

Isolation, Characterization and Biopesticide Formulation of a Native *Trichoderma* Isolate from Agricultural Soil of Janjgir-Champa District Area, Chhattisgarh.

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ABSTRACT

Trichoderma species are important fungal biocontrol agents because of their ability to suppress a broad range of soil and plant-associated pathogens. The present investigation focused on the isolation, characterization, antagonistic evaluation and formulation of native *Trichoderma* obtained from agricultural soil different areas of Janjgir–Champa district of Chhattisgarh, India using the serial dilution agar plate technique on *Trichoderma* Selective Medium (TSM). Three isolates were first characterized for antagonism against *Fusarium sp* and selected the potential one for further characterization and evaluated for its antagonistic activity against three plant pathogens- *Fusarium sp*, *Pestalotiopsis sp* and *Colletotrichum sp* using the dual culture assay. The most promising isolate, designated SM/AS/SG001 was subsequently characterized using morphological and molecular approaches and after evaluation the isolate showed strong inhibition against all the three plant pathogens, with mean radial-growth inhibition of 75.67%, 80.33% and 82.33%, respectively. Microscopic observations indicated close hyphal association and coiling with the test pathogens, supporting a role for mycoparasitic interaction. The isolate grew rapidly, with an average radial growth rate of approximately 6.93 mm day⁻¹, and produced abundant green conidia. Molecular analysis based on the ITS region placed the isolate (NCBI accession no- PZ943074) within the *Trichoderma* clade and showed close affinity with *T. hamatum* and *Trichoderma sp.* HBE11. For mass multiplication, a low-cost potato-based liquid medium supplemented with sugar and lemon juice was used. The resulting culture was incorporated into a liquid carrier containing carboxy-methyl cellulose and glycerol. Viable propagule counts decreased from 12 × 10⁶ to 7 × 10⁶ CFU during five months of storage, corresponding to retention of approximately 58.33% of the initial population. Overall, the findings indicate that SM/AS/SG001 is a promising native isolate with strong in-vitro antagonistic activity and potential for development as an economical microbial biocontrol formulation.

Keywords

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1. INTRODUCTION

Finding environmentally appropriate substitutes for industrial fertilizers and pesticides, has become more urgent due to the growing demand for sustainable agricultural output. One of the main obstacles to global agricultural productivity is soil-borne plant diseases, which lower crop yield and quality and result in significant financial losses. Chemical fungicides have long been used to control disease, but their careless application has resulted in the emergence of resistant pathogen populations, pollution of the environment, disturbance of beneficial soil microflora, and worries about the health of ecosystems and food safety (Howell, 2003; Harman, 2006). As a result, beneficial microorganism-based biological control has become a viable and successful approach to integrated disease management (IDM) (Harman *et al.*, 2004). Because of their quick growth, adaptability to a variety of soil conditions, and capacity to inhibit a

wide range of phytopathogenic fungi, species in the genus *Trichoderma* are regarded as some of the most effective microbial biocontrol agents (Betez *et al.*, 2004; Harman *et al.*, 2004). Naturally occurring filamentous fungi called *Trichoderma sp.* invade the rhizosphere and plant roots, where they use a variety of ways to exhibit antagonistic activity. Mycoparasitism, competition for nutrients and ecological niches, production of antifungal secondary metabolites, secretion of cell wall-degrading enzymes like proteases, β -1,3-glucanases, and chitinases, and induction of systemic resistance in plants are some of these (Howell, 2003; Harman, 2006; Lorito *et al.*, 2010). By increasing nutrient availability, promoting root development, improving nutrient uptake, and boosting tolerance to abiotic stresses including drought and salinity, *Trichoderma sp.* not only suppress disease but also stimulate plant growth (Shoresh *et al.*, 2010; Woo *et al.*, 2014). Because of *Trichoderma's* diversified

uses, it has become a very important part of integrated disease management in sustainable agriculture.

Environmental factors significantly impact on *Trichoderma*'s effectiveness as a biopesticide; because of it is extremely strain-dependent. The native strains can act more effectively in the soil and successfully compete with the native micro flora. It is because, may be they are more ecologically better adapted and fit to that native agricultural environment than commercial product (Kubicek *et al.*, 2019). In order to find superior strains, it is very difficult to identify and evaluate native *Trichoderma* isolates from agricultural soils.

It requires accurate identification of *Trichoderma* species with precession techniques. The traditional or customary identification process based on colony morphology and microscopic features often lacks enough taxonomic precision because of its significant interspecific similarities. So that, for accurate species identification and phylogenetic classification, different molecular techniques like, amplification and sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA the most prominent (Samuels & Hebbbar, 2015; Druzhinina *et al.*, 2018). Therefore the combination of biochemical, morphological, and molecular characterisation results the most potential reliable identification methodology.

In addition to its antagonistic potential, *Trichoderma*'s practical use in agriculture depends on the creation of stable, commercially feasible formulations that can sustain high propagule viability while being stored. According to John *et al.* (2010), the optimum formulation should have a long shelf life, high conidial viability, ease of application, cost-effectiveness, and environmental safety. Because they preserve better spore viability, allow for higher propagule concentrations, enable large-scale production, and work well with seed treatment, soil application, and foliar spraying, liquid formulations have grown in significance (Schuster & Schmoll, 2010; Woo *et al.*, 2014). Additionally, using low-cost, locally accessible substrates for bulk multiplication drastically lowers production costs without sacrificing formulation quality.

