and 7.5) by adding the appropriate amount of citrate phosphate buffer and adjusting the pH accordingly. Each 250 mL of conical flask was filled with 100 mL of PDB. The flasks were tightly sealed with nonabsorbent cotton plugs and covered with aluminum foil secured by rubber bands. The medium was sterilized by autoclaving at 15 psi (121.6°C) for 20 minutes. Under aseptic conditions in a laminar airflow, the PDB medium in the flasks was inoculated with 5 mm discs from 7-day-old cultures of *T. viride* isolates, with three replicates for each treatment. The inoculated flasks were then incubated in a biological oxygen demand (BOD) chamber at 28°C ± 2°C for 7 days. After incubation, the intensity of growth and sporulation of the different isolates of *T. viride* was assessed. The mycelial mats were harvested by filtering the culture through sterilized Whatman Filter Paper No. 4 and then dried in a hot air oven at 35°C for 48 h to obtain their dry weight. The biomass was calculated using the methods described.[16,17]

Antagonistic efficacy of *T. viride* isolates against soil borne pathogens by dual culture assay

The effectiveness of *T. viride* isolates was evaluated against *Fusarium oxysporum* f. sp. *ciceri*, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, *Rhizoctonia bataticola*, and *Pythium* sp. using the dual culture technique. Both the target pathogens and the fungal antagonist (*T. viride*) were cultured on the PDA medium. Five-millimeter-diameter bits were cut from the edges of five-day-old cultures of both the pathogens and the antagonist, and these were placed diametrically opposite each other on

Table 1. *Trichoderma viride* isolates with their accession numbers

Isolates Code	Accession No.	Place of Collection	Fungus identified
02CP	ITCC 9825	Etawah	*Trichoderma viride*
10CP	ITCC 9826	Kaushambi	
13CP	ITCC 9827	Kaushambi	
21CP	ITCC 9828	Kaushambi	
25CP	ITCC 9829	Kaushambi	
45CP	ITCC 9830	Faizabad	
49CP	ITCC 9831	Sultanpur	
64CP	ITCC 9832	Mirzapur	
70CP	ITCC 9833	Mirzapur	
117CP	ITCC 9834	Bhadohi	

the Petri dish, 5 mm from the edge. A control Petri dish was inoculated with each target pathogen alone without an antagonist. Each treatment was replicated three times and incubated at 28°C ± 2°C. After 7 days, the presence of inhibition zones between *T. viride* and the pathogens was observed. The radial growth of the pathogens on both the dual culture and control plates was measured, and the inhibition percentage of the pathogens was calculated using the methodology outlined.[18]

$$\text{Percent inhibition (I)} = C\text{-}T/C \times 100$$

Where,
C represent the mycelial growth of pathogens in control.

Table 2. Effect of various pH levels on growth and sporulation of *T. viride* isolates

T. viride isolates	Dry weight of mycelium (milligram)			
	pH 4.5	pH 5.5	pH 6.5	pH 7.5
02CP	238.667	257.333	269.333	262.667
10CP	242.000	262.667	271.667	263.667
13CP	235.000	255.000	265.667	261.000
21CP	240.333	258.000	266.000	262.000
25CP	244.667	263.000	272.667	269.000
45CP	250.000	266.000	275.333	273.000
49CP	252.667	269.667	278.667	275.000
64CP	232.667	243.000	263.667	256.667
70CP	230.667	240.000	257.667	250.000
117CP	227.667	239.000	251.667	245.000
CD @ 5%	7.100	13.516	7.709	5.505

T is the mycelial growth of pathogens in the dual culture plate.

RESULTS

Isolation and identification of *T. viride* isolates

Fifty soil samples were collected from the rhizosphere of chickpea fields across different districts of Uttar Pradesh. The bioagents were isolated and purified using the serial dilution technique, as described in the Materials and Methods section. These isolates were morphologically identified under microscopic observation, and out of the 50 isolates, 10 were confirmed as *T. viride* by the Indian Type Culture Collection (ITCC), Division of Plant Pathology, IARI (New Delhi). These 10 isolates were assigned accession numbers ITCC-9825 to ITCC-9834 (Table 1).

