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Harnessing the potential of *Trichoderma* spp. from rhizosphere of avenue trees for managing Collar Rot in Chickpea

HET U. PATEL, DARSHAN M. PARMAR, PUJA PANDEY* AND R. G. PARMAR
Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University
Anand 388110, Gujarat

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Chickpea (*Cicer arietinum* L.) is a crucial legume crop cultivated globally for its nutritional value and its role in sustainable agriculture. However, its production is severely impacted by collar rot caused by *Sclerotium rolfsii*, a destructive soil-borne fungal pathogen with a broad host range. Conventional chemical control methods pose environmental concerns, necessitating eco-friendly and sustainable alternatives. This study aimed to evaluate the antagonistic potential of *Trichoderma* spp. against *S. rolfsii* for integrated management of collar rot in chickpea. *Trichoderma* spp. were isolated from rhizosphere soils of various avenue trees, and their antagonistic activity was assessed using the dual culture method. Among the isolates, TrichoAAU2 from the rhizosphere of Asopalav exhibited the highest mycelial growth inhibition (83.06%), followed by the isolate TrichoAAU4 from the rhizosphere of Peepal (67.78%). Additionally, TrichoAAU2 and TrichoAAU4 showed maximum inhibition of sclerotia production, producing 5 and 21 sclerotia, respectively. Molecular characterization of the potent isolate, TrichoAAU2 was conducted through ITS rDNA sequencing, and it was identified as *T. asperellum.* The findings demonstrate that *T. asperellum* isolated from a rhizosphere region of Asopalav is an effective biocontrol agent against *S. rolfsii* causing collar rot in chickpea. These results highlight their potential for incorporation into sustainable and integrated disease management strategies for chickpea cultivation.

Keywords : *Cicer arietinum* L., Collar Rot, *Sclerotium rolfsii, Trichoderma* spp.,

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a vital legume crop, widely cultivated in arid and semi-arid regions of the world due to its significant nutritional value and its role in sustainable agriculture through nitrogen fixation (Khaitov and Abdiev, 2018). However, chickpea production is severely hindered by several diseases, among which collar rot, caused by *Sclerotium rolfsii* Sacc., is one of the most destructive (Singh *et al*. 2022). *S. rolfsii* Sacc. is a soil-borne fungal pathogen that causes southern blight, stem rot and sclerotial root rot in a wide range of crops, including groundnut, tomato, papaya, soybean and brinjal.

It is particularly destructive in tropical and subtropical regions with warm and humid climates, where it can cause severe yield losses. Taxonomically, it belongs to the Kingdom Fungi, Phylum Basidiomycota, Class Agaricomycetes, Order Agaricales and Family Sclerotiniaceae. Although it is classified under the genus *Sclerotium*, and its teleomorph is *Athelia rolfsii* (Kumari and Gatak, 2018). The pathogen is characterized by rapid growth of white, cottony mycelium and the formation of numerous brown, round sclerotia (survival structures) that allow it to persist in soil for long periods. Infection typically starts at the collar region of the plant, leading to stem rot, yellowing, wilting, and eventual death. Favourable conditions for disease development include high soil moisture, temperatures between 25–35°C, and the presence of organic matter. Due to its broad host range, saprophytic ability, and persistence in the soil, managing *S. rolfsii* is difficult. Effective control relies on integrated strategies such as crop rotation, soil solarization, biological control using *Trichoderma* spp., and the use of resistant cultivars (Katan, 2017).

*Correspondence: pujapandey41124@gmail.com

Traditional management strategies for collar rot, including chemical control, have raised concerns due to environmental impacts, the development of resistant strains, and food and soil residue (Nguyen *et al*., 2017). Consequently, there is a growing interest in exploring eco-friendly and sustainable alternatives for disease management. Biological control, particularly using antagonistic microorganisms, has emerged as a promising strategy (Heydari and Pessarakli, 2010).