Rich microbial diversity is supported by Chhattisgarh's vast agricultural fields and varied agro-climatic conditions. However, little is known about the native *Trichoderma* populations connected to the Janjgir-Champa district, Chattishgarh region's agricultural soils. Localized biocontrol agents for long-term crop protection may be produced by characterizing these native isolates and creating economical formulations. One of the most abundant sources of helpful microbes is found in agricultural soils. Because of their distinct physicochemical characteristics and vibrant microbial communities, paddy-cultivated soils provide as a significant ecological niche for a variety of *Trichoderma*

species. The identification of highly competitive strains with greater biocontrol efficacy and adaptation to local environmental circumstances may be made easier by investigating native populations from these soils.

In order to isolate local *Trichoderma* species from the paddy-cultivated soils of the Janjgir-Champa district area of Chhattisgarh and to describe the isolates using biochemical and molecular techniques, the current study was conducted. The most potent isolate was chosen for the creation of an inexpensive liquid formulation after the isolates' antagonistic potential against *Fusarium sp.*, *Pestalotiopsis sp.* and *Colletotrichum sp.* were assessed in vitro. The goal of the project is to find a native *Trichoderma* formulation that is stable, economically feasible, and environmentally sustainable for possible use in integrated disease management initiatives.

2. MATERIALS AND METHOD

2.1 Study site and isolation of *Trichoderma*

The present study was conducted in the Department of Botany, C. V. Raman University, Chhattisgarh, India. Soil samples were collected from agricultural fields at Jabalpur village (21°49'40" N, 82°13'20" E), Amartal village (22°00'20" N, 82°31'35" E), Kansaiband village (21°51'30" N, 82°49'40" E) and Pali village (21°28'10" N, 82°41'30" E) of Janjgir-Champa district, Chattishgarh, India (Figure-1). Isolation was performed using *Trichoderma* Selective Medium (TSM), while soil-borne phyto pathogenic fungi were maintained on Potato Dextrose Agar (PDA). The antagonistic potential of the isolates against selected soil-borne pathogens was evaluated using the dual culture technique on PDA.

2.2 Preparation of culture media

TSM was prepared following the formulation described by Akrami (2011), comprising MgSO₄ (0.2 g), K₂HPO₄ (0.9 g), KCl (0.15 g), NH₄NO₃ (1.0 g), anhydrous D-glucose (3.0 g), Rose Bengal (0.15 g), and agar (20.0 g) per litre of distilled water. The basal medium (950 mL) was sterilized at 121°C for 20 min, cooled, and supplemented aseptically with 50 mL sterile distilled water containing 0.25 g chloramphenicol. For mass multiplication of *Trichoderma*, a potato-based liquid medium containing peeled potato (200 g), commercial sugar (20 g), lemon juice (2 mL), and distilled water (1 L) adjusted to pH 6.5 was used.

2.3 Isolation of *Trichoderma sp.* by serial dilution-agar plating method

Serial dilution of soil samples was performed to isolate microorganisms. Briefly, 1 g of finely pulverized, air-dried soil transferred aseptically into 9 mL of autoclaved distilled water to

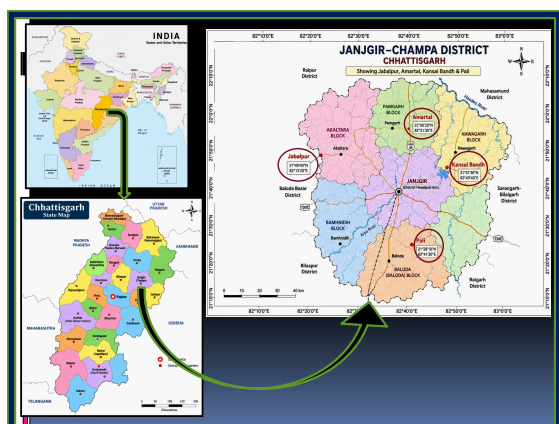


Figure 1. Showing the soil sampling site of Janjgir-Champa district and the area are Amartal, Jabalpur, Kansaiband and Pali.

maintain a 10^{-1} dilution. To ensure uniform mixing of microorganisms the suspension was vortexed thoroughly using a vortex mixer. After that, ten-fold serial dilutions 10^{-3} to 10^{-6} were prepared under aseptic conditions in test tubes by dispensing 1 mL of the initial dilution into 9 mL of autoclaved distilled water and after each transfer the suspension was mixed properly by tilting or vortexed for uniform distribution. After that, 0.1 mL aliquots from each dilution of 10^{-3} to 10^{-6} were spread aseptically onto Petri dishes containing sterilized culture medium using L-shaped glass spreader. All plates were made in triplicate subjected to incubation at $30 \pm 1^\circ\text{C}$ for 4-6 days for fungal growth and colony development.

