

Research Paper

Biocontrol potential of *Trichoderma asperellum* for management of collar rot incited by *Sclerotium rolfsii* Sacc. and growth enhancement in chickpea

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ABSTRACT

Collar rot caused by *Sclerotium rolfsii* Sacc. is a major constraint in chickpea (*Cicer arietinum* L.) production, especially in warm and humid regions. This study evaluated the biocontrol potential of fifteen *Trichoderma asperellum* isolates against *S. rolfsii* through *in vitro* dual culture assays and greenhouse pot trials. Among the isolates, IPR Tas-13 exhibited the highest mycelial inhibition (81.57%), followed by IIPR Tas-2 (77.43%) and IPR Tas-15 (75.94%). *In vivo* experiments with chickpea variety JG-62 confirmed that IPR Tas-13 significantly enhanced seed germination (96.44%) and plant height (45.34 cm), while reducing disease incidence (15.07%). These results were compared with commercial *T. asperellum* formulation Dalhanderma (14.11% disease incidence) and the fungicide Tebuconazole 50% (11.95%), which also showed effective disease suppression. The findings suggested that IPR Tas-13 is a promising biocontrol agent with strong antagonistic activity and growth-promoting effects, offering an eco-friendly alternative to chemical fungicides for managing collar rot in chickpea.

Key words: Biocontrol, Chickpea, Collar rot, Dalhanderma, *Trichoderma asperellum*, *Sclerotium rolfsii*

INTRODUCTION

Chickpea, also known as Bengal gram, garbanzo bean, Egyptian pea, channa, or chole, belongs to the Fabaceae family, and the genus *Cicer* within the tribe *Cicereae* (Bentham and Hooker 1972). Chickpea was first domesticated in present-day Turkey (part of Southwest Asia) and is now widely cultivated across Asia and Europe. It is considered one of the world's major pulse crops, with India accounting for nearly 75% of global production (Keote *et al.* 2019). In India, during 2023–24 Rabi season, Bengal gram (chickpea) was sown over approximately 101.92 lakh hectares, with major contributing states being Maharashtra (26.31 lakh ha), Madhya Pradesh (23.46 lakh ha), Rajasthan (19.38 lakh ha), Karnataka (9.62 lakh ha), Uttar Pradesh (6.82 lakh ha) and Gujarat (6.31 lakh ha) (Anonymous 2023–24).

Despite being a highly valuable crop, chickpea is susceptible to 172 pathogens globally, including 67 fungi, 22 viruses, 3 bacteria, 80 nematodes, and mycoplasma (Kamthe *et al.* 2023). Fusarium wilt (*Fusarium oxysporum* f. sp. *ciceri*), dry root rot (*Rhizoctonia bataticola*), and collar rot (*Sclerotium rolfsii*) are major diseases within this complex

that pose serious threats to chickpea production, potentially causing substantial yield losses (Nene *et al.* 1981). Soil-borne diseases, such as Fusarium wilt, dry root rot and collar rot are especially challenging and remain major constraints to chickpea cultivation (Kamthe *et al.* 2023).

Sclerotium rolfsii is a soil-borne saprophytic fungus that is responsible for causing various diseases such as collar-rot, sclerotium wilt, stem-rot, seedling blight, damping-off, foot-rot, stem blight and root rot in nearly 500 plant species, which includes several plants like chili, eggplant, tomato, soyabean, maize, groundnut, beans, watermelon, sunflower and chickpea (Vaniya *et al.* 2022, Chowdary *et al.* 2024). This pathogen requires a warm climate along with high moisture and high temperature (Singh *et al.* 2023). Seedling mortality caused by this pathogen ranges between 54.7% and 95%, while field-level yield losses have been reported between 22% and 50% (Ahsan *et al.* 2018).

