

***In vitro* bio efficacy of biocontrol agents against *Colletotrichum truncatum* associated with leaf blight disease of Linseed in India**

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Received:- 05 August 2026/ Revised:- 18 August 2026/ Accepted:- 24 August 2026/ Published: 31-08-2026

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Abstract— Leaf blight caused by *Colletotrichum truncatum* is an important fungal disease of linseed (*Linum usitatissimum* L.) that can reduce crop growth and productivity. Considering the ecological concerns associated with excessive fungicide use, the present study evaluated the *in vitro* efficacy of *Trichoderma asperellum* and *Pseudomonas fluorescens* against *C. truncatum* using the dual culture technique. Among *T. asperellum* treatments, *T*₁ showed the greatest antagonistic activity, restricting pathogen growth to 25.40 ± 0.52 mm with 70.80% inhibition, followed by *T*₂ (26.80 ± 0.63 mm; 69.20%) and *T*₃ (27.65 ± 0.58 mm; 68.22%), compared with 87.00 ± 0.00 mm in the control. Among *P. fluorescens* treatments, *T*₂ was most effective, limiting mycelial growth to 29.10 ± 0.56 mm and achieving 66.65% inhibition, followed by *T*₁ (31.25 ± 0.78 mm; 64.18%) and *T*₃ (32.53 ± 0.75 mm; 62.72%), against 87.25 ± 0.73 mm in the control. Overall, *T. asperellum* exhibited superior antagonistic activity. This study demonstrates that promising biocontrol agents identified through *in vitro* screening could serve as environmentally compatible components of an integrated disease management strategy for the management of *C. truncatum*-induced leaf blight in linseed.

Keywords— Antagonism, biocontrol agents, biological control, *Colletotrichum truncatum*, dual culture technique, leaf blight, linseed.

I. INTRODUCTION

Linseed (*Linum usitatissimum* L.), commonly known as flaxseed, is an important traditional *rabi* oilseed crop cultivated during the winter season. Its significance is attributed to its high-quality seed oil, nutritional value, and industrial applications. The crop also serves as a valuable component of diversified cropping systems because of its adaptability to relatively poor soils and water-limited conditions. Linseed oil is widely used in the food, cosmetic, and eco-material industries owing to its desirable fatty acid composition and functional properties (Zanetti et al., 2013). Based on morphological characteristics and end use, the crop is broadly classified into two types: seed type (linseed), primarily cultivated for oil production and fibre type (flax), grown mainly for fibre production. In India, cultivated varieties are predominantly seed types and are grown primarily for oil extraction (Singh, 2016; Biradar et al., 2016).

Linseed seeds generally contain approximately 33–47% oil, depending on genotype, environmental conditions, and other factors. The oil is particularly valued for its high concentration of essential fatty acids and is an important plant-based source of omega-3 fatty acids, especially alpha-linolenic acid (ALA), which constitutes approximately 55% of the total fatty acids. In India, a substantial proportion of linseed oil is utilized for industrial purposes, including the manufacture of boiled, borated, epoxidized, aluminate, urethane, and isomerized oils (Mueed et al., 2022). During 2021–22, linseed was cultivated in India on approximately 2.0 lakh hectares, producing about 1.2 lakh metric tonnes, with an average productivity of 659 kg ha⁻¹. The crop is cultivated extensively in Madhya Pradesh, Rajasthan, Bihar, Uttar Pradesh, Assam, Jharkhand, and several other states. Madhya Pradesh accounts for the largest area under cultivation, with approximately 1.16 lakh hectares and a production of about 0.55 lakh tonnes, although its productivity remains relatively low at approximately 474 kg ha⁻¹ (APEDA, 2022).

Despite its economic and nutritional importance, linseed production is affected by several biotic constraints, particularly fungal diseases that reduce plant growth, yield, and seed quality (Patel et al., 2023). Leaf blight is an important disease that reduces photosynthetic area and may cause premature defoliation and yield loss. *Colletotrichum truncatum* is an important fungal pathogen causing necrotic leaf lesions that may enlarge and coalesce under warm and humid conditions. Its survival in infected crop residues can contribute to disease persistence and subsequent outbreaks (Sampathkumar et al., 2025). A distinct leaf blight caused by *Colletotrichum truncatum* was recently observed in linseed fields of Karnataka, and this pathogen–disease association is newly reported in India (Sampathkumar et al., 2025).

