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Hemlata Sahu
 Department of Plant
 Pathology, AKS University,
 Satna, Madhya Pradesh, India

Premlata
 Department of Plant
 Pathology, AKS University,
 Satna, Madhya Pradesh, India

Doomar Singh
 Department of Plant
 Pathology, AKS University,
 Satna, Madhya Pradesh, India

In vitro evaluation of bioagents against *Fusarium oxysporum* F. sp. *lentis* causing wilt in lentil (*Lens culinaris* Medik)

Hemlata Sahu, Premlata and Doomar Singh

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Abstract

Lentil (*Lens culinaris* Medik), a crucial pulse crop, is significantly affected by wilt caused by *Fusarium oxysporum* f. sp. *lentis*. This study investigates the occurrence, isolation, and *in vitro* management of this pathogen using three bioagents: *Trichoderma viride*, Neem oil, and *Pseudomonas fluorescens*. Surveys conducted in the Sohawal block of Satna district during the Rabi season of 2024–25 confirmed variable wilt incidence. The pathogen was isolated, purified. *In vitro* evaluations using poisoned food technique showed that all three bioagents inhibited fungal growth, with *Trichoderma viride* (T3) and *Pseudomonas fluorescens* (2%) demonstrating the highest efficacy. These findings suggest promising eco-friendly strategies for managing lentil wilt.

Keywords: Lentil (*Lens culinaris* Medik), *Fusarium oxysporum* f. sp. *lentis*, wilt disease, biocontrol agents, *Trichoderma viride*, *Pseudomonas fluorescens*, neem oil, *In vitro* evaluation, soil-borne pathogen control

Introduction

Lentil (*Lens culinaris* Medik) is among the earliest domesticated pulse crops and holds significant importance in global agriculture due to its high protein content and rich nutritional profile (Sandhu & Singh, 2007; Zapata *et al.*, 2004)^[8, 12]. Pulses, including *lentis*, serve as a primary source of protein in Indian diets, particularly for the predominantly vegetarian population. However, India's pulse production has struggled to meet the increasing demand, necessitating enhanced cultivation strategies to bridge the supply gap. Lentil seeds are a nutrient-dense food source, comprising approximately 23.7% protein, 60% carbohydrates, and 11% water. In addition to macronutrients, lentils are rich in essential minerals such as calcium, iron, and niacin, making them a valuable component of a balanced diet. Beyond human consumption, lentil straw is widely utilized as a nutritious and cost-effective animal feed, further reinforcing the crop's agricultural and economic significance. The present investigation explores the use of bioagents as an eco-friendly approach to manage lentil wilt.

Material methods

Survey of disease incidence

A survey was conducted to record the occurrence and distribution of wilt of lentil in Sohawal block, Satna districts across one major lentil-growing block in central India - Madhya Pradesh, during the Rabi season of 2024-25. The number of fields in Sohawal block visited Satna district ranged from 10, with a distance of 5-7 km. A total of write numbers fields across Shohawal block of Satna districts were surveyed, and disease incidence, was recorded. In each field infected plant were randomly selected, and counted. Disease incidence was calculated based on the number of infected plants relative to the total number of plants. lentil plants displaying typical wilt symptoms were collected from the surveyed areas, packed in labelled polythene bags, and were brought to the laboratory for pathogen isolation.

$$\text{Percent disease incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100$$

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Corresponding Author:
Hemlata Sahu
 Department of Plant
 Pathology, AKS University,
 Satna, Madhya Pradesh, India

Isolation and purification of fungus from infected roots and stem

Plants showing typical wilt symptoms were washed under running tap water and blot dried. Infected roots and stems were cut into pieces of 5–6 mm in size. After thorough washing in sterile distilled water the pieces were transferred using forceps onto sterilized Potato Dextrose Agar (PDA) medium in Petri dishes and incubated at 27 °C to obtain mycelium, conidia, conidiophores, and chlamydospores growth. After 48 hours of incubation, the growing mycelium and spores were observed and marked. Mycelium and spores from the margins of the resulting colonies were cut with a sterilized 5 mm cork borer and transferred to a Petri dish containing PDA for re-isolation of the pathogen.