Effect of various pH levels on growth and sporulation of *T. viride*

All 10 confirmed *T. viride* isolates were tested at different pH levels to assess their impact on the growth and sporulation of the fungi. The pH levels of 4.5, 5.5, 6.5, and 7.5 were maintained across three replicate samples in PDB medium to determine the optimal conditions for the growth and biomass production of *T. viride*. The pH adjustments were made by adding an appropriate amount of citrate phosphate buffer. After a 7 day incubation period in the PDB medium with varying pH levels, the mycelial mats were harvested, and their average dry weights (measured in milligrams)

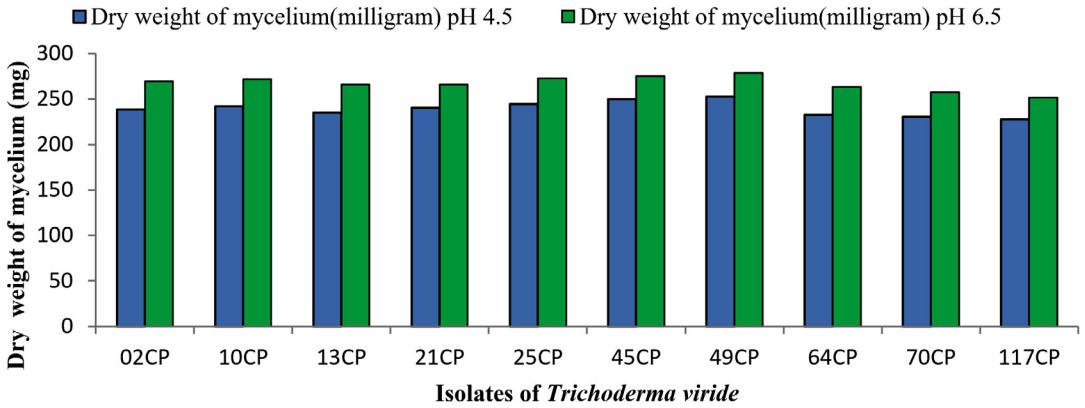

Figure 1. Effect of various pH levels on growth and sporulation of *Trichoderma viride* isolates

were recorded and presented in Table 2. A review of the data (Table 2) revealed notable disparities in biomass production across all isolates. The highest average dry weight, 278.67 mg, was consistently recorded at a pH of 6.5, whereas the lowest, 227.67 mg, was observed at a pH of 4.5. In addition, isolate 49CP consistently exhibited the highest dry weight production at all pH levels, whereas isolate 117CP consistently demonstrated the lowest (Figure 1).

Antagonistic evaluation of *T. viride* isolates against soil borne pathogens

The antagonistic effectiveness of 10 *T. viride* isolates against five phytopathogens, isolated from chickpea rhizospheric soil, was