Trichoderma spp. have long been recognized as effective biocontrol agents due to their strong antagonistic activity against a wide range of plant pathogens (Harman *et al.* 2004, Vinale *et al.*

2008). Their application to suppress the growth of *Sclerotium rolfsii*, a major soil-borne pathogen, represents a sustainable and eco-friendly alternative to chemical fungicides. *Trichoderma* spp. have been widely employed for decades in managing soil-borne plant diseases due to their multiple mechanisms of action, including competition, antibiosis, and mycoparasitism (Kucuk *et al.* 2003, Palmieri *et al.* 2022). In addition to their biocontrol potential, *Trichoderma* species are also well-known plant growth-promoting fungi (PGPF), which enhance nutrient uptake, stimulate plant defense responses, and improve crop productivity (Harman *et al.* 2004, Tyśkiewicz *et al.* 2022). Among these, *Trichoderma asperellum* has emerged as a particularly promising species, showing both strong biocontrol efficacy and growth-promoting abilities in various crop systems (Gajera *et al.* 2013, Mukherjee *et al.* 2013). These combined traits make *T. asperellum* a valuable component in integrated disease and crop management strategies.

The main objectives of the study were to isolate, identify, and purify the fungus *Sclerotium rolfsii* responsible for collar rot disease in chickpea, and to evaluate the antagonistic effect of different isolates of *Trichoderma asperellum* against *Sclerotium rolfsii* under laboratory conditions. Additionally, the study aimed to assess the effectiveness of these isolates in reducing disease incidence and improving plant growth parameters such as seed germination and plant height under greenhouse pot conditions.

MATERIALS AND METHODS

The present study, based on laboratory and

greenhouse experiments, was conducted in the Division of Crop Protection, ICAR-Indian Institute of Pulses Research, Kanpur (U.P.) during 2022-2023.

Collection of diseased collar rot samples

Infected chickpea plants were collected from the ICAR-IIPR Main Research Farm, Kanpur, at the seedling and adult stages. Diseased root pieces were surface sterilized with 1% sodium hypochlorite, rinsed with sterile water, and placed on Potato dextrose agar (PDA) in Petri dishes under aseptic conditions, following protocols by Butler (1918) and Narayanasamy (2012).

Purification of the pathogen

The pathogen culture was purified through multiple subcultures using the hyphal tip method. Inoculated Petri dishes were then placed in an incubator set at $25 \pm 2^\circ\text{C}$ for 3 to 5 days, with regular monitoring for growth. Identification of pathogen isolates relied on observing colony characteristics and sclerotial formation.

Collections of *Trichoderma asperellum*

Fifteen isolates of *Trichoderma asperellum* cultures were obtained from Crop Protection Division, ICAR-IIPR, Kanpur (Table 1).

In vitro efficacy of biocontrol agents: Dual culture technique

The antagonistic activity of 15 biocontrol agents against *Sclerotium rolfsii* was assessed using the dual culture technique in a completely randomized

Table 1. Fifteen isolates of *Trichoderma asperellum* from different locations used for the study