The cultures were then purified using the single spore isolation technique. Spores were collected from a sporulating fungal culture of 48 hrs old using a sterile loop and suspended in sterile distilled water in a test tube. Serial dilutions (10^{-1} to 10^{-5}) of the spore suspension were prepared to reduce the spore concentration. A small drop of the diluted suspension was pipetted onto PDA medium in a Petri dish and spread gently using a sterile bent glass rod.

The Petri dishes were incubated at an optimal temperature of 25–28 °C for 12–24 hours until germination was observed. Using a compound microscope, well-separated single germinating spores on the agar surface were located and transferred to fresh PDA plates. These plates were incubated at the appropriate temperature for fungal growth. Colony development was monitored, and the cultures were checked for purity by examining colony morphology and microscopic features to ensure separation of individual spores. The purified isolates were then transferred to PDA slants. The pure cultures of all isolates grown on PDA slants were stored at 8 °C for further studies.

In vitro evaluation of different bio-agents against the fungus

An *in vitro* study was conducted to assess the efficacy of different three Bio-agent: *Trichoderma viride*, Neem oil, *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *lentis* using the poisoned food technique with Potato Dextrose Agar (PDA) as the basal medium.

Bio-agents Tested and Their Concentrations:

1. *Trichoderma viride*
2. Neem Oil – 0.5%, 1.0%, 1.5%, 2.0% (Neemanjali)
3. *Pseudomonas fluorescens* – 1.0%, 1.5%, 2.0% (Multiplex)

Based on the active ingredient, the required amount of each bio-agents was weighted mixed thoroughly with autoclaved and cooled (40 °C) PDA in conical flasks to achieve the desired concentrations. Twenty milliliter of bioagents-amended PDA was poured aseptically into 90 mm sterile glass Petri plates and allowed to solidify at room temperature. Each plate was then inoculated at the center with a 5 mm disc of actively growing *Fusarium oxysporum* f. sp. *lentis* culture (7-day-old), placed in an inverted position. Control plates (without any bio-agents) were also inoculated with the same culture disc to serve as untreated controls. Three replications were maintained for each treatment. Plates were incubated at optimum temperature and suitable pH for 7 days. After incubation, radial mycelium growth (in mm) of the pathogen was measured. The percent inhibition of mycelium growth was calculated using the following formula

$$\text{Percent Growth Inhibition} = \frac{C-T}{C} \times 100$$

Where,

C= growth of the test fungus in control plates (mm)

T= growth of the test fungus in treated plates (mm)

3. Results and Discussion

3.1 Effect of *Pseudomonas fluorescens* on the Growth of *Fusarium oxysporum* f. sp. *lentis*

The radial growth of *Fusarium oxysporum* f. sp. *lentis* was significantly influenced by different concentrations of *Pseudomonas fluorescens* at 3, 5, and 7 days after inoculation (DAI). The results, as presented in Table 3.1, indicate a varied suppressive effect of the bacterial antagonist across different time intervals.

Table 3.1: Effect of *Pseudomonas fluorescens* on the growth of *Fusarium oxysporum* f. sp. *lentis*

Treatment (concentration)	Mean radial growth (in cm) <i>Fusarium oxysporum</i> f. sp.		
	3DAI	5DAI	7DAI
1%	1.400	5.750	8.338
1.5%	1.038	5.588	8.063
2%	1.263	5.500	6.013
Control	0.700	3.725	5.875
SE(m) ±	0.188	0.277	0.568
C.V%	34.104	10.764	11.355
C.D(P=0.01)	0.81	1.19	2.45
F- Tabulated	5.95	5.95	5.95

At 3 DAI, the highest radial growth was observed in the 1% treatment (1.400 cm), followed by 1.5% (1.038 cm) and 2% (1.263 cm). Interestingly, the control (0.700 cm) recorded the lowest growth, likely due to slower initial colonization of the pathogen in the absence of bioagent competition. However, by 5 DAI, all treated plates exhibited increased growth compared to the control (3.725 cm), with the highest growth again at 1% (5.750 cm), followed closely by 1.5% (5.588 cm) and 2% (5.500 cm). This trend suggests that at

early stages, *P. fluorescens* may not inhibit the pathogen significantly but exerts a stronger effect over time.

