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Research Article

Nutrient-independent antagonism of an arid-adapted *Trichoderma asperellum* isolate against causative agent of the *Fusarium* wilt in tomato

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ABSTRACT: *Fusarium* wilt, caused by *Fusarium* spp., is a major agricultural bottleneck threatening the United Nations' Sustainable Development Goal of Zero Hunger. While synthetic fungicides are standard practice, their environmental toxicity necessitates the prioritization of sustainable, ecologically competent native biocontrol agents. This study aimed to isolate an arid-adapted indigenous biocontrol agent and evaluate its nutrient-independent antagonism against *Fusarium oxysporum* f. sp. *lycopersici* (FOL), the causative agent of tomato wilt. To ensure methodological rigor, the virulence of a reference FOL strain was first validated *in vivo* on tomato (cv. BASF Arya). Subsequently, to guarantee nutritional impartiality during *in vitro* interaction studies, FOL growth kinetics were evaluated across six solid media with broad compositional variety. As no significant growth differences were observed among organic nutrient-rich media ($p>0.05$), Potato Dextrose Agar (PDA) was selected as an unbiased baseline medium. Concurrently, ten native fungal isolates sourced from the extreme pedoclimatic conditions of the Kachchh region were screened in dual-culture assays. Isolate UBN14 demonstrated superior, true biological antagonism, exhibiting a significant time-dependent increase in pathogen inhibition that peaked at 64.22% at 7 Days After Inoculation (DAI). Molecular identification via ITS1-ITS4 genomic sequencing confirmed UBN14 as *Trichoderma asperellum*. By validating its efficacy against a virulent pathogen under nutritionally unbiased conditions, this study establishes the highly resilient, arid-adapted *T. asperellum* UBN14 as a potent candidate for integration into sustainable disease management protocols.

KEYWORDS: Biological control, dual culture assay, ecological competence, *Fusarium oxysporum*, media optimisation

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated vegetable crops across the globe due to its economic, nutritional, and culinary value. In India, tomato is grown extensively across diverse agro-climatic regions, with an annual production exceeding 20 million metric tonnes, and India ranks 2nd globally (FAO, 2023). Despite this substantial output, production is frequently constrained by various biotic, abiotic, and post-harvest factors. Among these, biotic stress caused by soil-borne fungal pathogens is a primary limiting factor responsible for extensive yield losses, ranging from 30 to 80% in severely affected fields (Sahoo *et al.,* 2023; Vignesh *et al.,* 2020). FOL is a devastating vascular wilt pathogen that damages the tomato plant by causing *Fusarium* wilt. It infects plants through rootlets, invading xylem and leading to vascular obscuring, leaf wilting, and eventually plant death. To overcome the impact of soil-borne phytopathogens, farmers are extensively dependent on chemical pesticides and or fungicides. Their indiscriminate use resulted in severe ecological and health consequences (Sharma & Pandey, 2021). The residues of these recalcitrant compounds can contaminate soil and water sources and can also affect non-target organisms. There are also reports indicating the development of resistance in the phytopathogens. So, there is an urgent need to shift towards sustainable, eco-friendly alternatives, specifically Biocontrol Agents (BCAs). Biocontrol agents, particularly antagonistic fungi such as *Trichoderma* spp., offer a promising strategy for managing *Fusarium* wilt (Pandey *et al.*, 2021). However, while these fungi can suppress pathogens through multiple mechanisms, commercial bio-inoculants frequently fail under field conditions due to a lack of ecological competence in harsh pedoclimatic environments. Furthermore, standard *in vitro*

dual-culture screening protocols often fail to account for the differential nutrient preferences of interacting fungi, leaving it ambiguous whether pathogen inhibition is a true antagonistic response or merely an artifact of nutrient competition. Therefore, this study aimed to comprehensively evaluate an arid-adapted, indigenous isolate of *Trichoderma asperellum* (UBN14) sourced from the extreme climate of the Kachchh region. By first validating pathogen virulence to satisfy Koch's postulates and subsequently establishing a nutritionally impartial baseline through rigorous media optimization, this research wanted to demonstrate the true, nutrient-independent biological antagonism of a highly resilient native biocontrol candidate against FOL.

MATERIALS AND METHODS

The identified phytopathogen, FOL ITCC No. 8111, was obtained from the Indian Type Culture Collection (ITCC) of Division of Plant Pathology, Indian Council of Agricultural Research-Indian Agricultural Research Institute (ICAR-IARI), New Delhi, India. All the chemicals used in the study were analytical grade. All media used in the study were purchased from HiMedia Laboratory Pvt. Ltd. (Mumbai, India). BASF Arya (Hy. F1) variety of tomato seeds were purchased from a local agricultural store. For the statistical analysis, IBM's SPSS Software Version 27.0 by International Business Machines, New Orchard Road, Armonk, New York, United States was used.