2.4 Purification and maintenance of isolates

After growth of the fungal colonies the pure colonies of *Trichoderma* were spotted and transferred aseptically again onto potato dextrose agar (PDA) plates and kept for incubation at $30 \pm 1^\circ\text{C}$ for 5-6 days to obtain pure culture. Subsequently the pure colonies of *Trichoderma* were aseptically transferred from the culture plates onto potato dextrose agar (PDA) slants supplemented with Mox-500 with the help of a sterile inoculating needle. After that, the inoculated tubes were incubated at $30 \pm 1^\circ\text{C}$ for 5-6 days and subsequently kept in refrigerator for further use. The *Trichoderma* isolates in the test tube kept for preservation were sub-cultured onto sterile PDA plates at a regular intervals to obtain subsequent morphological, physiological, and biochemical analyses.

2.5 Morphological characterization of the *Trichoderma* isolates

The *Trichoderma* isolates were examined under a compound microscope and identified based on their morphological characteristics, including colony growth pattern, colony colour, and the shape and size of conidiophores, conidia, and phialides, with reference to standard taxonomic literature. Microscopic observations were carried out at 45X magnification. The dimensions of the fungal structures were determined using a calibrated stage

and ocular micrometer and expressed in micrometres/ μm (Raeder and Broda, 1985).

The observations were made from cultures that were grown on PDA plate the following features were measured: the width of the phialides at their widest point, the length of the phialides, the length and width of the conidia, and the presence of chlamydospores. Each of these features was measured after the samples were initially wetted with 3% KOH.

2.6 DNA extraction and ITS amplification

For molecular analysis, the cultures were maintained on PDA at 25°C for DNA extraction and subsequently incubated in PDA for four-five days. After that mycelial portion was concentrated by light centrifugation and then kept on filter paper, two to three times washed with distilled water, frozen, and then used for DNA extraction. Genomic DNA was extracted using the method described by Raeder and Broda (Samual *et al.*, 2002). The DNA was again resuspended in 50 μL of TE buffer and its quantity was determined using ethidium bromide fluorescence. Primers ITS1(50-TCCGTAGGTGAACCTGCGG-30) and ITS4 (50-TCCTCCGCTTA TTGATATGC-30), as described by White *et al.* (1990) were used to amplify a part of the rDNA that includes ITS1 and ITS2 regions along with the 5.8S rDNA gene. The PCR amplification of the ITS gene was carried out using 156 ng of extracted DNA, along with 10 pM of each primer. The TAQ Master mix used contained a high-fidelity DNA polymerase, 2.5 mM dNTPs, 3.2 mM MgCl_2 , and PCR enzyme buffer. The reactions underwent an initial denaturation step of 3 minutes at 94°C , followed by 30 cycles of 1 minute at 94°C , 1 minute at 50°C , and 2 minutes at 72°C . A final extension step of 7 minutes at 72°C was performed using the Gene Amp PCR system. The PCR products were then separated on a 1% agarose gel. Sequencing of the purified PCR product using the ITS1 forward primer was carried out at Biokart, Bangalore, India.

2.7 Sequence analysis and phylogenetic reconstruction

The amplified product (~600 bp) was sequenced and submitted to NCBI BLAST for similarity search. The 20 closest sequences of *Trichoderma* spp. were retrieved from GenBank and aligned with the isolate sequence using Clustal W. Evolutionary relationships were inferred using the Neighbor-Joining (NJ) method (Saitou & Nei, 1987). The evolutionary distances were computed using the Kimura 2-parameter model (Kimura, 1980), expressed as the number of base substitutions per site. Bootstrap analysis (1000 replicates) was performed to assess the reliability of branching patterns (Felsenstein, 1985). All ambiguous positions were removed using the pair wise deletion option. The final dataset contained 628 positions.

Phylogenetic analysis was conducted in MEGA11 (Tamura et al., 2021).

2.8 Assessment of Radial Growth Rate

4-6 days old mycelial discs (5 mm diameter) of the selected *Trichoderma* isolate was aseptically cut using a sterile cork borer and transferred individually onto freshly prepared, autoclaved PDA plates with the aid of a sterile inoculation needle. The inoculated plates were incubated at $30 \pm 1^\circ\text{C}$. Radial mycelial growth was recorded after 24 h of incubation as the initial observation and subsequently at 12 h intervals until the colonies completely covered the Petri plates. Colony diameter was measured in millimetres (mm) to determine the growth rate of each isolate.

2.9 Evaluation of antagonism by Duel-culture assay

The antagonistic activity of the selected *Trichoderma* isolate was evaluated against *Fusarium sp.*, *Pestalotiopsis sp.* and *Colletotrichum sp.* by a dual-culture assay. The experiment carried out following completely a randomized design with three replications in each case. A 3-mm mycelial disc of the pathogen and an equivalent disc of *Trichoderma* were positioned on opposite sides of a PDA plate at equal distances from the centre. The control plates contain only the pathogen and all the plates were incubated at $30 \pm 1^\circ\text{C}$. The growth of the test pathogen and the *Trichoderma* isolate was recorded after every 24-h intervals. Inhibition percentage of the radial growth was calculated as-

$$I = [(C - T)/C] \times 100,$$

Here, C is the pathogenic growth in the control and T is the pathogenic growth in dual culture.

2.10 Antagonism classification

The intensity of antagonism was evaluated qualitatively and labelled as strong, moderate, weak, or absent by analyzing the degree of pathogen growth inhibition in the plates. The Isolate which shows more than 50% inhibition was labelled as showing strong antagonism; those who show 20–50% inhibition were labelled as moderate antagonism. Isolates resulting in 1–19% inhibition were categorized as showing weak antagonism, while 0% inhibition was considered no antagonism, indicating no observable inhibitory effect on pathogen growth.