Isolate Name	Sampling location	State	Latitude	Longitude	Species	Crop	GENBANK ACC. No. (ITS)	GENBANK ACC. No. (Tef)
IIPR-79	Basti	Uttar Pradesh	26.8140° N	82.7630° E	<i>T. asperellum</i>	CHICKPEA	OP783867	OP886827
IIPRTh-31	ANGRAU	Telangana	24.8773° N	79.0193° E	<i>T. asperellum</i>	CHICKPEA	MK968811	MN232100
IIPR-9	Kanker	Chhattisgarh	20.1990° N	81.0755° E	<i>T. asperellum</i>	CHICKPEA	OP783870	OP886825
IIPR-78	Kanker	Chhattisgarh	20.1990° N	81.0755° E	<i>T. asperellum</i>	CHICKPEA	OP783868	
IIPR-74	Kanker	Chhattisgarh	20.1990° N	81.0755° E	<i>T. asperellum</i>	CHICKPEA	MK849905	MN226937
IIPRTas-2	Dharwad	Karnataka	15.4589° N	75.0078° E	<i>T. asperellum</i>	PIGEONPEA	KX681711	MT239396
IIPRTas-4	ANGRAU	Telangana	16.3665° N	80.4369° E	<i>T. asperellum</i>	CHICKPEA	KX681713	MH822533
IIPRTas-5	Kanker	Chhattisgarh	20.1990° N	81.0755° E	<i>T. asperellum</i>	CHICKPEA	KX681715	MH822533
IIPRTas-6	Meerut	Uttar Pradesh	28.9845° N	77.7064° E	<i>T. asperellum</i>	CHICKPEA	KX681717	MT239393
IIPRTas-7	Ayodhya	Uttar Pradesh	26.7922° N	82.1998° E	<i>T. asperellum</i>	CHICKPEA	KX681718	MH822532
IIPRUTK-100	Andhra Pradesh	Andhra Pradesh	15.9129° N	79.7400° E	<i>T. asperellum</i>	CHICKPEA	OP783881	OP886837
IIPRCPT-91	Hamirpur	Uttar Pradesh	31.6862° N	76.5213° E	<i>T. asperellum</i>	CHICKPEA	MK841021	MN226919
IPRTas-13	Ayodhya	Uttar Pradesh	26.7922° N	82.1998° E	<i>T. asperellum</i>	PIGEONPEA	KX681733	MT239392
IPRTas-12	Nagpur	Maharashtra	21.1458° N	79.0882° E	<i>T. asperellum</i>	PIGEONPEA	MH511666	MT239394
IPRTas-15	ANGRAU	Telangana	16.3665° N	80.4369° E	<i>T. asperellum</i>	PIGEONPEA	MH511671	MH822529

design with three replications (Fokkema 1978). PDA supplemented with streptomycin was poured into 9 cm Petri plates under aseptic conditions. Discs (5 mm) of 5–7 days old cultures of the pathogen and biocontrol agents were placed opposite to each other, and plates were incubated at 25 °C for 7 days. Controls had the pathogen alone. Mycelial inhibition was calculated using Vincent's (1947) formula:

$$\text{Inhibition \%} = [C - T / C] \times 100$$

Where, C = Diameter of fungus colony (mm) in control plate

T = Diameter of fungus colony (mm) in treated plate

Assessment of agronomic traits and disease incidence under *in vivo* conditions

A total of 19 treatments, including 15 *Trichoderma asperellum* isolates, a chemical fungicide (Tebuconazole 50%), a commercial formulation of *Trichoderma* (Dalhanderma), and two controls (untreated and pathogen-inoculated), were evaluated under greenhouse conditions to assess their effects on seed germination, plant height, and collar rot incidence in chickpea. The experiment was conducted in grow bags filled with sterilized soil, vermicompost, and coco peat (2:1:1) (Swarup *et al.* 2019). Spore suspensions (1×10^7 /ml) of *T. asperellum* and the pathogen (*Sclerotium rolfsii*) were prepared (Khaleedi & Kaheri, 2016). Surface-sterilized JG 62 seeds were soaked in *Trichoderma* and pathogen suspensions for 4 hours, air-dried, and sown (Monalisa *et al.* 2017). Each grow bag received 5 seeds, with regular irrigation and thinning after 2 weeks. Observations were recorded at 45 DAS (Table 3, Figure 4).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants} \times 100}{\text{Total number of plants}}$$

RESULTS AND DISCUSSION

Symptomatology

The pathogen primarily caused collar rot in seedlings, marked by yellowing of leaves that often remained attached (Figure 1). Young seedlings typically collapsed, while older plants showed drying symptoms without collapsing. Infected tissue decayed from the collar region, often covered with white cottony mycelium and brown sclerotia clustered at the stem base. Affected seedlings were easily uprooted due to weakened tissues.