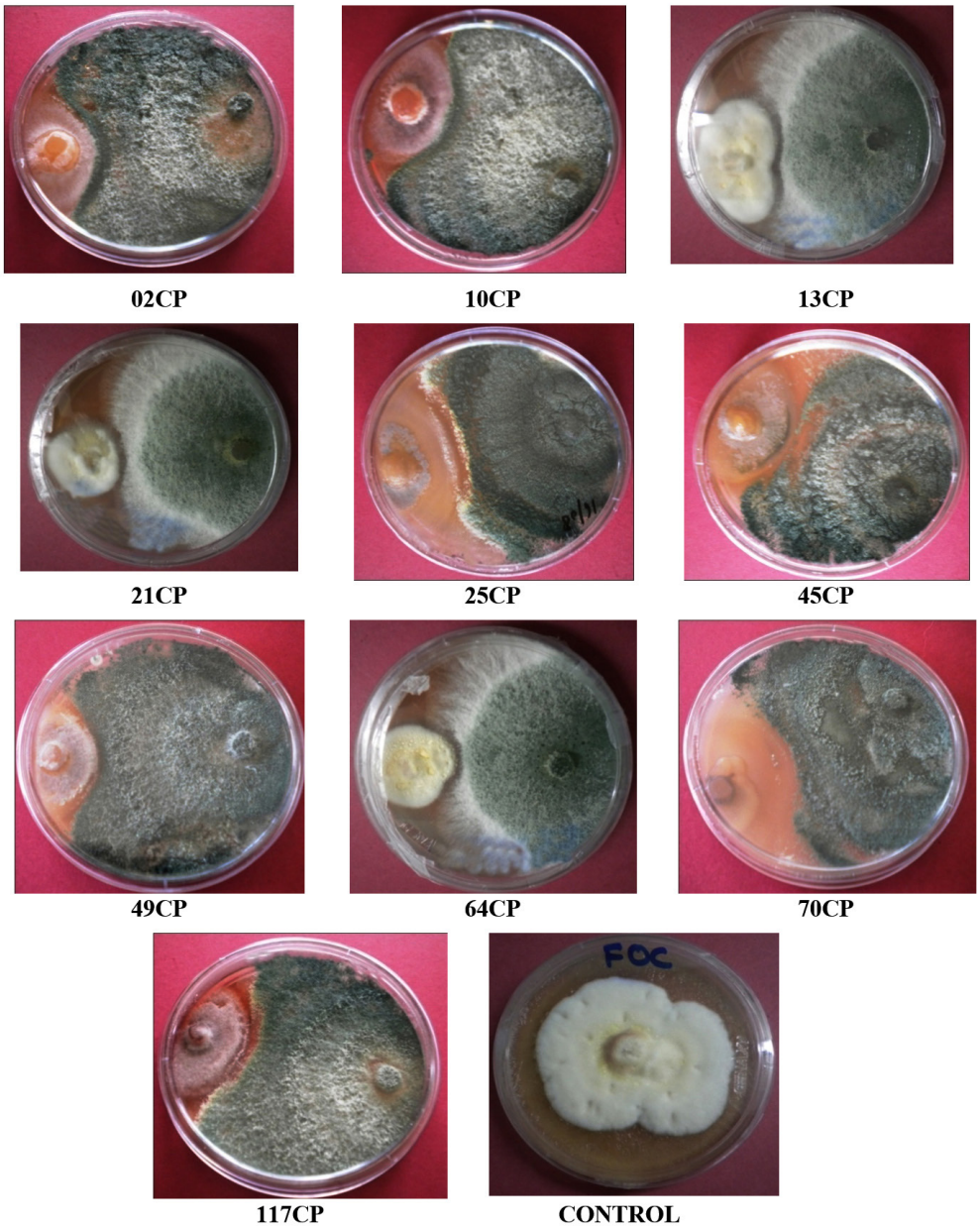

Figure 2. Antagonistic activity of *T. viride* isolates against *Fusarium oxysporum* f. sp. *ciceri*

Table 3. Antagonistic activity of *T. viride* isolates against soil borne pathogens

S. No. *T. viride* isolates	Foc		*S. rolfsii*		*S. sclerotiorum*		*Pythium* sp.		*R. bataticola*	
	Growth (mm)	I.P.	Growth (mm)	I.P.	Growth (mm)	I.P.	Growth (mm)	I.P.	Growth (mm)	I.P.
02CP	28.0	58.82	38.0	53.65	33.0	51.11	36.0	44.61	39.5	36.80
10CP	26.0	61.76	34.0	58.53	35.0	48.14	38.0	41.53	44.5	28.80
13CP	32.0	52.94	36.0	56.09	41.0	39.25	32.0	50.76	39.5	36.80
21CP	27.0	60.29	39.0	54.43	37.0	45.18	32.0	50.76	41.0	34.40
25CP	30.0	55.88	37.0	54.87	34.5	48.88	33.0	49.92	40.5	35.20
45CP	29.0	57.35	32.0	60.97	36.0	46.66	39.0	40.00	35.5	43.20
49CP	25.0	63.23	28.0	65.85	31.5	53.33	30.0	53.84	32.5	48.00
64CP	27.0	60.29	40.0	51.21	36.0	46.66	37.0	43.07	39.0	37.60
70CP	35.0	48.52	34.0	58.53	32.5	51.58	38.0	41.53	37.0	40.80
117CP	30.0	55.88	40.0	51.21	33.5	50.37	36.0	44.61	37.0	40.80
Control	68.0	00	82.0	00	67.5	00	65.0	00	62.5	00
CD @ 5%	2.861		4.013		4.274		4.268		6.062	