2.11 Low cost formulated media preparation and mass multiplication

To prepare 1 L of potato broth medium, 250 gm of fresh potatoes were peeled and cut into approximately 1 cm³ cubes. The potato pieces were boiled in 500 ml of distilled water for 30–40 minutes. The resulting extract was filtered through sterile gauze cloth to remove the potato residues. The filtrate was then diluted with tap water to a final volume of 1 L. Subsequently, 20 g of sugar and half teaspoons of lemon juice were added and mixed thoroughly until completely dissolved. The prepared medium was sterilized in a stainless-steel container

by autoclaving at 121°C for 20–30 minutes. After sterilization, the medium was allowed to cool to room temperature after that, the selected *Trichoderma* isolate were aseptically inoculated with the help of a sterile needle and incubated at $30 \pm 1^\circ\text{C}$ for 5-7 days.

2.12 Preparation of the liquid formulation and shelf life assessment

The liquid formulation was prepared according to the method attributed to Chandra *et al.* (2005). The mass-multiplied low cost culture broth was incorporated into a liquid carrier containing 0.5% carboxymethyl cellulose (CMC), 1% glycerol and distilled water. The preparation was mixed aseptically and filled into sterile food-grade plastic containers for storage under cool conditions.

The quality and shelf life of the liquid-based *Trichoderma* formulation were evaluated by estimating the viable propagule count (colony-forming units; CFU) at three storage intervals: immediately after formulation (0 month), one month, and fifth months after storage under room conditions in cool place. The viable counts were found out by employing the serial dilution plate technique. Six numbers of sterile test tubes were taken and labelled as 10^{-1} to 10^{-6} and filled with 9 mL of sterile distilled water each containing. After that, One ml of the low cost formulation was transferred aseptically to the first test tube (10^{-1}) and mixed properly to acquire a uniform suspension. After that, in each successive dilution tubes up to 10^{-6} , 1 mL of the suspension was aseptically serially transferred from the previous one.

With the help of a sterile L-shaped glass spreader an aliquot (0.1 mL) from the 10^{-4} , 10^{-5} , and 10^{-6} dilutions separately was transferred onto sterile Potato Dextrose Agar (PDA) plates and spread. All the plates were then incubated at $30 \pm 1^\circ\text{C}$ until the colony formation in the plates. Colony Forming Units (CFU ml⁻¹ of formulation) were calculated using the standard formula:

$$\text{CFU ml}^{-1} = (\text{Number of colonies} \times \text{Dilution factor}) / \text{Volume plated (mL)}$$

2.13 Statistical analysis

The experiments were conducted using a Completely Randomized Design with appropriate replication. Analysis of variance was performed using Agres and Agdata software. Treatment means were compared using Fisher's protected least significant difference test, and effects were considered statistically significant at $P \leq 0.05$ based on the calculated F-values.

3. RESULT

Soil samples were collected from various locations including Jabalpur village ($21^\circ 49' 40''$ N, $82^\circ 13' 20''$ E), Amartal village ($22^\circ 00' 20''$ N, $82^\circ 31' 35''$ E), Kansaiband village ($21^\circ 51' 30''$ N, $82^\circ 49' 40''$ E), and Pali village ($21^\circ 28' 10''$ N, $82^\circ 41' 30''$ E) in Janjgir-Champa district, Chhattisgarh, India. Three *Trichoderma* isolates

were obtained with the designation SM/AS/SG00, SM/AS/SG002, SM/AS/SG003 respectively and among them; the isolate SM/AS/SG001 demonstrated strong antagonistic activity against plant pathogens and was chosen for further study.

3.1 Colony morphology and growth

The isolate demonstrate fast growth rates and exhibit green pigmentation with various textures and margins, the differences in the macroscopic characteristics and growth speeds highlight its unique adaptations to the respective rhizosphere environments. As seen in Table 1 and Figure 2(a, b, c), the isolate exhibited colonies that were starts white colour to pale or vibrant green or bright green, mature colonies showed dark green, fluffy texture with fringed margin. On the reverse side, the colonies showed a pale yellow to dark green concentric ring. The growth rate of the isolate was approximately 6.93 mm per day, indicating a fast growth speed. *Trichoderma* growth rate of is a key factor always influenced by different types of environmental conditions like pH, abundance of nutrient, and how it interacts with neighbouring microbes in the rhizosphere. Typically, *Trichoderma* strains are distinguished by degree of growth rates and the secondary metabolites production profile, which determine the role and characteristics of their antagonism property to pathogens. Beyond serving as weapons against diseases, these chemical compounds prompt spore formation and stimulate growth, which alters the physical development of the fungi (Li *et al.*, 2016; Filizola *et al.*, 2019). The colony appearance or the growth patterns of *Trichoderma* are always affected by different variables like nutrients, ph and temperature etc. The distinction among *Trichoderma* strains according physical macroscopic appearance like colony appearance, mycelia growth rate. The microscopic morphological characteristics like phialides structure and phialospores determines the understanding about the adaptability and diversity within the genus (Mukherjee *et al.*, 2013; Carvalho *et al.*, 2018). Microscopic characteristics of the isolate SM/AS/SG001, as is shown in Table 2 and Figure 2 (d-f) disclose definite morphological characteristics which were very much important for the identification purpose. In this case, the isolate shows smooth-walled, green conidia and indicated variableness in conidial shape, size, phialid dimensions, and conidiophores characteristics. SM/AS/SG001 conidia were ellipsoidal to cylindrical, smooth-walled, and varied from hyaline to bright green in colour. They measured between 3.28-5.22 μm in length and 2.37-3.81 μm in width, with average dimensions of 4.24 μm in length and 3.09 μm in width. The phialides of SM/AS/SG001 were flask-shaped, hyaline, and ranged in length from 13.98-21.06 μm and in width from 4.11-7.93