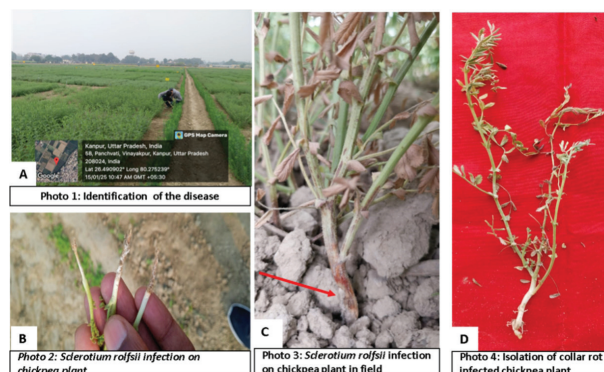


Fig. 1. Collection of plant disease samples from the field (A). Symptoms of collar rot disease caused by *Sclerotium rolfsii* in chickpea (B, C, D)

Morphology

On potato dextrose agar, the isolated *Sclerotium rolfsii* exhibited robust growth characteristics. It developed dense and fluffy mycelium that appeared snowy white with a silky texture (Figure 2). Approximately nine days after inoculation, the fungus started forming brown, firm sclerotia. These sclerotia initially appeared white but matured into light or dark brown, varying in diameter from 0.5 to 1.5 mm. The colony efficiently covered a 90 mm Petriplate within three to five days. Based on distinctive cultural characteristics and sclerotial formation patterns, the pathogen was confirmed to be *Sclerotium rolfsii* (Barnett and Hunter 1972). The aforesaid isolation and identification of *Sclerotium rolfsii* attempted in the present study are in accordance with the earlier reports of several researchers (Grover and Chona 1960, Gunaseeli and Muthukumar 2025, Kumar *et al.* 2025).

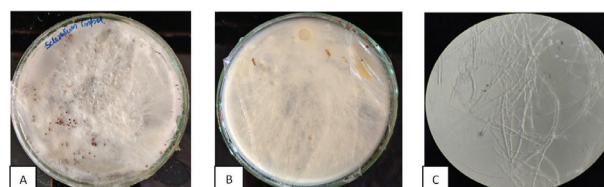


Fig. 2. Culture plate of *Sclerotium rolfsii* and microscopic image. Front side (A), backside (B) and microscopic image (C)

Evaluation of antagonistic activity of *Trichoderma* isolates against *Sclerotium rolfsii* through dual culture assay

A dual culture assay was conducted to assess the antagonistic potential of fifteen *Trichoderma asperellum* isolates against *Sclerotium rolfsii* (Table 3, Figure 4). Among all isolates, IPR Tas-13

showed the highest inhibition percentage (81.57%), significantly reducing the pathogen's growth (16.33 mm) compared to the control (88.66 mm). This was followed by IIPR Tas-2 (77.43%), IIPR Tas-15 (75.94%), IIPR-9 (75.93%), and IPR Tas-12 (75.18%), all of which demonstrated strong antagonistic activity. Other effective isolates included IIPR Tas-7 (72.54%), IIPR Tas-4 (72.17%), IIPR-78 (70.30%), IIPR-79 (70.67%), and IIPR UTK-100 (70.29%), showing over 70% inhibition. Moderate inhibition was observed in IIPR Tas-6 (68.79%), IIPRCPT-91 (67.67%), IIPR Th-31 (62.78%), and IIPR-74 (62.78%). The least effective isolate was IIPR Tas-5, recording the lowest inhibition (59.39%).

Statistical analysis revealed significant differences ($P < 0.01$) among the treatments. The results clearly indicate that several *Trichoderma asperellum* isolates, particularly IPR Tas-13, exhibited strong antagonism against *Sclerotium rolfii*, and can be further evaluated for their biocontrol potential under greenhouse and field conditions. These results align with prior findings demonstrating the antagonistic activity of *Trichoderma* spp. through competition, hyperparasitism, and production of antifungal compounds like gliotoxin, viridin, and peptaibols (Harman *et al.* 2004, Vinale *et al.* 2008, Mukherjee *et al.* 2012). Moreover, the variability among isolates highlights the importance of local

screening to identify the most effective strains for biocontrol application.