*I.P. = Inhibition Percentage

assessed in vitro using the dual culture technique. The data summarized in Table 3 clearly indicate that all the isolates significantly impeded the radial growth of the tested pathogens. Among them, isolate 49CP exhibited the highest inhibition of mycelial growth, with percentages of 65.85%, 63.23%, 53.84%, 53.33%, and 48.00% against *S. rolfsii*, *F. oxysporum* f. sp. *ciceri*, *Pythium* sp., *S. sclerotiorum*, and *R. bataticola*, respectively. Conversely, the lowest inhibition rates were observed with isolates 64CP (51.21%), 70CP (48.50%), 45CP (40.00%), 13CP (39.25%), and 10CP (28.80%) against the same pathogens. The dual culture technique was applied to assess the antagonistic activity of *T. viride* against soil-borne pathogens under laboratory conditions, with the goal of identifying the most effective isolate among the ten tested. The results are shown in Figure 2 to Figure 6.

DISCUSSION

Fluctuations in pH significantly affected the growth and biomass production (sporulation) of *T. viride* isolates. Statistical analysis revealed that isolate 49CP exhibited superior growth compared with the other isolates, whereas the others performed similarly to each other. An optimal pH of 6.5 was identified for biomass production, yielding 278.67 mg of dry weight. This finding aligns with the results,[16] who conducted experiments to determine the optimal pH conditions for biomass production of *Trichoderma* spp., exploring various pH levels and temperatures. They observed significant differences in biomass production across pH levels ranging from 4.0 to 8.0, with the most favorable pH range being between 5.5 and 7.5, where mycelium dry weight varied from 1.41 to 1.35 g. Similarly,[17] investigated the physiological characteristics of *Trichoderma* sp. across different liquid media, temperatures, and pH levels. They found the pH range of 6.5 to 7.5 to be the most favorable, with mycelium dry weight ranging from 144.8 to 142.4 mg.[19] Studied the effects of temperature (10°C, 15°C, 25°C, and 35°C) and pH levels (5.5, 7.0, and 8.5) on the growth and development of 26 native strains of *Trichoderma* spp. Most of these strains exhibited optimal growth and development at 25°C and pH 5.5.

The antagonistic evaluation of *T. viride* isolates against soil-borne pathogens in laboratory conditions aimed to identify the most efficient isolate. Similar findings were reported,[20] who tested six strains of *Trichoderma* spp. to assess their ability to inhibit soil-borne pathogens, including *Rhizoctonia solani*, *S. rolfsii*, and *S. sclerotiorum*. In their study, co-culturing these pathogens with

Trichoderma spp. revealed that *T. viride* (Tv-2) significantly reduced the mycelial growth of *R. solani* by 71.41% compared with the control. Similarly, *T. viride* (Tv-1) was the most effective isolate against *S. rolfsii* and *S. sclerotiorum*, exhibiting inhibition rates of 67.91% and 66.21%, respectively, over the control,[21] evaluated the antagonistic capabilities of different *Trichoderma* species against *F. oxysporum* f. sp. *ciceri*, *F. oxysporum* f. sp. *udum*, and *P. aphanidermatum*. The highest inhibition of mycelial growth (65.00%) was observed against *P. aphanidermatum*, followed by 63.66% against *Fusarium oxysporum* f. sp. *udum* and 62.00% against *Fusarium oxysporum* f. sp. *ciceri*. However, *T. virens* (T.vi (CSAU)) demonstrated the least effectiveness against the tested pathogens.[22] explored the antagonistic effects of *T. viride* on mycelial proliferation of