Management of fungal leaf blight diseases mainly depends on synthetic fungicides. However, their repeated and excessive use can lead to fungicide resistance, chemical residues, environmental pollution, disruption of beneficial microorganisms, and increased production costs. Therefore, sustainable and eco-friendly disease-management methods are needed. Biological control using beneficial microorganisms such as *Trichoderma*, *Pseudomonas*, and *Bacillus* offers a promising alternative. These microorganisms suppress plant pathogens through competition, production of antimicrobial compounds and cell-wall-degrading enzymes, mycoparasitism, and inhibition by volatile compounds. Some biocontrol agents also promote plant growth and induce systemic resistance, making them useful components of integrated disease management (Islam et al., 2024).

However, the efficacy of biocontrol agents varies depending on the antagonist, pathogen isolate, environmental conditions, and interactions between the organisms. Therefore, preliminary *in vitro* screening is an important step in identifying efficient antagonists before their evaluation under greenhouse and field conditions. The dual-culture technique provides a simple and reliable method for assessing direct antagonistic interactions by measuring the ability of a biocontrol agent to restrict the radial growth of a target pathogen under controlled conditions. Such screening enables the selection of promising antagonists for subsequent evaluation and integration into sustainable disease-management programmes (Nguyen et al., 2025; Tyagi et al., 2024).

As this pathogen–disease association is newly reported in India and information on its management is limited, the present study was undertaken to evaluate the *in vitro* bio-efficacy of selected biocontrol agents against *C. truncatum* by assessing their ability to inhibit mycelial growth and identifying the most effective antagonists.

II. MATERIALS AND METHODS

2.1 Survey and Collection of Diseased Samples

A systematic roving field survey was conducted during the *rabi* seasons of 2023–24 and 2024–25 in major linseed-growing districts of North Karnataka. During the survey, linseed plants exhibiting characteristic symptoms of leaf blight were recorded. Diseased leaf samples representing typical symptoms were collected from different locations, placed in sterile paper bags, transported to the laboratory, and processed for isolation and identification of the associated fungal pathogen and subsequent studies.

2.2 Isolation and Identification of the Fungal Pathogen

Diseased linseed leaves exhibiting characteristic leaf blight symptoms were gathered from different locations during the roving field survey and transported to the laboratory in sterile paper bags for further processing. The samples were washed thoroughly under running tap water to remove adhering soil and debris. Small pieces of diseased leaf tissue measuring approximately 5 × 5 mm were excised from the advancing margin of the lesions, ensuring that both healthy and infected tissues were included. The tissue pieces were surface sterilized with 2% sodium hypochlorite for 1–2 min, followed by three rinses with sterile distilled water. The sterilized pieces were blotted dry on sterile filter paper and aseptically placed on potato dextrose agar (PDA) plates supplemented with streptomycin to suppress bacterial contamination. The plates were incubated at 28 ± 2 °C and monitored periodically for fungal growth. The fungal colonies emerging from the infected tissues were subcultured onto fresh PDA plates to obtain pure cultures. The purified isolates were maintained on PDA slants at 4 °C for further studies (Sampathkumar et al., 2026). Identification of the fungal pathogen was initially based on cultural and morphological characteristics, including colony colour, texture, growth pattern, pigmentation, hyphal characteristics, and reproductive structures. The fungal isolates were examined microscopically using a compound microscope to record their morphological characteristics. The observed features were compared with published taxonomic descriptions and relevant literature for preliminary identification (He et al., 2016). Molecular confirmation of the pathogen identity was performed as part of an earlier study (Sampathkumar et al., 2025), where the isolates were confirmed as *C. truncatum* using multi-gene phylogeny (ITS, *ACT*, *TUB2*, and *GAPDH* sequences).