μm , with mean values of 17.52 μm and 6.02 μm , respectively. The conidiophores were branched and colourless to pale yellow, producing conidia in whorls or clusters on the flask-shaped phialides. These observations are crucial for accurate identification and understanding the biological behaviour of the fungi, which have implications for disease management and ecological studies. The dimensions of conidia, including their length and diameter, vary significantly among different *Trichoderma* species, and these morphological traits are often used to classify and identify isolates. A study demonstrated that conidial dimensions can be measured accurately, allowing for differentiation among closely related species based on these metrics (López-Quintero *et al.*, 2013). Additionally, in species identification and ecological adaptation the density of conidial clusters plays a significant role. The amount of higher conidial clusters can determine particular species' adaptations to environmental conditions, effecting their reproductive strategies and growth (Calcáneo-Hernández *et al.*, 2023). The temperature, UV radiation, chemical pressure, cold stress etc such environmental factors influence conidial density and germination (Qu *et al.*, 2017). The expression networks of gene regulation also affect the conidial cluster formation and density, which can affect asexual lifecycles (Xu *et al.*, 2024).

Isolate	Soil source	Colony		Growth rate mm/day	Growth speed
		Front	Reverse		
SM/AS/SG001	Agricultural soil	Colonies first appeared whitish and turns to pale or vibrant	The colour ranges from pale yellow to light green, featuring a concentric ring with dark green in the centre.	6.93	Fast

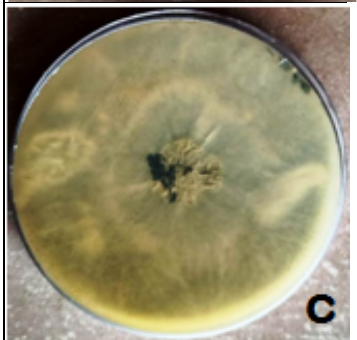
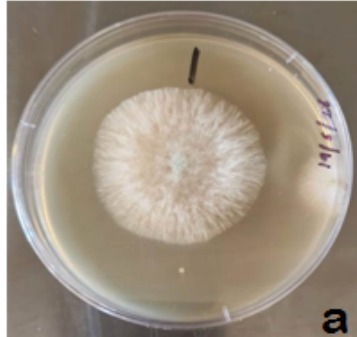
		t gre en or bri ght gre en, ma tur e col oni es sho we d dar k gre en, flu ffy tex tur e wit h fri ng ed ma rgi n.			
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Table 1. Macroscopic characteristics of Trichoderma sp. isolate SM/AS/SG001.

Isolate	Conidia				Phialid				Conid iophore
	Shap e and col our	R a n g e S i z e L × W (μ m)	L e n g t h ± S. D . (μ m)	W i d t h ± S. D . (μ m)	Shap e an d col our	R a n g e S i z e L x W (μ m)	L e n g t h ± S. D . (μ m)	W i d t h ± S. D . (μ m)	
S M / A S / S G 0	Ell ips oi dal to cyl in dri	3. 2 8 - 5. 2 2 X	4. 2 4 ± 0. 4 3	3. 0 9 ± 0. 5 2	Sle nd er, fla sk-sh ap ed	1 3. 9 8 - 2 1. 3	1 7. 5 2 ± 2. 1 3	6. 0 2 ± 1. 7 2	bra nch ed and col orle ss to

0 1	cal , gr ee n, sm oo th wa lle d an d pr od uc e de ns e clu ste rs	2. 3 7 - 3. 8 1			str uct ure s tha t pr od uc e co nid ia an d t ran sp- are nt i n col or.	0 6 X 4. 1 1 - 7. 9 3			pal e yell ow, pro duc ing con idia in wh orls or clus ters on the flas k-sha ped phi alid es
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Table 2. Microscopic characteristics of Trichoderma sp. isolate SM/AS/SG001.