Isolation and identification of fungal isolates

Isolation and morphological identification

For the isolation of fungal isolates, various soil samples were collected from virgin soil as well as the tomato rhizosphere area from 2 different tehsils of Kachchh district, *i.e.*, (i) Mandvi and (ii) Anjar. Samples were collected in sterile ziplock bags and were stored at 4°C within 4-8 h from the collection till further use. Serial dilutions were performed using Potato Dextrose Agar (PDA) to get isolates. 0.1 mL was inoculated and incubated at 27°C till distinct growth was visible. Obtained isolates were studied for their morphological characteristics and were subjected to microscopic observation by performing the lactophenol cotton blue staining method.

Molecular identification

For molecular identification of a potent isolate, the sample was outsourced (GeneXplore, Ahmedabad, India). Brief methodology: the genomic DNA was isolated, and purity was verified via 1.0% agarose gel electrophoresis. The Internal Transcribed Spacer (ITS) region, the universal DNA barcode for fungi, was amplified using ITS1 and ITS4 primers. Sequencing was performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit on an ABI 3500xL Genetic Analyzer. The resulting sequences were analyzed using NCBI BLAST, and taxonomic identity was confirmed based on maximum identity scores with reference sequences in GenBank.

Cultural and pathogenic characterization of FOL

Isolated fungal cultures were initially grown on PDA, and for the subsequent *in vitro* antagonism assay, both the isolate and FOL needed to grow on a common medium in a single plate (Heflish *et al.,* 2021). Therefore, a preliminary experiment was conducted to determine the most suitable solid medium for the optimal growth of the phytopathogen. For that, 6 different solid media, *viz*. Potato Dextrose Agar (PDA), Rose Bengal Agar (RBA), Sabouraud Dextrose Agar (SDA), Czapek Dox Agar (CDA), Mycological Agar (MYA), and Water Agar (WA) were selected to provide a broad nutritional and compositional variety, ranging from rich, undefined substrates to chemically defined medium to a nutrient-deprived stress medium. This range allowed assessment of the growth of FOL under varied carbon/nitrogen sources, pH conditions, and nutrient availability, thereby enabling selection of an optimal medium for subsequent antagonism assays (Nirmaladevi & Srinivas, 2012).

Growth on various solid media

An actively growing culture of FOL from the PDA slant was received, and using the direct streak plate method, the culture was transferred to fresh PDA plates and slants for further study as well as storage purposes, respectively. All inoculated plates and slants were incubated at 27 ± 2°C. To assess its mycelial growth, the six different solid media listed above were prepared according to the procedure provided by the manufacturer. The medium was sterilized by autoclaving and was poured into 90 mm pre-sterilized petri plates. A 5 mm mycelial disc, taken from the periphery of a previously activated FOL culture, was aseptically inoculated in the centre of each petri plate. The plates were incubated at 27 ± 2°C for 7 days. Each treatment was replicated three times. The radial growth of the fungal colony was measured in millimetres (mm) by taking the average colony diameter.

Statistical analysis

To recognize the best medium as well as incubation time for the growth of FOL, the recorded data were subjected to statistical analysis using repeated-measure Analysis of Variance (ANOVA). The culture medium was

treated as the between-subjects factor, and incubation time (days) was treated as the within-subjects factor.

Pathogenicity test

A pathogenicity test was conducted to confirm the virulence of the FOL isolate procured from ITCC on the selected tomato variety. The experiment was designed to satisfy Koch's postulates and involved two methods of inoculation and a negative control group for both tests, *i.e.*, (i) Seed dipping, and (ii) Foliar spray method. For each method, the inoculum from actively growing FOL was prepared, and it contained an initial concentration of 1×10^6 spores/mL (Avin, 2020; Caligiore-Gei & Valdez, 2015).

Preparation of conidial suspension

To prepare a conidial suspension, FOL was inoculated on PDA plates and incubated at 27 ± 2°C. To harvest the conidia, plates were flooded with sterile 20mL distilled water and scraped, then the resulting suspension was filtered through cheesecloth. 0.05% Tween 80 was used for the proper dispersion. Prepared suspension was used for counting of spores by Neubauer's counting chamber, as well as checked for O.D. at 530 nm, and diluted the suspension to achieve a final concentration of 1×10^6 spores/mL.