2.3 Collection and Isolation of Biocontrol Agents

Trichoderma asperellum and *Pseudomonas fluorescens* were obtained from commercially available talc-based formulations developed by the University of Horticultural Sciences, Bagalkot. These formulations were used as sources for isolating the biocontrol agents. The biocontrol agents were isolated from the formulations and cultured on suitable media to obtain pure cultures. *T. asperellum* was identified based on colony characteristics such as colour, texture, growth pattern and pigmentation, along with microscopic features such as conidiophores, phialides and conidia. *P. fluorescens* was identified based on its colony characteristics, pigmentation and microscopic features. The observed characteristics were compared with standard taxonomic descriptions and published literature to confirm their identity.

2.4 In vitro Antagonistic Activity of Biocontrol Agents against the Fungal Pathogen

The antagonistic efficacy of the biocontrol agents against the pathogen was evaluated using the dual culture technique described by Dennis and Webster (1971) on potato dextrose agar (PDA) medium.

2.4.1 Dual Culture Assay of *Trichoderma asperellum*

Approximately 20 mL of molten PDA medium was poured into sterile Petri plates and allowed to solidify. A 5-mm-diameter mycelial disc was cut from a 10-day-old culture of the pathogen using a sterile cork borer and placed near the periphery of one side of the PDA plate. Similarly, a 5-mm-diameter disc of the antagonist, *T. asperellum*, was placed near the opposite periphery of the same plate. Plates inoculated only with the pathogen served as controls. All inoculated plates were incubated at 28 ± 2 °C for 7 days. Each treatment was maintained with three replicates. After incubation, when the pathogen colony in the control plates reached approximately 90 mm in diameter, the radial growth of the pathogen was measured.

2.4.2 Dual Culture Assay of *Pseudomonas fluorescens*

For evaluation of the bacterial antagonist, a 5-mm-diameter mycelial disc of the test fungus was placed at the centre of a PDA plate. *Pseudomonas fluorescens* was streaked on either side of the fungal inoculum at an appropriate distance. Plates inoculated only with the pathogen served as controls. All plates were incubated at 28 ± 2 °C for 7 days. Following incubation, the radial growth of the pathogen was measured and compared with that of the control.

2.5 Calculation of Mycelial Growth Inhibition

The percentage inhibition of mycelial growth of the pathogen by the antagonists was calculated using the formula described by Vincent (1927).

$$\text{Mycelial inhibition (\%)} = \frac{C-T}{C} \times 100 \quad (1)$$

where:

C = radial growth of the pathogen in the control plate

T = radial growth of the pathogen in the treatment plate containing the antagonist

The percentage inhibition of mycelial growth was calculated using the same formula for both *Trichoderma asperellum* and *Pseudomonas fluorescens* treatments.

2.6 Statistical Analysis

The data on the *in vitro* efficacy of the biocontrol agents were statistically analysed using one-way analysis of variance (ANOVA) at the $P < 0.05$ significance level in SPSS version 16.0 (SPSS Inc., USA). Treatment means were separated using Tukey's honestly significant difference (HSD) test.

III. RESULTS

3.1 Identification of *Colletotrichum truncatum*

A systematic field survey was conducted during the *rabi* seasons of 2023–24 and 2024–25 across the linseed-growing districts of North Karnataka. During the survey, linseed plants exhibiting characteristic symptoms of leaf blight were observed. The disease was characterized by dark, sunken, circular to irregular lesions that frequently developed into shot-hole-like necrotic areas on the leaves (Fig. 1). Symptomatic leaf samples were collected from the surveyed locations and subjected to pathogen isolation. After 7 days of incubation, the developing fungal mycelium was transferred to fresh potato dextrose agar (PDA) plates, followed by single-spore isolation, which resulted in the recovery of four pure fungal isolates.

On PDA, the fungal colonies were circular with smooth margins. Initially, the colonies exhibited white to light-gray mycelial growth, which gradually turned dark gray with increasing incubation time. The reverse side of the colonies showed dense, dark-gray pigmentation with distinct zonation and well-defined concentric rings. After 10 days of incubation, numerous black acervuli developed abundantly on the colony surface, measuring 117–153 μm in diameter (Fig. 2A). The acervuli were spherical and produced numerous setae. The setae were rigid, linear, dark brown to black, straight at the base, and rounded at the apex, measuring 49–53 μm in length and 1.5–2.0 μm in width ($n = 10$). The conidia were hyaline, aseptate, smooth-walled, and characteristically curved, with nearly parallel sides. Conidial dimensions ranged from 15.8–20.1 μm in length and 2.6–3.9 μm in width ($n = 50$). The conidia possessed a truncate basal end and gradually tapered towards an acute, distinctly curved apical end. Granular cytoplasmic contents were clearly visible within the conidia (Fig. 2B).