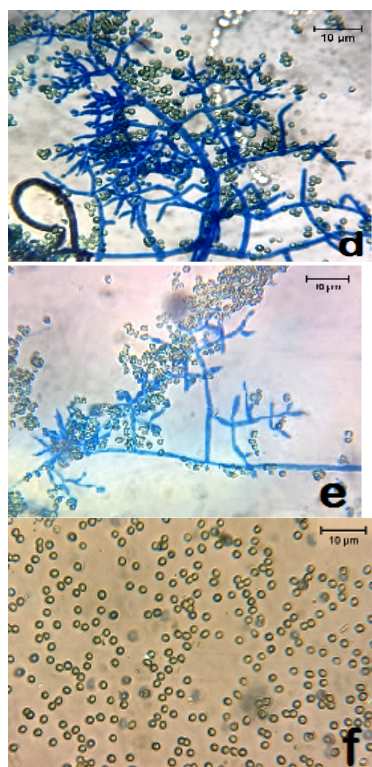


Figure 2. Macroscopic and microscopic characteristics of *Trichoderma* spp. isolate SM/AS/SG001. (a) front colony view after three days of inoculation; (b) front colony view after nine days; (c) back view of the culture plate; (d-e) conidiophore and phialide; (f) conidia of the isolate. The black scale bar is 10 μ m

3.2 Molecular characterization

BLAST analysis confirmed high similarity of the isolate sequence with *Trichoderma* sp., particularly *T. hamatum* and *T. sp. HBE11*. The resulting tree (Figure 3) shows SM/AS/SG001 clustering within the *Trichoderma* clade. SM/AS/SG001 grouped closely with *Trichoderma hamatum* strain CEN1350 and *Trichoderma* sp. strain HBE11, supported by high bootstrap values (>95%). Branch lengths were short, indicating low genetic divergence among these strains. Other reference sequences formed distinct clades representing related *Trichoderma* species, confirming the taxonomic placement of SM/AS/SG001. The phylogenetic analysis validated the molecular identity of the native isolate as *Trichoderma* sp., consistent with morphological observations. High bootstrap support values demonstrate the reliability of the inferred branching patterns, while short branch lengths suggest close genetic similarity. The clustering of SM/AS/SG001 with *T. hamatum* and *T. sp. HBE11* indicates that the isolate shares a recent common ancestor with these strains, which are known for their biocontrol potential. This molecular characterization complements the morphological observations and antagonistic assays. The placement of SM/AS/SG001 within a well supported *Trichoderma* clade strengthens its identification and

highlights its ecological adaptability to local agro-climatic conditions.

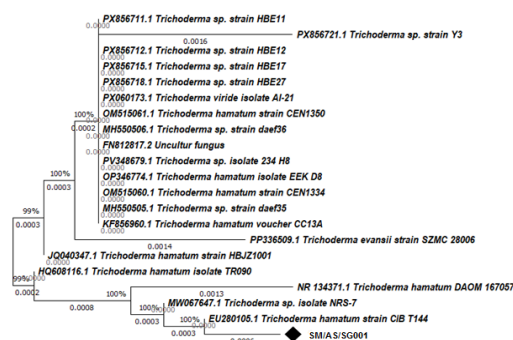


Figure 3. Neighbor-Joining phylogenetic tree based on ITS rDNA sequences of *Trichoderma* isolates, including native isolate SM/AS/SG001 (diamond).

3.3 Antagonistic activity

The dual culture assay demonstrated strong antagonistic activity of *Trichoderma* sp. against all the three tested plant pathogenic fungi. The percentage inhibition of radial growth varied among the three replications (Table-3). Against *Fusarium* sp., inhibition ranged from 69–81%, with a mean inhibition of 75.67%. *Pestalotiopsis* sp. showed comparatively higher inhibition, ranging from 76–83%, with a mean of 80.33%. The highest antagonistic effect was observed against *Colletotrichum* sp., where inhibition ranged from 79–85%, giving a mean inhibition of 82.33%. Based on the inhibition degree, the *Trichoderma* isolate exhibited strong antagonistic activity against all three pathogens. These findings indicate that the tested *Trichoderma* isolate have considerable potential as biological control agents for managing fungal plant diseases.

3.4 Microscopic evidence of interaction

The antagonistic mechanism was determined through microscopic examination, which shows intimate physical contact between the *Trichoderma* hyphae and all three test pathogens. The *Trichoderma* hyphae were observed to coil around and closely associate with the pathogen hyphae, a characteristic feature of mycoparasitic interactions thus confirm mycoparasitism (Picture-4). In contrast, the absence of distinct inhibition zones in the dual-culture assays suggested that antibiosis was not the predominant mechanism during the experimental conditions. The pathogenic growth inhibition observed in dual culture test was therefore assigned primarily to direct mycoparasitic interaction and competition for nutrients and space, as indicated by the reduced pathogen biomass in the vicinity of *Trichoderma* growth. These results show that *Trichoderma* suppresses pathogens through several pathways, relying heavily on physical contact and resource competition instead of antibiosis. Because these fungi are mostly saprophytic, they thrive in nutrient-dense settings and quickly claim open ecological niches. This rapid colonization allows them to outcompete harmful

fungi for vital space and nutrients. By locking down these niches early, *Trichoderma* effectively blocks pathogens from establishing and spreading (Pfordt *et al.*, 2023). This fierce rivalry for space and food acts as a primary driver of *Trichoderma*'s biocontrol success, especially in the rhizosphere where swift colonization leaves soil-borne pathogens with very few resources.

Test Species		<i>Fusarium</i> sp.	<i>Pestalotiopsis</i> sp.	<i>Colletotrichum</i> sp.
Inhibition %	R 1	77	82	83
	R 2	69	76	85
	R 3	81	83	79
	Mean	75.67	80.33	82.33
Inhibition degree		Strong	Strong	Strong

Table 3. Antagonism of *Trichoderma* spp. against *Fusarium*, *Pestalotiopsis*, *Colletotrichum* sp. in the dual culture test. R- indicates the replicates.