By 7 DAI, a clear antagonistic effect was observed at the 2% concentration, which recorded the lowest radial growth (6.013 cm), significantly less than both 1% (8.338 cm), 1.5% (8.063 cm), and the control (5.875 cm). These findings imply that while lower concentrations may initially stimulate fungal growth due to the presence of additional nutrients or by competitive stress induction, higher

concentrations exert an inhibitory influence as the bacterial population stabilizes and produces antifungal metabolites. These observations align with those reported by Raghuchander *et al.* (2004) [4] and Sharma and Sharma (2008) [10], who demonstrated that *P. fluorescens* significantly inhibits *Fusarium* spp. *in vitro* through the production of antibiotics (such as pyoluteorin and 2,4-

diacetylphloroglucinol), siderophores, and lytic enzymes. Gopalakrishnan *et al.* (2011) [3] also confirmed the suppressive effects of *Pseudomonas* species against soil-borne pathogens including *F. oxysporum* in chickpea and lentil systems, suggesting that strain specificity and application dosage are key determinants of efficacy.

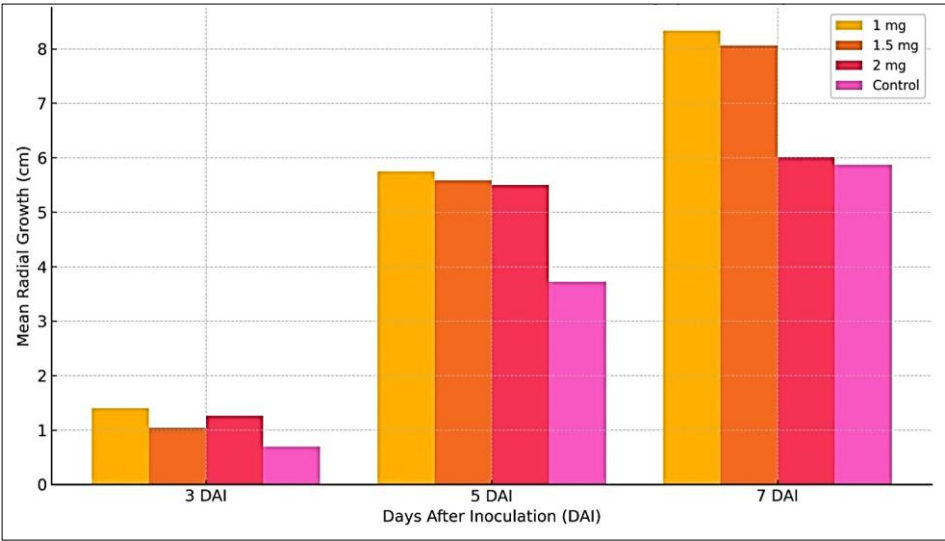
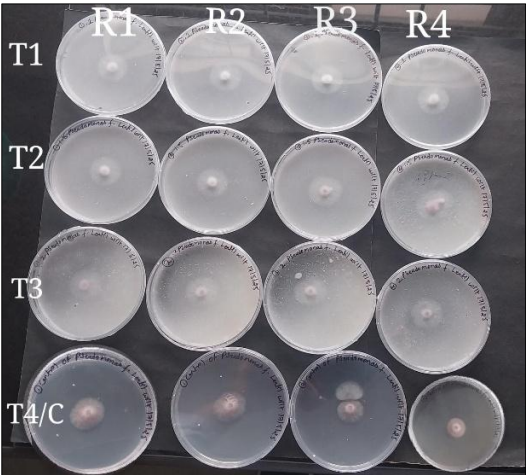
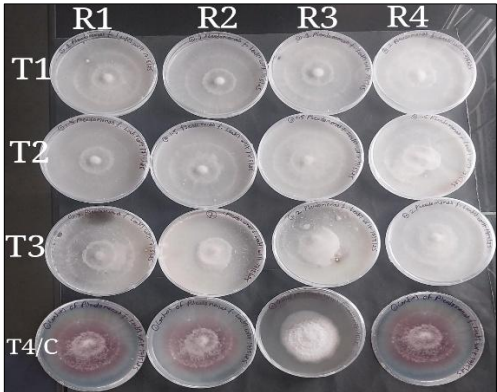