Based on the cultural and morphological characteristics, the fungal pathogen associated with leaf blight of linseed was identified as *Colletotrichum truncatum* (He et al., 2016). The identity was further confirmed using multi-gene phylogeny (ITS, *ACT*, *TUB2*, and *GAPDH* sequences) in an earlier study (Sampathkumar et al., 2025).



FIGURE 1: Leaf blight of linseed. A: Field symptoms showing brown to dark brown, necrotic lesions on leaves with progressive drying and blighting; B: Close-up view of characteristic leaf lesions; C: Microscopic view of the associated fungal structures.

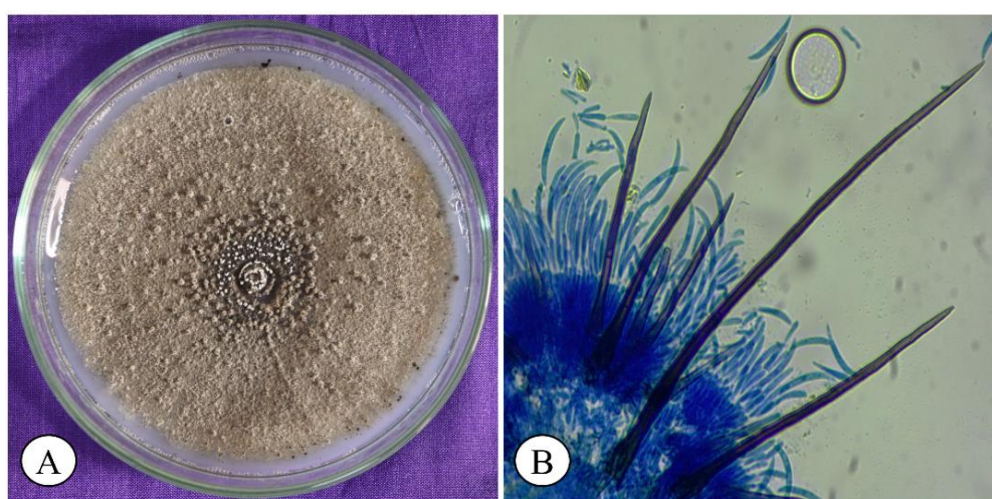


FIGURE 2: Cultural and microscopic characteristics of *Colletotrichum truncatum*. A: Colony morphology of *C. truncatum* on potato dextrose agar (PDA), showing characteristic colony growth and pigmentation after incubation; B: Microscopic view showing acervuli/conidiophores and numerous falcate, sickle-shaped conidia characteristic of *C. truncatum*.

3.2 In vitro Antagonistic Activity of Biocontrol Agents against the Fungal Pathogen

The biocontrol agents were successfully isolated from their respective formulations and obtained as pure cultures. Based on cultural and microscopic characteristics, the isolates were identified as *Trichoderma asperellum* and *Pseudomonas fluorescens*. Their observed characteristics were consistent with standard taxonomic descriptions, confirming their identity.

3.2.1 Dual Culture Assay of *Trichoderma asperellum* Against *Colletotrichum truncatum*

The *in vitro* assay demonstrated that *Trichoderma asperellum* effectively suppressed the mycelial growth of *Colletotrichum truncatum* compared with the untreated control (Table 1). Among the treatments, T₁ exhibited the highest antagonistic activity, recording the lowest mycelial growth (25.40 ± 0.52 mm) and maximum inhibition (70.80%). This was followed by T₂, which restricted pathogen growth to 26.80 ± 0.63 mm and resulted in 69.20% inhibition, while T₃ recorded 27.65 ± 0.58 mm growth with 68.22% inhibition. In contrast, the untreated control showed maximum mycelial growth (87.00 ± 0.00 mm) and no inhibition. These findings demonstrate the considerable antagonistic potential of *T. asperellum* against *C. truncatum*, with T₁ being the most effective treatment under *in vitro* conditions (Fig. 3).