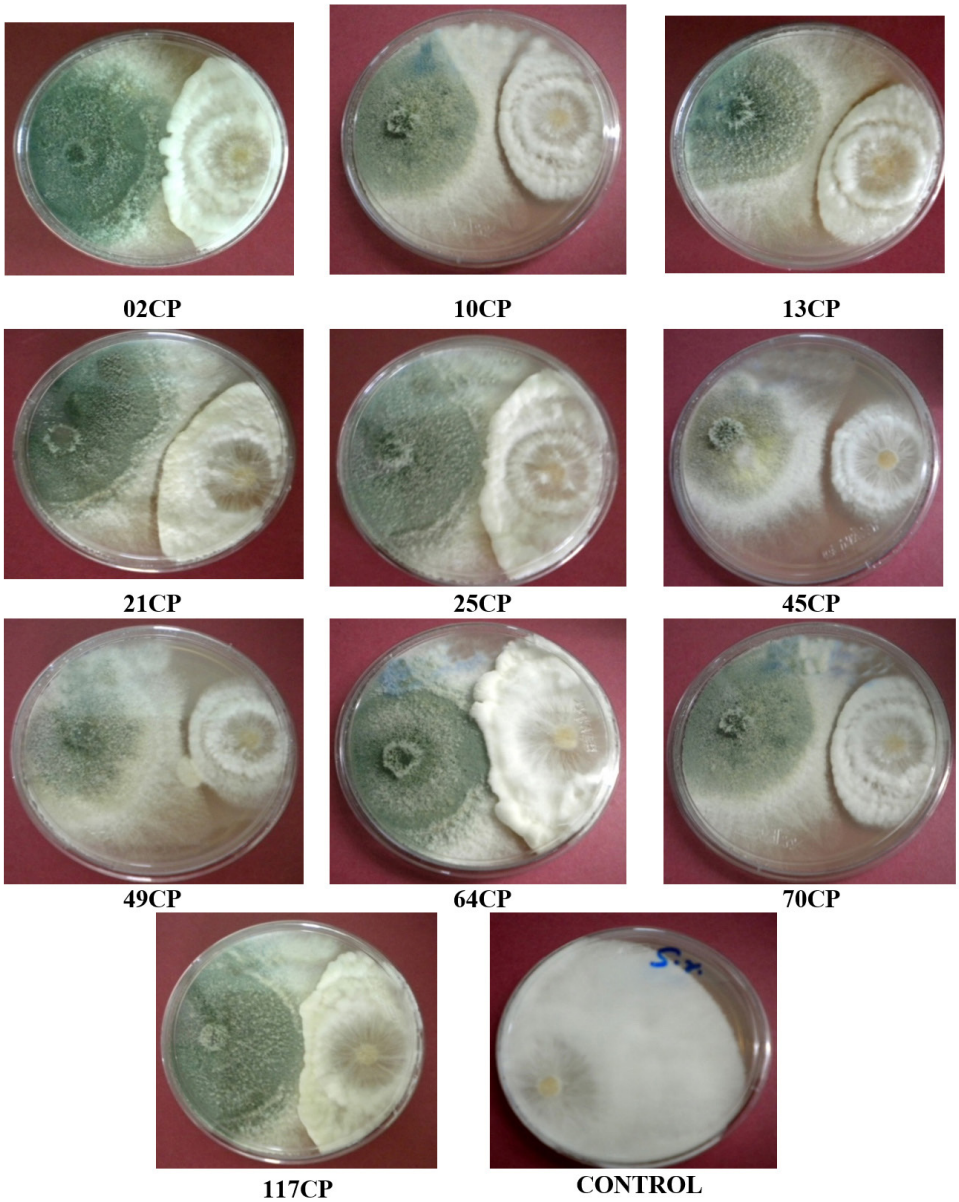

Figure 3. Antagonistic activity of *T. viride* isolates against *Sclerotium rolfsii*

Fusarium oxysporum f. sp. *ciceri*, reporting a maximum inhibition of mycelial growth at 59.25% and the lowest average colony growth recorded at 11 mm by the 01PP isolate.[23] Investigated eight *Trichoderma* species against prevalent phytopathogens, finding that *Trichoderma viride* (01PP) exhibited inhibition percentages of 50.00%, 70.42%, 78.88%, and 76.00% against *S. rolfsii*, *R. solani*, *P. aphanidermatum*, and *F. oxysporum* f. sp. *ciceri*, respectively. Similarly,[24] evaluated five *Trichoderma* species-*T. aggressivum, T. citrinoviride, T. erinaceum, T. harzianum,* and *T. koningiopsis* isolated from various regions in India, for their antagonistic activity against *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia bataticola,* which cause wilt complex in chickpea. *T. koningiopsis* stood out with high tolerance, achieving the highest mycelial inhibition rates of