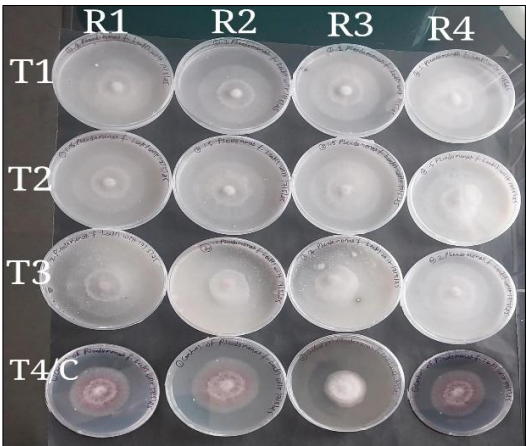
Fig 1: Effect of *Pseudomonas fluorescens* on *Fusarium oxysporum* f. sp. *lentis*



Effect of *Pseudomonas fluorescens* on the growth of *Fusarium oxysporum* f. sp. *lentis* on 3 DAI



Effect of *Pseudomonas fluorescens* on the growth of *Fusarium oxysporum* f. sp. *lentis* on 7 DAI



Effect of *Pseudomonas fluorescens* on the growth of *Fusarium oxysporum* f. sp. *lentis* on 5 DAI

3.2 Effect of Neem Oil on the Growth of *Fusarium oxysporum* f. sp. *lentis*

Neem oil treatments at 0.5%, 1.0%, and 1.5% concentrations exhibited a moderate inhibitory effect on *F. oxysporum* f. sp. *lentis*, with the highest suppression generally observed at the 1.0% concentration.

Table 3.2: Effect of Neem oil on the Growth of *Fusarium oxysporum* f. sp. *lentis*

Treatment (concentration)	Mean radial growth (in cm) <i>Fusarium oxysporum</i> f. sp.		
	3DAI	5DAI	7DAI
0.5 ml	1.000	2.650	4.125
1 ml	1.063	2.388	3.750
1.5 ml	1.038	2.500	4.000
Control	1.225	2.463	4.163
SE(m) ±	0.073	0.112	0.068
C.V%	13.515	8.972	3.411
C.D(P=0.01)	0.31	0.48	0.29
F- Tabulated	5.95	5.95	5.95

At 3 DAI, all Neem oil treatments showed slight inhibition of fungal growth compared to the control (1.225 cm), with the lowest growth observed at 0.5% (1.000 cm). The difference, although minor, was statistically significant ($CD = 0.31$ at $P = 0.01$). By 5 DAI, 1% Neem oil recorded the least radial growth (2.388 cm), followed by 1.5% (2.500 cm) and 0.5% (2.650 cm), while the control remained at 2.463 cm. At 7 DAI, the 1% treatment continued to perform best, with growth limited to 3.750 cm, lower than the control (4.163 cm).

These results suggest that Neem oil at 1% concentration exerts a consistent fungistatic effect on *Fusarium* growth. The antifungal action is likely due to azadirachtin and other

limonoids, which interfere with fungal membrane function and enzyme activity.

Similar inhibitory effects of Neem-based products have been reported by Patel *et al.* (2015)^[6] and Manjunatha *et al.* (2012) against *Fusarium* spp. and *Rhizoctonia bataticola* in legumes. Saxena and Pandey (2001)^[9] also documented that Neem oil inhibits sporulation and conidial germination, contributing to disease suppression under laboratory conditions. However, it was also noted in these studies that excessive concentrations (>2%) may result in phytotoxicity or reduced antagonistic efficiency, reinforcing the effectiveness of the 1% dose observed in the current experiment.