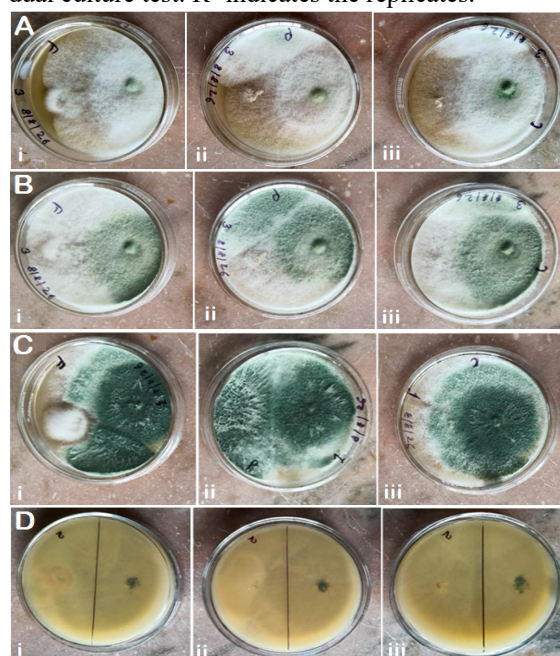


Figure 4. Dual culture test of *Trichoderma* sp. SM/AS/SG001 against *Fusarium*, *Pestalotiopsis*, *Colletotrichum* sp. (right side of the plate-SM/AS/SG001 and left side is the test fungus); (i) *Fusarium* sp.; (ii) *Pestalotiopsis* sp.; (iii) *Colletotrichum* sp.; A. picture after 4 days of inoculation; B. after six days; C. after eight days; D. the back side of the dual culture test plates.

3.5 Mass multiplication and formulation

The successful application of *Trichoderma* sp. as biological control agents depends on their effective isolation, identification, characterization, evaluation of antagonistic activity, and subsequent mass multiplication and formulation under laboratory conditions. Among the *Trichoderma* isolates evaluated, *Trichoderma* isolate SM/AS/SG001

exhibited the highest antagonistic activity against all three tested plant pathogens. Based on its superior biocontrol potential, the isolate was selected for mass multiplication in formulated low cost boiled Potato extract juice (250 gm/lit) supplemented with sugar (20gm/lit) and lemon juice (5ml/lit), which showed a promising growth of the *Trichoderma* isolate SM/AS/SG001. The formulated culture produced a dense white to green mycelial mat with abundant sporulation, indicating satisfactory mycelial growth and conidial production in that low-cost formulation medium (Figure- 5). After that the biopesticide product was formulated as liquid-based containing 0.5% CMC, 1% glycerol, and distilled water along with the *Trichoderma* broth.

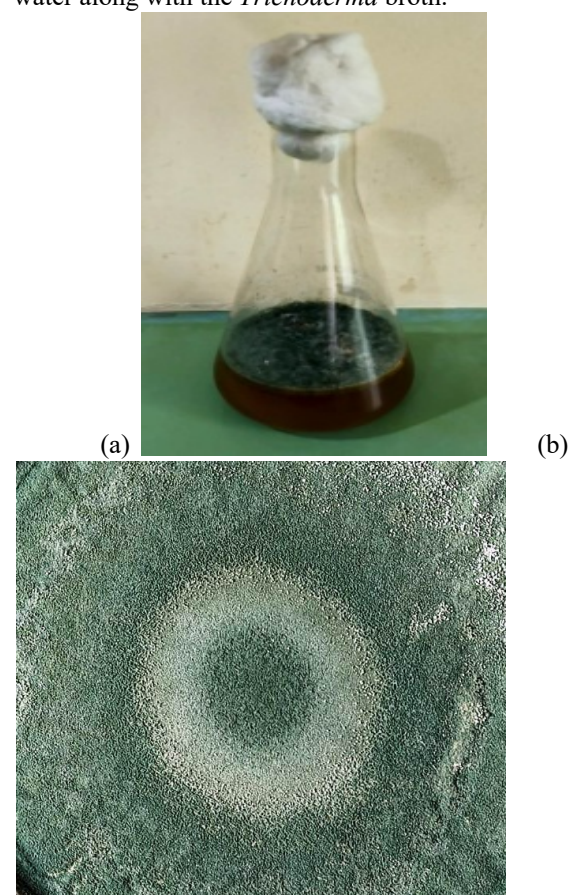


Figure- 5 (a) *Trichoderma* growth in low cost media; (b) The formulated culture produced a dense white to green mycelial mat with abundant sporulation

3.6 Shelf life study

The liquid formulation of *Trichoderma* isolate SM/AS/SG001 contained a substantial number of viable conidia and demonstrated good storage stability. The shelf-life of the formulation was determined on monthly basis up to five months by Colony-Forming Unit (CFU) counts which showed a slight gradual decrease from 12×10^6 CFU to 7×10^6 CFU after five months of storage. In this study, it has been found a reduction of approximately 41.67% in CFU, while 58.33% of the initial CFU was maintained. Not with standing this decrease, the

prepared low cost formulation maintained a appreciable population of viable CFU even after five months of storage, which indicate a satisfactory stability and perseverance of the biocontrol agent (Martinez *et al.*, 2023). Therefore, the formulated *Trichoderma* isolate SM/AS/SG001 can be considered suitable for use even after five months of storage, demonstrating its potential as a stable and economical liquid formulation for biological disease management.