TABLE 1

IN VITRO EVALUATION OF *Trichoderma asperellum* AGAINST *Colletotrichum truncatum*

Treatment	Mycelial growth of <i>C. truncatum</i> (mm) (Mean $\pm$ SE)	Growth Inhibition (%)
T ₁	25.40 ± 0.52	70.8
T ₂	26.80 ± 0.63	69.2
T ₃	27.65 ± 0.58	68.22
Control	87.00 ± 0.00	0.00
SE $\pm$	0.58	—
CD at 5%	1.84	—

Mycelial growth of *C. truncatum* was recorded after 7 days of incubation. Each treatment was replicated three times.

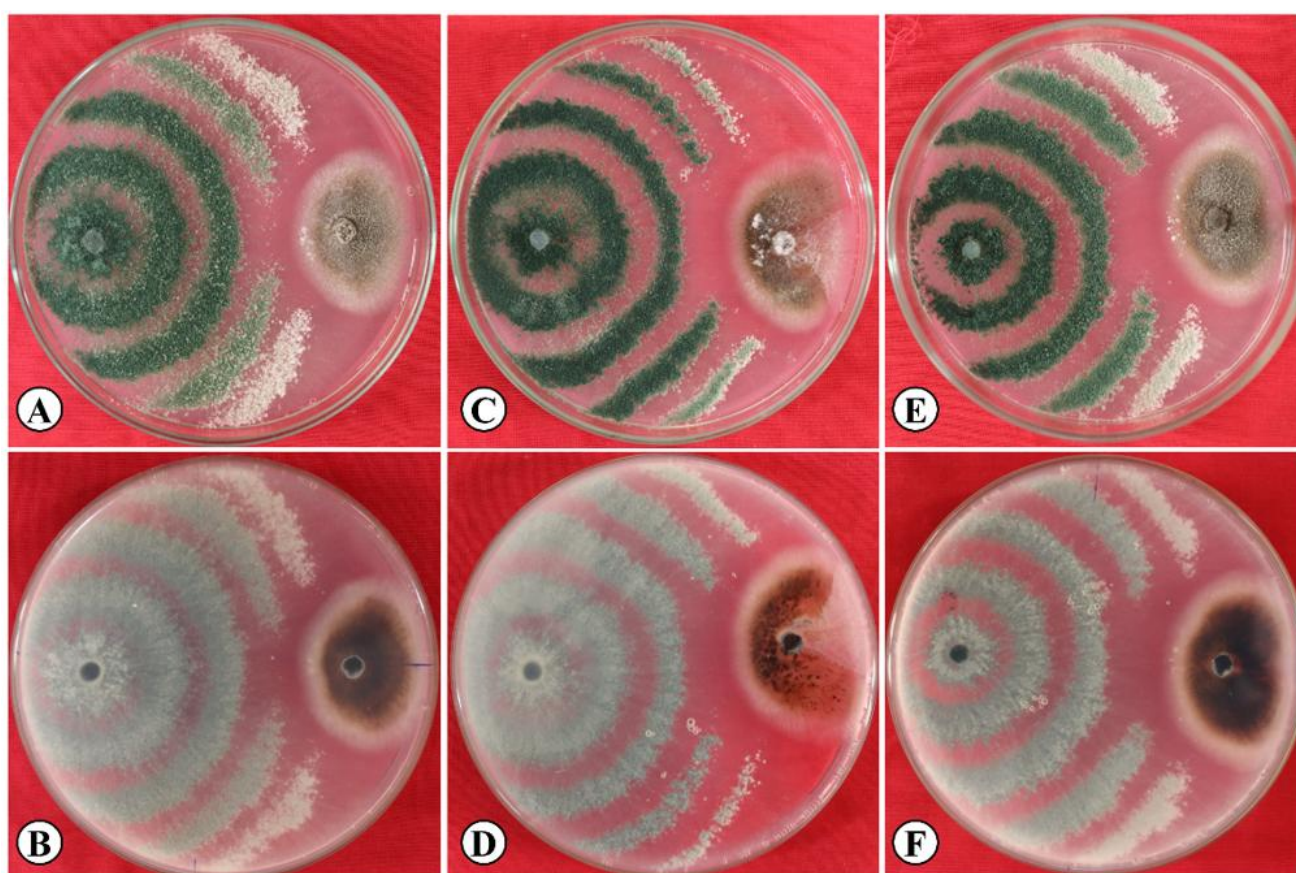


FIGURE 3: Antagonistic activity of *Trichoderma asperellum* against *Colletotrichum truncatum* in a dual culture assay, showing inhibition of the radial growth of the pathogen by the biocontrol agent.

3.2.2 Dual culture assay of *Pseudomonas fluorescens* against *Colletotrichum truncatum*

Similarly, the *in vitro* evaluation of *Pseudomonas fluorescens* revealed substantial suppression of *C. truncatum* growth in all treatments compared with the untreated control (Table 2). Mycelial growth ranged from 29.10 to 32.53 mm across the treatments, whereas the control recorded 87.25 ± 0.73 mm. T₂ was the most effective treatment, resulting in the lowest mycelial growth (29.10 ± 0.56 mm) and highest inhibition (66.65%). T₁ followed with 31.25 ± 0.78 mm growth and 64.18% inhibition, whereas T₃ recorded 32.53 ± 0.75 mm growth and 62.72% inhibition. Overall, *P. fluorescens* exhibited appreciable antagonistic activity against *C. truncatum*, with T₂ showing the greatest suppression of pathogen growth under *in vitro* conditions (Fig. 4).

TABLE 2
IN VITRO EVALUATION OF *Pseudomonas fluorescens* AGAINST *Colletotrichum truncatum*

Treatment	Mycelial Growth of <i>C. truncatum</i> (mm) (Mean $\pm$ SE)	Growth Inhibition (%)
T ₁	31.25 ± 0.78	64.18
T ₂	29.10 ± 0.56	66.65
T ₃	32.53 ± 0.75	62.72
Control	87.25 ± 0.73	0.00
SE $\pm$	0.41	—
CD at 5%	1.33	—

Mycelial growth of *C. truncatum* recorded after 7 days of incubation. Each treatment is replicated three times.