Among the various biocontrol agents, *Trichoderma* spp. has gained considerable attention due to their robust antagonistic properties against a wide array of plant pathogens, including *S. rolfsii* (Ghazanfar *et al*. 2018). *Trichoderma* spp. are well-known for their ability to suppress pathogens through multiple mechanisms such as mycoparasitism, production of antifungal metabolites, competition for nutrients and space, and induction of systemic resistance in host plants (Monfil and Casas-Flores, 2014).

This research paper aims to evaluate the bio-efficacy of *Trichoderma* spp. against *S. rolfsii* in controlling the collar rot of chickpea. The study focuses on understanding the interaction between *Trichoderma* spp. and *S. rolfsii* and assessing the potential of *Trichoderma* spp. as a sustainable and effective biocontrol agent in integrated disease management programs for chickpea cultivation.

MATERIALS AND METHODS

Isolation of Trichoderma spp. and Sclerotium rolfsii

Rhizosphere soil samples from avenue trees *viz*., Banyan (*Ficus bengalensis*), Asopalav (*Polyalthia longifolia*), Gulmohar (*Delonix regia*), Peepal (*Ficus religiosa*), Neem (*Azadiracta indica*), Casuarina (*Casuarina equisetifolia*), Palash (*Butea monosperma*) and Ashoka (*Saraca asoca*) were collected and used to isolate *Trichoderma* spp. by soil dilution plating using *Trichoderma* selective medium (TSM) (Elad and Chet, 1983). The isolates from Banyan, Asopalav, Gulmohar, Peepal, Neem, Casuarina, Palash and Ashoka were named as TrichoAAU1, TrichoAAU2, TrichoAAU3, TrichoAAU4, TrichoAAU5, TrichoAAU6, TrichoAAU7 and TrichoAAU8, respectively. These *Trichoderma* isolates were purified using the single hyphal tip method and used in further investigations. The *S. rolfsii* was isolated from the infected chickpea plant on a PDA medium.

Antagonistic activity of Trichoderma spp. against S. rolfsii

A dual culture approach (Dennis and Webster, 1971) was employed to determine the antagonistic potential of *Trichoderma* spp. against *S. rolfsii*. The antagonists and pathogen were cultured on PDA medium. Sterilized PDA medium (20 mL) was aseptically put into a sterilized Petri plate with a diameter of 90 mm. A 5 mm mycelial disc from a seven-day-old, actively growing *Trichoderma* spp. culture was inoculated to one side of the Petri plate. Similarly, the other side of the Petri plate was inoculated with *S. rolfsii* culture that had been grown for seven days. To facilitate comparability, the test pathogen was cultured alone in a Petri plate. All infected plates were incubated in a BOD incubator at 27±2 ºC. Each treatment was performed three times, and the percentage of mycelial growth of the pathogen was measured after seven days of incubation. The per cent growth inhibition over control was computed using Vincent's (1947) formula.

$$PGI = \frac{DC-DT}{DC} \times 100$$

Where, PGI = Per cent growth inhibition

DC = Mean diameter of mycelial colony in control treatment (mm)

DT = Mean diameter of mycelial colony in treated set (mm)

Effect of Trichoderma spp*. on sclerotia formation by S. rolfsii*

Following the evaluation of the previous experiment, the plates were incubated under the same conditions until the 14th day, at which point mature sclerotia were observed in the treatments. The sclerotia from each treatment were carefully collected using forceps and quantified manually. The experiment was conducted using a completely randomized design, with four replicates per treatment.

Molecular identification of potent Trichoderma isolate

Isolate Tricho AAU has shown highest growth inhibition of *S. rolfsii* in dual culture technique so it was considered as a potent isolate.