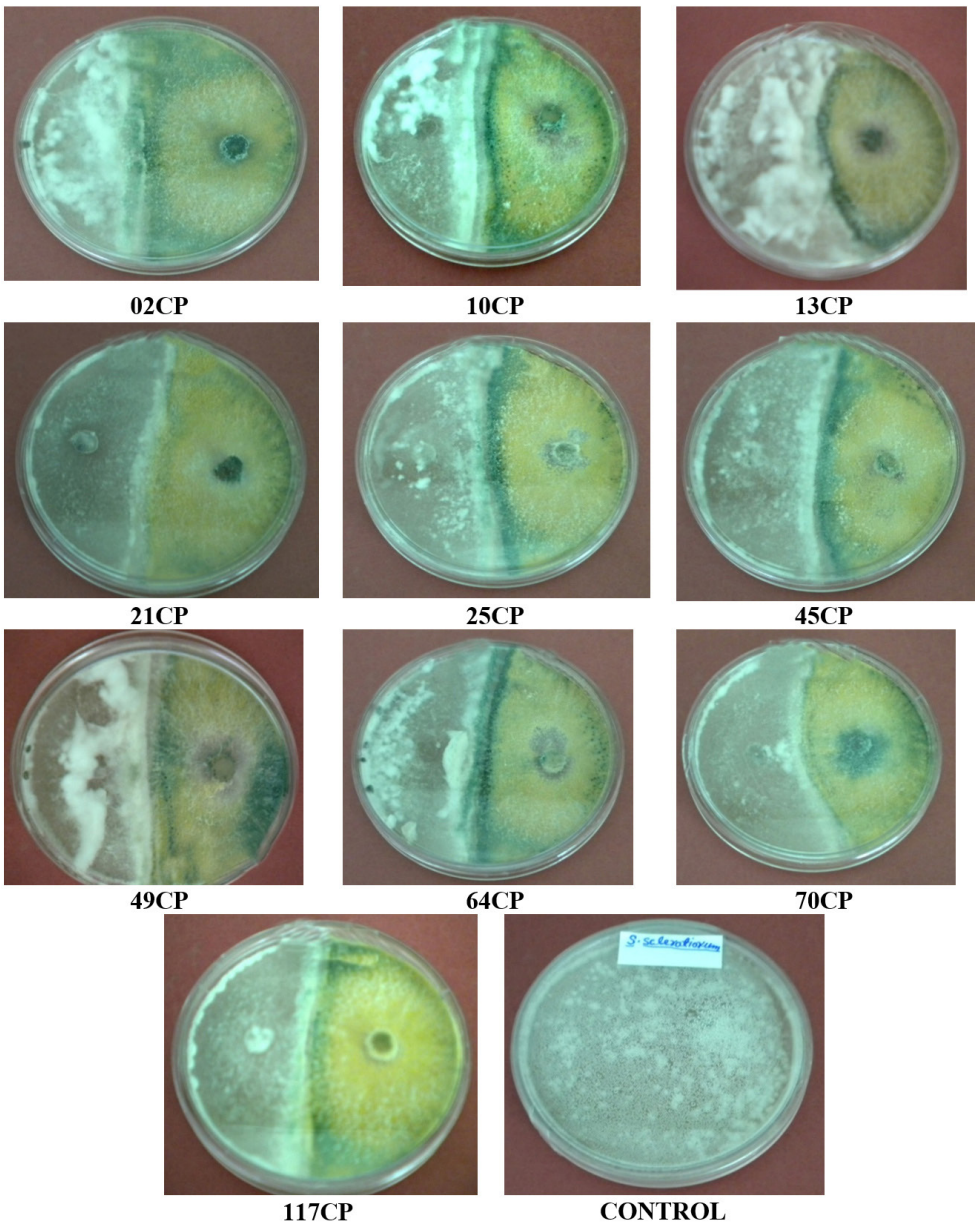

Figure 4. Antagonistic activity of *T. viride* isolates against *Sclerotinia sclerotiorum*

55.0% against *F. oxysporum* f. sp. *ciceri* and 36.3% against *R. bataticola*.[25] Assessed the antagonistic efficacy of *T. viride* and *T. harzianum* strains against *Fusarium proliferatum* and *Fusarium verticillioides* using a dual culture assay. *T. viride* exhibited strong antagonistic activity, with inhibition rates of 80.17% against *F. proliferatum* and 70.46% against *F. verticillioides*. The silmiar study supported with Cyriac *et al*,[26] investigated the antagonistic properties of *Trichoderma* isolates TRKR1, TRPN3, TRPN7, TRPN10, and TRPN18 against soil-borne pathogens *Pythium aphanidermatum* and *Rhizoctonia solani*, with TRPN7, TRPN15, and TRKR2 showing the highest mycelial inhibition (89.71%) against both pathogens. Similarly,[27] evaluated nine native *Trichoderma* isolates for bio-efficacy against *Sclerotium rolfsii*, with isolate ARS K-21 achieving the highest inhibition (89.26%),