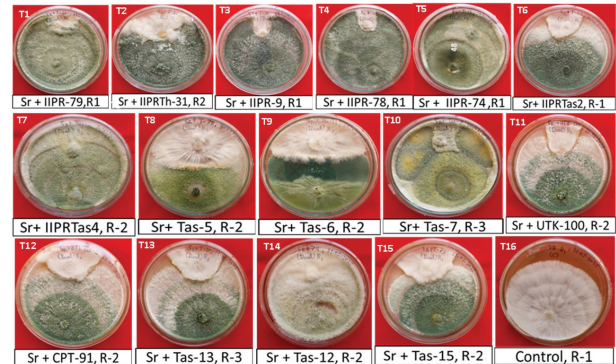


Fig. 3. Dual culture assay for *Trichoderma asperellum* isolates against *Sclerotium rolfii*

Effect of Trichoderma asperellum isolates on seed germination, plant height, and collar rot disease incidence in chickpea under pot trial

The data revealed that the maximum seed germination was recorded in IPR Tas-13 (96.44%), followed closely by IPR Tas-15 (94.96%) and IIPR Tas-4 (94.95%). Among the isolates, IPR Tas-12 showed the lowest germination (87.10%), while the pathogen-inoculated control (Sr) recorded only 41.00%, indicating a significant inhibitory effect of *S. rolfii*. In terms of plant height, IPR

Table 2. Radial growth and inhibition percentage of *Sclerotium rolfii* against isolates of *Trichoderma asperellum*

Treatments	Isolate code	Radial growth of pathogen (mm)			% inhibition (7 Days)
		3DAI	5DAI	7DAI	
T1	IIPR-79	13.00±0	19.00±0.81	26.00±0	70.67(57.21)
T2	IIPR Th-31	22.00±1	26.33±0.47	33.00±0.81	62.78(52.41)
T3	IIPR-9	15.00±1	18.33±1.24	21.33±0.47	75.93(60.63)
T4	IIPR-78	11.67±0.57	21.00±0.81	26.33±0.47	70.30(56.98)
T5	IIPR-74	18.00±1	27.33±0.94	33.00±0.81	62.78(52.41)
T6	IIPR Tas-2	12.33±0.57	16.33±0.47	20.00±0.81	77.43(61.65)
T7	IIPR Tas-4	13.33±0.57	20.66±0.47	24.66±0.94	72.17(58.17)
T8	IIPR Tas-5	20.33±0.57	28.33±0.47	36.00±0.81	59.39(50.42)
T9	IIPR Tas-6	15.66±0.57	20.66±0.47	27.66±0.47	68.79(56.04)
T10	IIPR Tas-7	11.00±0	21.00±0.81	24.33±1.24	72.54(58.41)
T11	IIPR UTK-100	12.33±0.57	19.00±0	26.33±0.94	70.29(56.98)
T12	IIPRCPT-91	12.00±1	22.33±0.94	28.66±0.47	67.67(55.35)
T13	IPR Tas-13	7.00±0	10.33±0.47	16.33±0.47	81.57(64.59)
T14	IPR Tas-12	12.33±0.57	16.00±0.81	22.00±0.81	75.18(60.13)
T15	IPR Tas-15	11.33±0.57	18.66±0.47	21.33±0.47	75.94(60.63)
T16	Control	48.33±0.57	73.00±0	88.66±0.47	
C.D.		1.105	1.456	1.467	1.128
SE(m)		0.382	0.502	0.507	0.389
SE(d)		0.54	0.71	0.717	0.55
C.V.		4.139	4.27	2.953	1.171

Data in parenthesis are Arcsine transformed values

Tas-13 performed better (45.34 cm), followed by Dalhanderma (44.57 cm) and Tebuconazole 50% (43.54 cm), suggesting that these treatments effectively enhanced vegetative growth. Conversely, the lowest plant height was observed in IIPR Tas-7 (32.16 cm) and Control (Sr) (24.33 cm). Regarding disease incidence, Tebuconazole 50% recorded the least infection (11.95%), followed by Dalhanderma (14.11%) and IPR Tas-13 (15.07%). These treatments showed strong suppression of collar rot. On the other hand, the highest disease incidence was found in IIPR Tas-5 (38.54%), followed by IIPR-74 (35.03%) and IIPR Th-31 (31.91%), indicating weaker antagonistic performance. The pathogen-inoculated control recorded 50.27%, confirming the virulence of *Sclerotium rolfsii* and the effectiveness of certain treatments in reducing disease pressure.