4. DISCUSSION

The present study demonstrates the potential of a locally obtained *Trichoderma* isolate as an in-vitro antagonist of several plant pathogenic fungi. The strong inhibition recorded against *Fusarium sp.*, *Pestalotiopsis sp.* and *Colletotrichum sp.* indicates that SM/AS/SG001 has a relatively broad antagonistic spectrum under the experimental conditions. The differences among pathogens are also noteworthy: *Colletotrichum* was most strongly inhibited, followed by *Pestalotiopsis* and *Fusarium*. Such pathogen-dependent variation is expected because the outcome of *Trichoderma*–pathogen interactions is influenced by the physiological properties of both organisms as well as by environmental conditions (Kubicek *et al.*, 2001).

Trichoderma is well-known to conquer the phytopathogens mainly through mycoparasitism, competition, antibiosis, plant-mediated defence responses and extracellular hydrolytic enzymes etc. The result of inhibition property should be considered as a combination of diversified possible interactions rather than being imputed to a particular mechanism. The importance of each mechanism varies with strain and target organism (Benítez *et al.*, 2004; Harman *et al.*, 2004). In the present assay, the rapid growth of SM/AS/SG001, together with the absence of a pronounced inhibition zone, suggests that rapid colonization and competition for space and nutrients may have contributed substantially to pathogen suppression.

The microscopic observations provide additional evidence for a direct interaction. Hyphal coiling and close association between *Trichoderma* and pathogen hyphae are recognized features of mycoparasitic interactions. Mycoparasitism is a complex process involving recognition and attachment of the target fungus, physical interaction and, in suitable systems, enzymatic degradation of the host cell wall. Chitinases, glucanases and proteases are among the enzymes associated with this process (Mukherjee *et al.*, 2022). Therefore, the hyphal behaviour observed for SM/AS/SG001 is consistent with a possible mycoparasitic contribution to antagonism. However, direct demonstration of enzymatic degradation or penetration would require additional microscopic, biochemical or molecular experiments and was beyond the scope of the present study.

The growth characteristics of SM/AS/SG001 further support its suitability for competitive interactions. With a recorded radial growth rate of 6.93 mm day⁻¹, the isolate demonstrated swift colonization of the available substrate. Such rapid development frequently offers a distinct competitive edge when two fungi vie for the same ecological niche, even though speed of growth by itself does not fully explain the mechanics of biocontrol (Calcáneo *et al.*, 2023). Physically, the isolate displayed features typical of the genus *Trichoderma*, including branched conidiophores, green conidia, and distinct phialides, all of which aligned well with its molecular classification.

According to the ITS sequence analysis, strain SM/AS/SG001 clustered closely alongside *T. hamatum* and *Trichoderma sp.* HBE11, backed by strong bootstrap values over 95%. This clear harmony between physical traits and genetic profiling reinforces the taxonomic classification up to the resolution offered by the current sequence data. All the same, ITS sequencing sometimes lacks the depth needed to separate closely related *Trichoderma* species without ambiguity. For a definitive, formal identification at the species level, tracking down additional genetic loci or running whole-genome analyses would be the logical next step (Gomathi *et al.*, 2023).

From a practical standpoint, the study successfully grew SM/AS/SG001 on an affordable, potato-based medium. Formulating a growth medium out of common ingredients like potatoes, commercial sugar, and lemon juice yields an accessible substrate for generating biomass and conidia. These budget-friendly alternatives prove highly beneficial for smaller laboratories and operations running on limited resources. Even so, variables like contamination risks, production costs, final spore concentrations, and batch uniformity must be thoroughly checked before moving this method into full-scale commercial manufacturing (Kumar *et al.*, 2023).

The formulation maintained a viable count of 7×10^6 CFU after a five-month period, down from an initial 12×10^6 CFU. While this 41.67% drop marks a noticeable dip in viability, over half of the original fungal population managed to survive. This outcome points to a reasonable level of storage stability under the tested conditions.

CONCLUSION

During our study, we isolated a promising native strain of *Trichoderma*, designated SM/AS/SG001, from farming soil in Chhattisgarh's Janjgir-Champa region. This specific isolate grew quickly, matched typical *Trichoderma* physical traits, and showed close genetic links to *T. hamatum* and its relatives. When tested in dual-culture plates against common plant pathogens, the strain heavily suppressed *Fusarium sp.* (75.67%), *Pestalotiopsis sp.* (80.33%), and *Colletotrichum sp.* (82.33%). Under the

microscope, we observed direct hyphal contact, pointing clearly to a mycoparasitic mechanism. Furthermore, we successfully mass-produced the isolate on an inexpensive, potato-based medium; the resulting liquid formulation kept a high viable count of 7×10^6 CFU even after five months on the shelf. Taken together, these results show that SM/AS/SG001 works well as a locally adapted biocontrol agent. However, we still need to run greenhouse and field trials, alongside deeper biochemical, molecular, and shelf-life testing, before farmers can use it commercially.

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