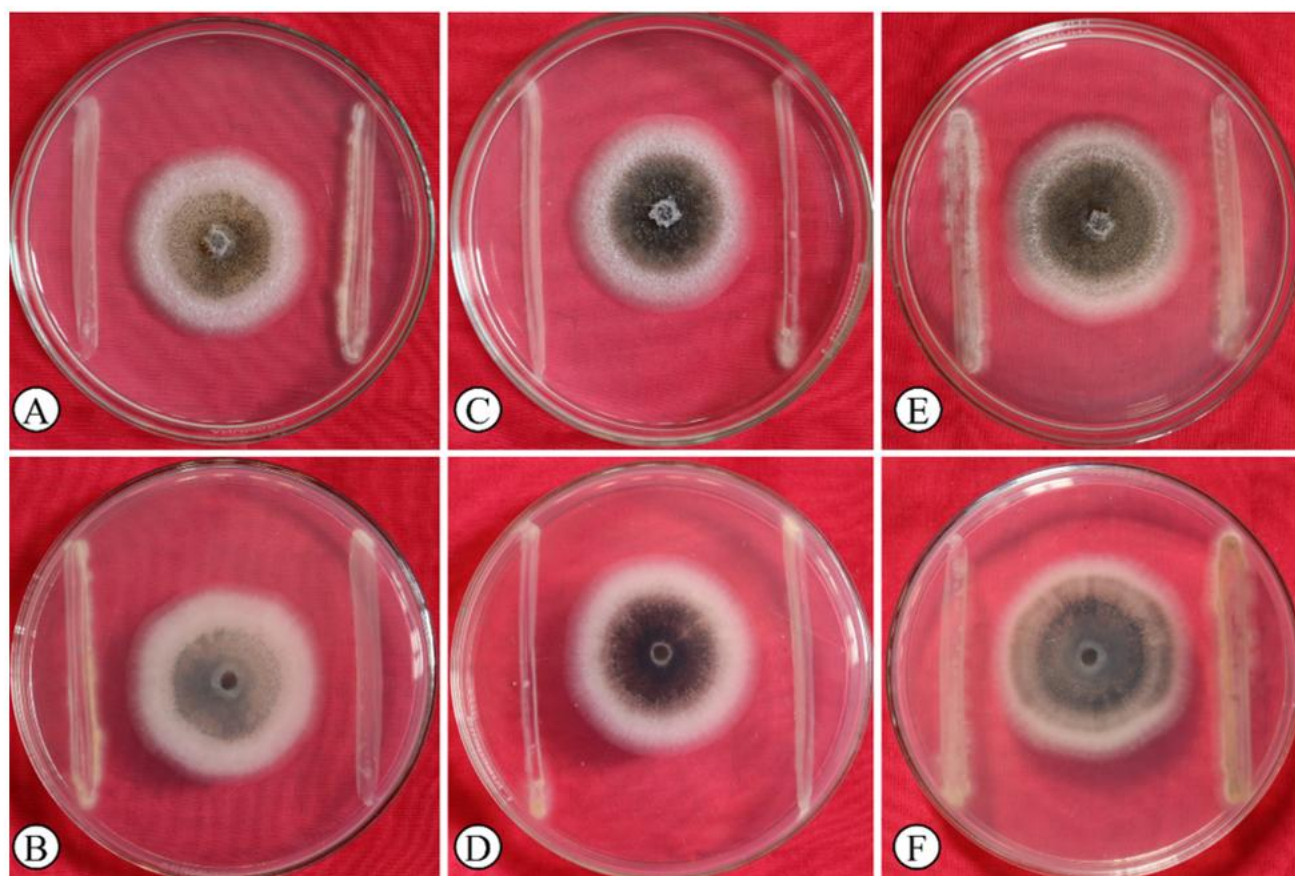


FIGURE 4: Antagonistic activity of *Pseudomonas fluorescens* against *Colletotrichum truncatum* in a dual culture assay, showing inhibition of the radial growth of the pathogen by the biocontrol agent.

IV. DISCUSSION

The *in vitro* evaluation of biocontrol agents against *Colletotrichum truncatum*, the pathogen responsible for leaf blight of linseed (*Linum usitatissimum* L.), revealed that *Trichoderma asperellum* and *Pseudomonas fluorescens* effectively inhibited the mycelial growth of the pathogen. These results suggest that both biocontrol agents have good antagonistic potential and could be used as eco-friendly components in the integrated management of linseed leaf blight.

Among the tested biocontrol agents, *Trichoderma asperellum* exhibited considerable antagonistic activity against *C. truncatum*. Reduction in radial growth of the pathogen in dual-culture assays may be attributed to several mechanisms operating either individually or simultaneously. *Trichoderma* is well known for its rapid growth and competitive ability, enabling it to efficiently colonize the available space and utilize nutrients that would otherwise be accessible to the pathogen. Such competition restricts the establishment and subsequent growth of *C. truncatum*. The findings agree with Aljabali et al. (2025) and Kumar and Tripathi (2018), who reported the strong antagonistic potential of *Trichoderma* spp. against phytopathogenic fungi, further supporting the efficacy of *T. asperellum* as a promising biocontrol agent. The superior performance of T₁ among the *Trichoderma* treatments may be attributed to isolate-specific differences in growth rate, sporulation capacity, or production of antifungal metabolites.

The antagonistic effect exhibited by *Pseudomonas fluorescens* against *C. truncatum* also highlights their potential as bacterial biocontrol agents. Fluorescent *Pseudomonas* species are efficient colonizers and are known to suppress several plant-pathogenic fungi through multiple mechanisms (Tamin et al., 2019). Their inhibitory effect may be associated with the production of antifungal metabolites, antibiotics, siderophores, hydrolytic enzymes and other secondary metabolites. Siderophore production is particularly important because it enables *Pseudomonas* to efficiently sequester iron from the surrounding environment, thereby reducing its availability to competing microorganisms, including fungal pathogens (Vanitha & Ramjegathesh, 2014). This nutrient competition can consequently restrict the growth and development of *C. truncatum*. The variation in inhibition among the *Pseudomonas* treatments (T₁, T₂, T₃) likely reflects differences in the production of these antagonistic compounds or differences in growth rate and colonization efficiency.