DNA extraction

DNA was isolated from TrichoAAU2 using the CTAB (Cetyl Trimethyl Ammonium Bromide) extraction method. One gram of mycelium was pulverized in liquid nitrogen and transferred into 7.5 mL of pre-warmed (65 °C) DNA extraction buffer consisting of 1 M Tris-HCl (pH 8.0), 5 M NaCl, 0.5 M EDTA (pH 8.0), and 2% CTAB. The mixture was thoroughly homogenized and incubated at 65 °C with gentle agitation for 45 minutes. Following incubation, an equal volume of chloroform:isoamyl alcohol (24:1 %v/v) was added, mixed gently to facilitate protein denaturation, and centrifuged at 12,000 rpm for 15 minutes at 4 °C. DNA was precipitated by adding 0.6 volumes of chilled ethanol and 0.1 volume of 3 M sodium acetate (pH 5.2), followed by centrifugation at 15,000 rpm for 15 minutes. The resulting pellet was washed twice with chilled 70% ethanol, air-dried at room temperature, and resuspended in 100 µL of sterile TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). To remove RNA contamination, 2 µL of DNase-free RNase was added to the resuspended DNA and incubated at 37 °C for 45 minutes, followed by enzyme inactivation at 65 °C for 10 minutes. The purity and concentration of the extracted DNA were assessed using a NanoDrop spectrophotometer and agarose gel electrophoresis. The DNA sample was stored at –20 °C for long-term use.

Amplification and sequencing of rDNA ITS region

PCR amplification of the pathogen was performed using the ITS1 and ITS4 primer pair. Each 50 µL PCR reaction contained 50 ng of genomic DNA, 1X PCR buffer (Thermo Scientific), 1.5 mM MgCl, , 0.2 mM dNTPs, 0.25 µM of each primer, and 1 unit of DreamTaq DNA Polymerase (Thermo Scientific). The thermal cycling conditions were as follows: an initial denaturation at 95 °C for 2 minutes, followed by 40 cycles of denaturation at 94 °C for 2 minutes, annealing at 55 °C for 1 minutes and extension at 72 °C for 2 minutes. This was followed by a final extension at 72 °C for 10 minutes, after which the reaction was held at 4 °C. The amplified PCR product was sequenced using an automated ABI 3730 Genetic Analyzer at SLS Research Pvt. Ltd., Surat, Gujarat, India. The sequence was analyzed using the BLAST (Basic Local Alignment Search Tool) algorithm against the GenBank database maintained by the National Center for Biotechnology Information (NCBI), USA, to assess the similarity of the newly sequenced samples with existing entries in the database

RESULTS AND DISCUSSION

Antagonistic activity of Trichoderma spp. *against S. rolfsii*

The highest per cent mycelial growth inhibition (83.06%) with the lowest mycelial growth (15.25 mm) of *S. rolfsiii* was recorded with TrichoAAU2 after 7 days of incubation followed by TrichoAAU4 with 67.78% mycelial growth inhibition and 29 mm mycelial growth. However, the lowest per cent mycelial growth inhibition (49.72%) with the maximum mycelial growth (45.25 mm) of *S. rolfsiii* was recorded with TrichoAAU5 (Fig. 1). Present findings corroborate with the results reported by Prajapati *et al.* (2015). They found the highest per cent mycelial growth inhibition (73.33%) of *S. rolfsii* by *T. asperellum*.

Effect of Trichoderma spp. *on sclerotia Formation by S. rolfsii*

After assessing mycelial growth in dual culture, the plates were incubated for an additional 14 days. Following this incubation period, the formation of mature brown sclerotia was observed in all treatments. Notably, all strains exhibited a reduction in sclerotia production compared to the control treatment (Table 1). The control treatment (Pathogen only) produced an average of 192 sclerotia, while the most effective outcomes were observed with TrichoAAU2 and TrichoAAU4, which produced averages of 5 and 21 mature sclerotia, respectively (Fig. 2). These results align with those reported by Silva *et al*.