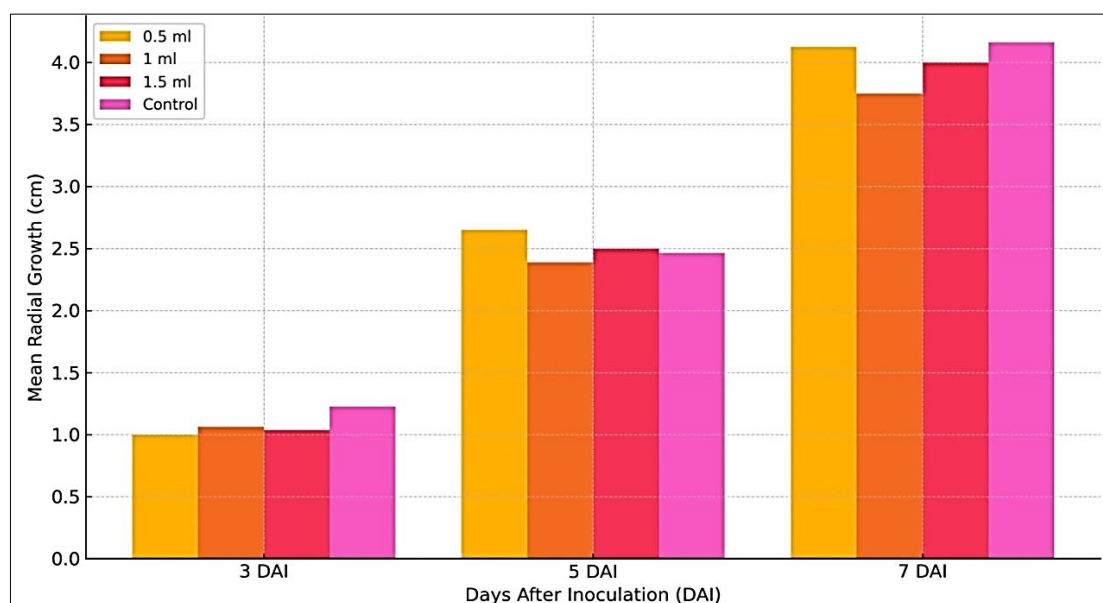
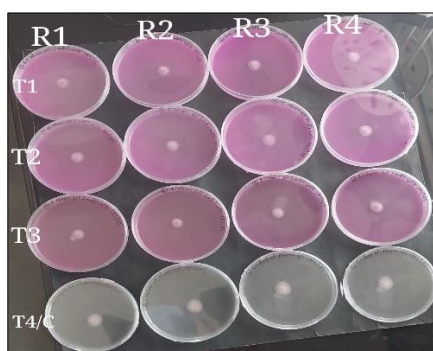
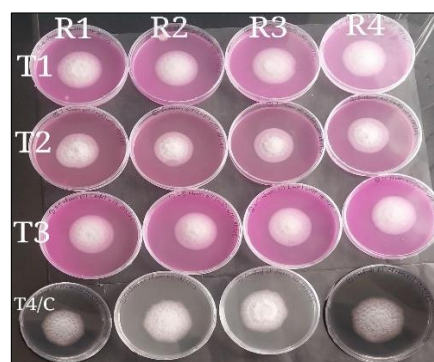


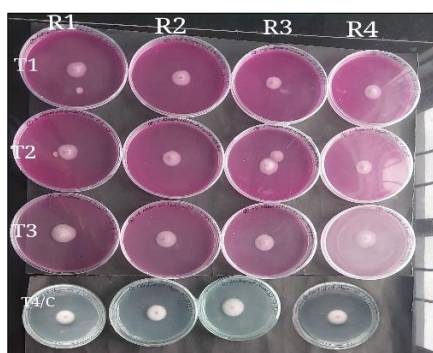
Fig 2: Effect of Neem Oil on *Fusarium oxysporum* f. sp. *lentis*



Effect of Neem oil on the Growth of *Fusarium oxysporum* f. sp. *lentis* on 3 DAI



Effect of Neem oil on the Growth of *Fusarium oxysporum* f. sp. *lentis* on 7DAI



Effect of Neem oil on the Growth of *Fusarium oxysporum* f. sp. *lentis* on 5 DAI

3.3 Effect of *Trichoderma viride* on the Growth of *Fusarium oxysporum* f. sp. *lentis*

The antagonistic effect of *Trichoderma viride* was assessed across five time intervals (1–5 DAI), and the data indicate a significant suppression of pathogen growth, particularly in the T3 treatment. At 1 and 2 DAI, negligible differences were recorded among all treatments, with growth ranging from 0.500 cm to 0.567 cm. However, from 3 DAI onward, treatment T3 consistently showed superior inhibitory activity.

Table 3.3: Effect of *Trichoderma viride* on the Growth of *Fusarium oxysporum* f. sp.