The present study revealed that *Trichoderma asperellum* IPR Tas-13 significantly enhanced the seed germination (96.44%), plant height (45.34 cm), and reduced the collar rot incidence (15.07%) in chickpea. These results are in consonance with earlier findings by Javaid and Ali (2016), who reported that *Trichoderma* spp. not only suppressed *Sclerotium rolfsii* but also improved plant growth parameters in legumes. Similarly, Adnani *et al.* (2024)

observed a notable reduction in disease incidence and improved germination when chickpea seeds were treated with *T. asperellum* under greenhouse conditions. These outcomes affirm that *T. asperellum* functions not only as a strong antagonist to soil-borne pathogens but also as a plant growth-promoting fungus (PGPF), supporting its dual role in sustainable crop protection (Harman *et al.* 2004, Mukherjee *et al.* 2013).

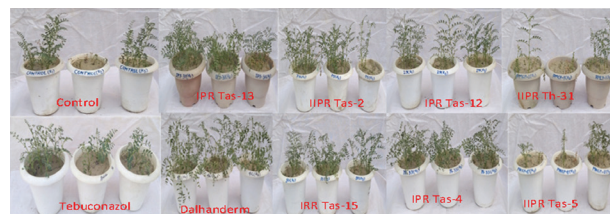


Fig. 4. Efficacy of *Trichoderma asperellum* against *Sclerotium rolfsii*

CONCLUSION

Among all treatments, *Trichoderma asperellum* IPR Tas-13 proved to be the most effective in suppressing collar rot incidence and enhancing plant growth in chickpea. Further field trials are recommended to validate its efficacy under diverse agro-ecological conditions. Future research

Table 3. Effect of different treatments on seed germination, plant height and collar rot disease incidence in chickpea

Sl. No.	Treatments	Germination (%)	Plant height (cm)	Disease incidence (%)
1	IIPR-79	93.09±0.77	38.94±0.49	26.87±0.86
2	IIPRTh-31	92.13±0.55	34.19±0.82	31.91±0.33
3	IIPR-9	92.16±0.67	43.17±0.75	24.70±0.35
4	IIPR-78	90.23±1.63	40.55±0.32	30.14±0.44
5	IIPR-74	94.50±1.66	33.11±0.95	35.03±0.72
6	IIPRTas-2	92.45±1.71	41.05±0.76	20.01±0.69
7	IIPRTas-4	94.95±0.65	35.37±0.70	22.48±0.45
8	IIPRTas-5	91.85±0.71	33.90±1.08	38.54±0.65
9	IIPRTas-6	89.77±0.55	38.44±0.62	24.74±0.28
10	IIPRTas-7	90.21±0.49	32.16±1.71	26.96±0.80
11	IIPRUTK-100	94.39±0.95	34.24±1.10	25.91±0.72
12	IIPRCPT-91	90.43±2.19	38.05±1.48	32.65±0.32
13	IPRTas-13	96.44±0.59	45.34±0.90	15.07±0.31
14	IPRTas-12	87.10±0.74	39.96±1.90	22.46±0.29
15	IPRTas-15	94.96±2.27	33.37±0.67	22.23±0.21
16	Tebuconazole 50%	94.96±0.45	43.54±0.90	11.95±0.36
17	Dalhanderma	93.15±0.58	44.57±0.66	14.11±0.65
18	Control (Sr) *	41.00±0.82	24.33	50.27
19	Control	90.33±0.47	39.18±0.72	0.00
	C.D.	2.299	1.824	1.08
	SE(m)	0.8	0.635	0.376
	SE(d)	1.131	0.898	0.531
	C.V.	1.545	2.928	2.598

*Sr: *Sclerotium rolfsii*

should focus on developing a stable commercial formulation, evaluating its compatibility with commonly used fungicides and biofertilizers, and integrating it into existing Integrated Disease Management (IDM) strategies. These efforts may help in the establishment of IPR Tas-13 as a sustainable and eco-friendly biocontrol agent for chickpea cultivation.

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