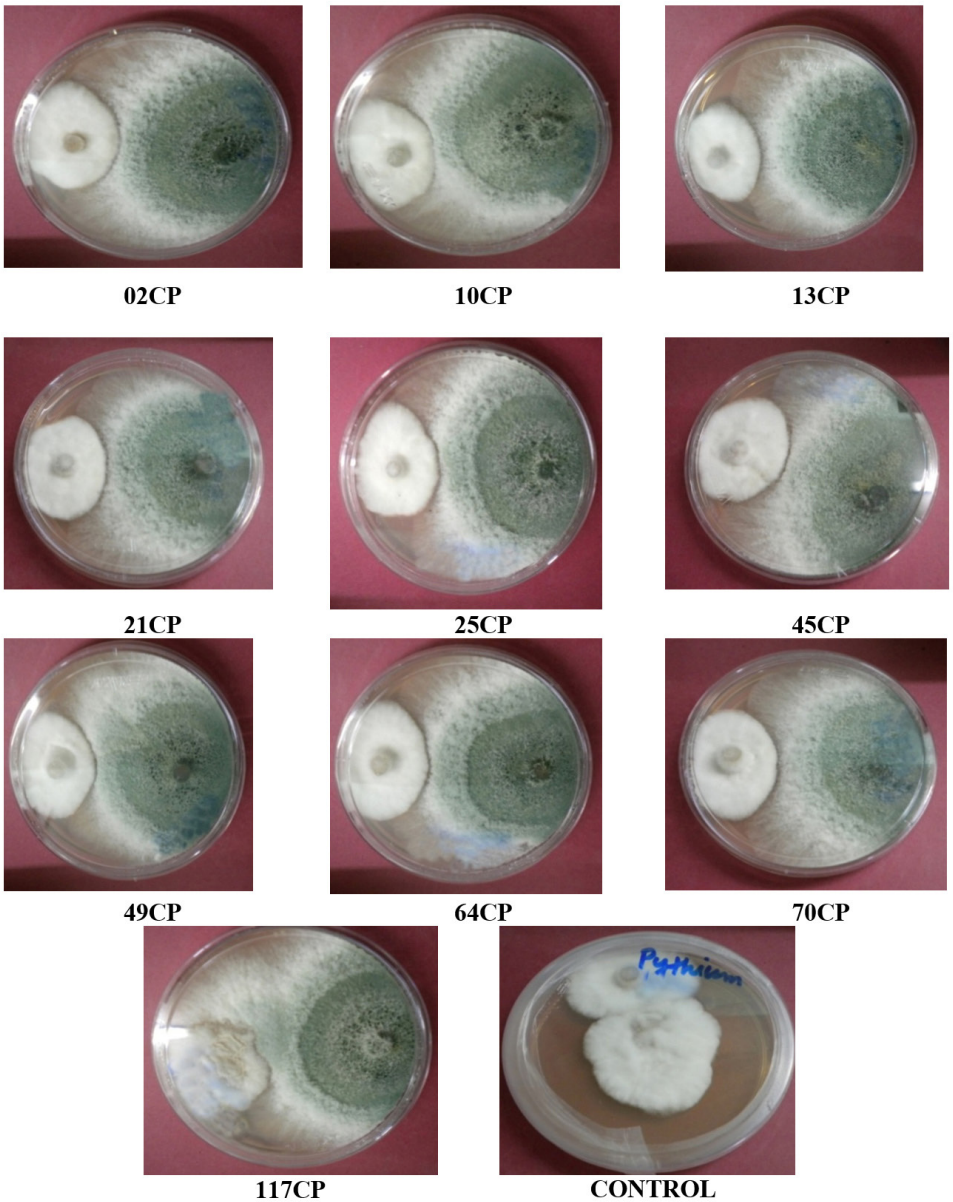

Figure 5. Antagonistic activity of *T. viride* isolates against *Pythium* spp.