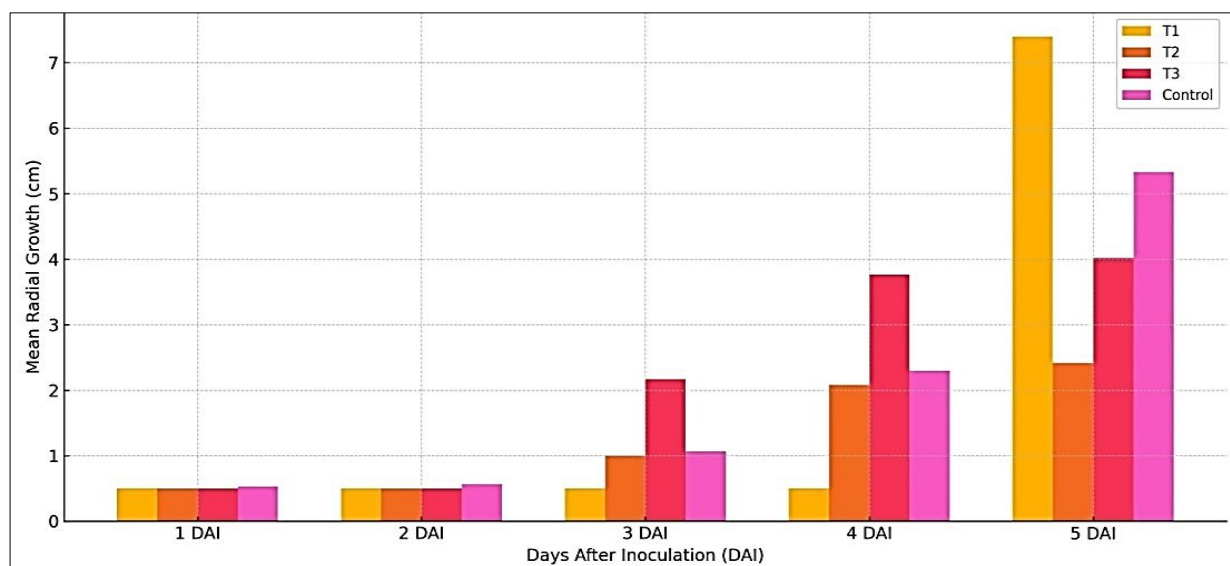
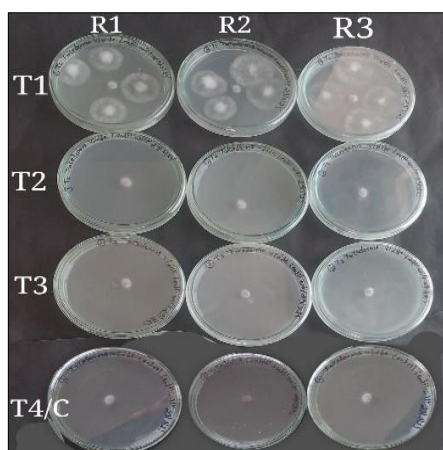
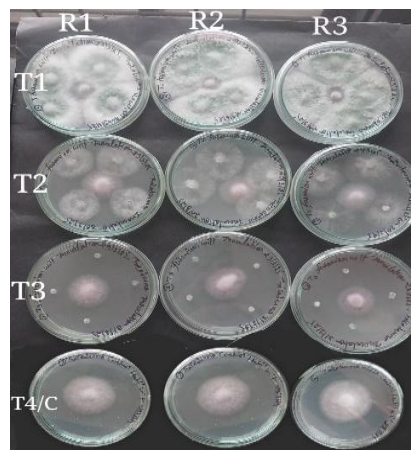
Treatment	Average growth Mean (in cm) <i>Fusarium oxysporum</i> f. sp.				
	1 DAI	2DAI	3DAI	4DAI	5DAI
T1	0.500	0.500	0.500	0.500	7.400
T2	0.500	0.500	1.000	2.083	2.417
T3	0.500	0.500	2.167	3.767	4.017
Control	0.533	0.567	1.067	2.300	5.333
SE(m) ±	0.017	0.033	0.061	0.127	0.179
C.V%	5.679	11.175	8.880	10.188	6.475
C.D(P=0.01)	0.08	0.15	0.28	0.60	0.84
F- Tabulated	7.95	7.95	7.95	7.95	7.95