Table. 1: Evaluation of *Trichoderma* spp. against *S. rolfsii* through dual culture technique

Tr. No.	Treatments	Mycelial growth (mm)	Growth inhibition (%)	No. of Sclerotia
T_1	TrichoAAU1	34.00	62.22	49.00
T_2	TrichoAAU2	15.25	83.06	5.00
T_3	TrichoAAU3	38.25	57.50	80.00
T_4	TrichoAAU4	29.00	67.78	21.00
T_5	TrichoAAU5	45.25	49.72	101.00
T_6	TrichoAAU6	38.00	57.78	74.00
T_7	TrichoAAU7	35.00	60.00	63.00
T_8	TrichoAAU8	32.67	64.17	35.00
T_9	Control (Pathogen only)	90.00	-	192.00
	S. Em. ±	0.52	-	0.42
	C. D. at 5%	1.51	-	1.24
	C.V. %	5.23	-	4.87

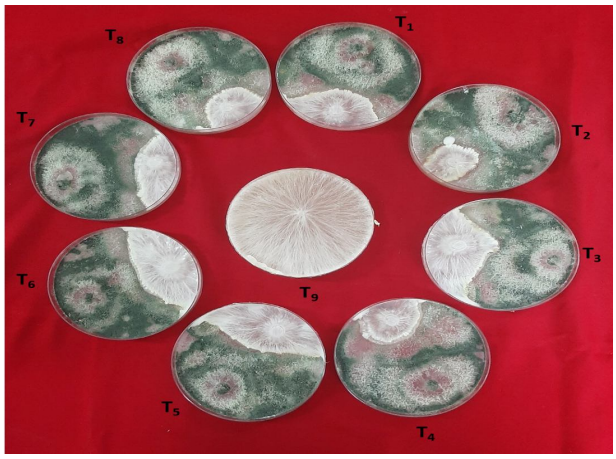

Fig.1: *In vitro* evaluation of antagonistic activity of *Trichoderma* spp. against *S. rolfsii*

Fig.2: Effect of *Trichoderma* spp. on sclerotia formation by *S. rolfsii*

(2022), who recorded 211 sclerotia in the control treatment (Pathogen only). In their study, the most favourable outcomes were achieved using *T. azevedoi* CEN1242 and *T. afroharzianum* CEN287, with mean values of 13 and 16 mature sclerotia, respectively.

Molecular identification of potent Trichoderma isolate

The DNA concentration was quantified using a NanoDrop spectrophotometer with the sample yielding 2190.82 ng/µL. Samples exhibiting high DNA concentrations and minimal RNA or protein contamination were selected for subsequent analyses. The PCR products were purified and sequenced by SLS Research Pvt. Ltd., Gujarat, India. The resulting sequences were aligned and analyzed using the NCBI BLAST tool to assess similarity with sequences available in the GenBank database. The sequences were matched at various global similarity levels, confirming their identity. The ITS rDNA sequence of the sample was identified as *Trichoderma asperellum* and submitted to the NCBI GenBank under accession number PQ675704. This sequence encompasses the internal transcribed spacer 1 (ITS1, partial sequence), the 5.8S ribosomal RNA gene (complete sequence), the internal transcribed spacer 2 (ITS2, complete sequence), and a partial sequence of the 28S ribosomal RNA gene.

CONCLUSION

The study demonstrates the efficacy of *Trichoderma* spp. isolated from a rhizosphere soil of avenue trees as a potent biocontrol agent against *S. rolfsii*, the causative agent of collar rot in chickpea. Among the tested isolates, TrichoAAU2 isolated from the rhizosphere soil of

Asopalav exhibited the highest antagonistic activity, achieving 83.06% mycelial growth inhibition of *S. rolfsii* and significant suppression of sclerotia production. Molecular identification confirmed the isolate TrichoAAU2 as *T. asperellum*, and the ITS rDNA sequence was deposited in the NCBI GenBank (Accession No. PQ675704). The findings underscore the potential of *T. asperellum* as a sustainable and eco-friendly alternative to chemical control methods, aligning with integrated disease management strategies for chickpea cultivation.

DECLARATION

Conflict of Interrest. Authors declare no conflict of interest

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