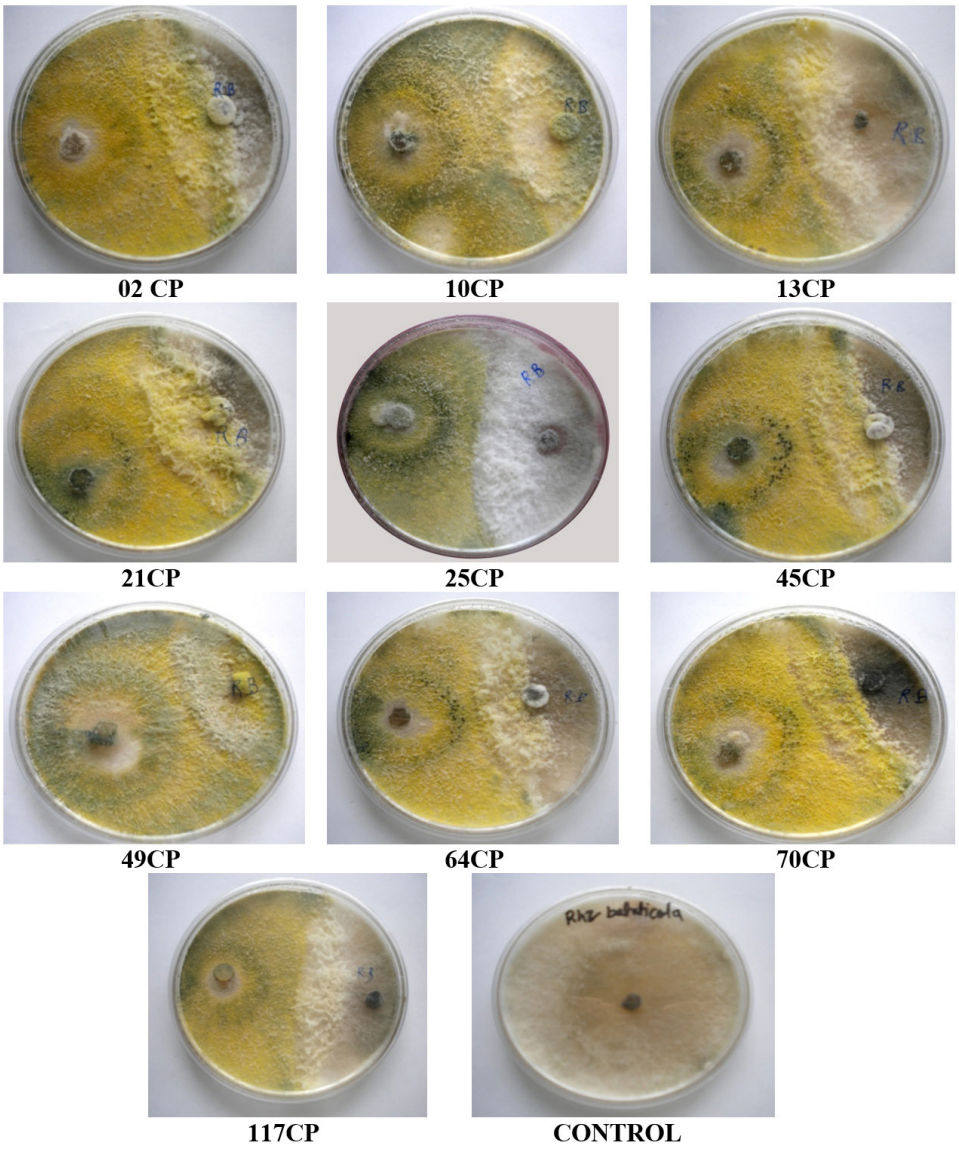

Figure 6. Antagonistic activity of *T. viride* isolates against *Rhizoctonia bataticola*

followed by ARS K-11 at 83.70% in a dual culture assay.

CONCLUSION

A pH level of 6.5 (slightly acidic) was found to be the most conducive for the growth and biomass (sporulation) production of *T. viride* isolates. Among the ten *T. viride* isolates investigated in this study, the 49CP isolate demonstrated superior performance across all parameters, particularly in terms of antagonistic activity. Therefore, the 49CP isolate of *T. viride* is recommended for further analysis, including the preparation of bioformulations and both *in vitro* and *in vivo* trial experiments for managing soil-borne pathogens. *T. viride*-based bioformulations could serve as an effective alternative to chemical treatments for the management of plant diseases.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This article does not contain any studies on human participants or animals performed by any of the authors.

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