Differences in percentage inhibition among the tested *Trichoderma* and *Pseudomonas* isolates may be due to differences in their antagonistic ability, growth rate, competition and production of antifungal compounds and lytic enzymes (Kuzmanovska et al., 2018). The sensitivity of *C. truncatum* to different antagonists may also affect the level of inhibition. Therefore, selecting highly effective isolates is important for successful biological control.

The strong *in vitro* suppression of *C. truncatum* by the effective *Trichoderma* and *Pseudomonas* isolates is particularly important from the perspective of sustainable disease management. Biological control agents can potentially reduce dependence on fungicides and thereby minimize problems associated with chemical residues, environmental contamination and the development of fungicide-resistant pathogen populations. Moreover, *Trichoderma* and plant growth-promoting *Pseudomonas* strains may provide additional benefits through rhizosphere competence, nutrient mobilization, plant-growth promotion and induction of host defence responses (Lahlali et al., 2022; Ayaz et al., 2023).

Although the *in vitro* results showed good antagonistic activity, they should be considered only as an initial indication of biocontrol potential. The effectiveness of biocontrol agents under greenhouse and field conditions may vary due to factors such as temperature, soil conditions, moisture, microbial competition, inoculum level, formulation and method of application (Ahmad et al., 2026). The present study showed that *Trichoderma asperellum* and *Pseudomonas fluorescens* were effective against *Colletotrichum truncatum*. Therefore, the most effective isolates identified in the *in vitro* study should be further tested under pot and field conditions to confirm their ability to manage leaf blight of linseed. Future research should also investigate the compatibility of these biocontrol agents with other disease management practices and their effects on crop growth and yield.

V. CONCLUSION

This study confirms the *in vitro* efficacy of *Trichoderma asperellum* and *Pseudomonas fluorescens* against *Colletotrichum truncatum*, the causal agent of leaf blight in linseed. Among the tested biocontrol agents, *T. asperellum* (T₁) exhibited the highest antagonistic activity, restricting pathogen growth to 25.40 ± 0.52 mm with 70.80% inhibition, followed by *P. fluorescens* (T₂), which limited mycelial growth to 29.10 ± 0.56 mm with 66.65% inhibition. Both biocontrol agents significantly suppressed the mycelial growth of the pathogen compared to untreated controls.

The study provides baseline information for the biological management of linseed leaf blight caused by *C. truncatum*, a newly reported pathogen–disease association in India. The promising results obtained from *in vitro* screening warrant further evaluation of the most effective isolates under greenhouse and field conditions. Integration of these biocontrol agents into sustainable disease management strategies could reduce dependence on synthetic fungicides and contribute to environmentally friendly linseed production.

- **Limitations of the Study:** This study was conducted under controlled *in vitro* conditions, and the efficacy of the biocontrol agents may vary under field conditions due to environmental factors, microbial competition, and other variables. The mechanisms of antagonism were not specifically investigated, and pathogenicity testing was not performed in this study (though the pathogen identity was confirmed molecularly in earlier work). Future research should focus on evaluating the selected biocontrol agents under greenhouse and field conditions and investigating their mechanisms of action.
- **Future Research Directions:** Future studies should include (i) evaluation of the most effective isolates under pot and field conditions, (ii) investigation of the mechanisms of antagonism (e.g., production of volatile compounds, chitinase activity, siderophore production), (iii) compatibility testing with other disease management practices, (iv) formulation and application optimization, and (v) assessment of the impact of biocontrol agents on crop growth and yield.

ACKNOWLEDGEMENT

The author gratefully acknowledges the Backward Classes Welfare Department, Government of Karnataka, for awarding the OBC Ph.D. Fellowship and providing financial support for this research. The author also sincerely thanks the Department of Studies in Microbiology, University of Mysore, for providing the necessary facilities, support, and permission to carry out the research work.

CONFLICT OF INTEREST

The author declares no conflict of interest regarding the publication of this manuscript.

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