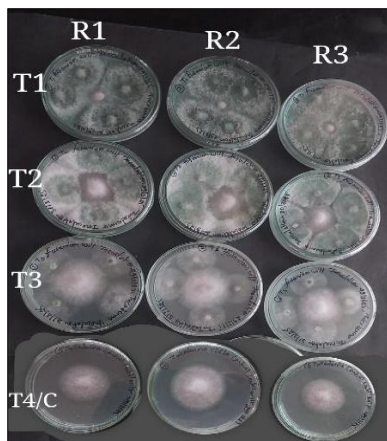
At 3 DAI, T3 restricted growth to 2.167 cm compared to 1.067 cm in the control. By 4 DAI, T3 continued to suppress growth (3.767 cm), while the control reached 2.300 cm. T1, however, showed a sharp increase in fungal growth (7.400 cm), indicating poor biocontrol performance, possibly due to insufficient colonization or low spore viability. At 5 DAI, T3 still recorded the lowest radial growth (4.017 cm), followed by T2 (2.417 cm) and control (5.333 cm), while T1 remained ineffective.

The superior performance of T3 treatment demonstrates that higher concentrations or more aggressive strains of *Trichoderma viride* are capable of significantly inhibiting

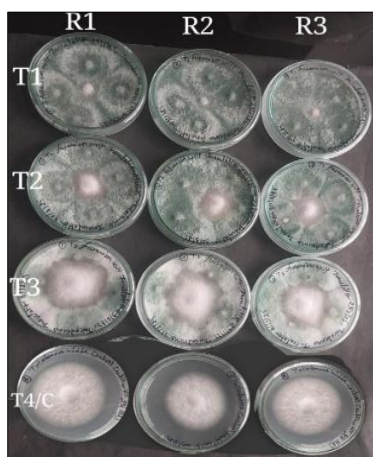
Fusarium growth. This is attributed to mechanisms such as mycoparasitism, antibiosis, and competition for nutrients and space.

These findings corroborate earlier studies by Elad *et al.* (1983) ^[2] and Papavizas (1985) ^[5], who described the strong antagonistic potential of *Trichoderma* spp. against several phytopathogens through production of cell wall-degrading enzymes (chitinases, glucanases) and volatile inhibitors. Dubey and Patel (2001) ^[1] and Singh *et al.* (2010) ^[11] also reported similar trends in lentil and chickpea pathosystems, affirming *Trichoderma* as a potent biocontrol agent under both *in vitro* and field conditions.

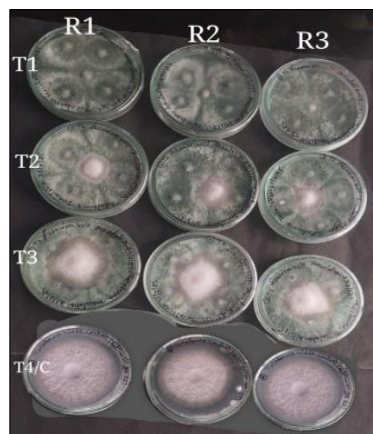
Effect of *Trichoderma viride* on *Fusarium oxysporum* GrowthEffect of *Trichoderma viride* on the Growth of *Fusarium oxysporum* f. sp. on 1 DAIEffect of *Trichoderma viride* on the Growth of *Fusarium oxysporum* f. sp. on 2 DAI



Effect of *Trichoderma viride* on the Growth of *Fusarium oxysporum* f. sp. on 3 DAI



Effect of *Trichoderma viride* on the Growth of *Fusarium oxysporum* f. sp. on 4 DAI



Effect of *Trichoderma viride* on the Growth of *Fusarium oxysporum* f. sp. on 5 DAI

Conclusion

The present study reveals the differential inhibitory effects of three bioagents on the growth of *Fusarium oxysporum* f. sp. *lentis*. Among the tested agents:

1. *Trichoderma viride* (T3) showed highest suppression
2. *Pseudomonas fluorescens* at 2% was also highly effective
3. Neem oil at 1% had moderate efficacy

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