Plant inoculation

The seeds of BASF Arya (Hy. F1) variety were surface sterilized by dipping in 1% sodium hypochlorite solution for 20 min, followed by three washes with sterile distilled water to remove residual disinfectant and dried seeds using blotting paper. For the seed dipping procedure, the method suggested by Prasad *et al.,* (2021) was adopted with modifications. Sterilized seeds were soaked in the 1×10^6 conidia/mL suspension for 30 min with gentle agitation. The seeds were then air-dried under aseptic conditions before being sown in pots with a diameter of 18 cm and a height of 17 cm, each containing sterilized soil. Three seeds per pot containing a sterilized soil-peat mixture (3:1 W/W) were sown in triplicate. For the foliar spray method, previously dried seeds were sown directly into the pot containing sterilized soil-peat mixture (3:1 W/W). At the 3-4 leaf stage, conidial suspension of 1×10^6 spores/mL was sprayed on the aerial part of the plant using the sprayer. A negative control was established for both methods, where seeds were dipped in or plants were sprayed with sterile distilled water containing 0.05% Tween 80, but no fungal conidia. Periodic irrigation was done to provide a proper water supply.

Symptom assessment and re-isolation of FOL

Plants were monitored daily for the appearance of wilt symptoms, such as leaf chlorosis, wilting, and vascular discoloration. To satisfy Koch's postulate, the symptomatic leaf from both test groups was removed, surface sterilized aseptically and placed on a PDA plate. The plates were incubated at 27 ± 2°C and observed for the growth of FOL. Morphological and microscopic structures were compared with the original culture to confirm its pathogenicity.

In vitro biocontrol activity assessment

Dual culture plate assay

Isolates showing distinct morphologies were used according to the method described by Heflish *et al.,* (2021). In which, A 5 mm mycelial disc from a 7-day-old culture of FOL was placed on one side of a 90 mm PDA plate, near the periphery and 1 cm away from the edge. On the opposite side, a 5 mm disc from a 7-day-old culture of one of the UBN isolates was placed. For the control, a PDA plate was inoculated with only a 5 mm disc of FOL at the centre of the plate. Each treatment and the control were replicated three times. After the inoculation, all plates were incubated at 27 ± 2°C and observed daily for 7 days for the radial growth of fungi. The percent inhibition of mycelial growth of FOL was calculated using the formula provided by (Swain *et al.,* 2018).

$$I = \left[\frac{(C - T)}{C}\right] \times 100 \qquad (1)$$

where: I = inhibition, C = measured radial growth of FOL in control, T = radial growth of FOL in dual culture plate.

Statistical analysis for dual culture plate assay

To assess the effect of different UBN isolates and the duration of incubation on biocontrol activity, the daily percent inhibition data were subjected to a Two-Way ANOVA. The UBN isolate and incubation time were treated as the independent variables. Upon detection of a significant difference, a Tukey's HSD test was performed for post-hoc pairwise comparisons ($p<0.05$).

RESULTS AND DISCUSSIONS

Isolation and identification

Isolation and morphological identification

A total of 16 fungal isolates were obtained from various samples cultured on PDA. Based on unique colony morphology and microscopic features, 10 of these isolates

were selected for further study. Lactophenol cotton blue staining was used to examine the microscopic structures. Five selected isolates, which produced green-pigmented sporulating colonies, displayed branched, septate hyphae with flask-shaped phialides from which globose conidia were produced. The other five isolates were highly diverse. They exhibited a wide range of macroscopic characteristics, including variations in colony colour, texture, and margin. Microscopically, these isolates also showed significant differences in the size and shape of their conidia. All 10 unique isolates were subcultured onto PDA slants for preservation and future use.

Molecular identification of potent isolate

Phylogenetic analysis based on sequence produce using ITS1-ITS4 universal primers was performed using the Neighbor-Joining method to determine the taxonomic position of the isolate UBN 14. The isolate clustered with reference strains of *Trichoderma asperellum*, forming a distinct clade separate from closely related species such as *Trichoderma viride*. The phylogenetic tree depicting a distinct clade is given in Figure 1. However, the bootstrap value supporting this clustering was 59%, indicating low statistical confidence in the phylogenetic placement. Due to this weak bootstrap support, the phylogenetic inference alone was insufficient for definitive species-level identification. Therefore, sequence similarity analysis was performed using BLAST, which revealed 100% nucleotide identity with *Trichoderma asperellum* sequences in GenBank (*e.g.*, Accession Nos. OM280451.1 and MW368691.1; details given in Table 1. Based on the combined evidence from phylogenetic clustering and high sequence similarity, the isolate was identified as *Trichoderma asperellum*. The sequence has been submitted to the NCBI database and assigned the accession number PX845989.

FOL growth and behaviour

All the media equally supported the growth of FOL except WA, which indicates that the pathogen is capable of growing on diverse substances. In the case of WA, the comparatively reduced growth observed is likely due to the absence of additional nutrient sources, as this medium contains only agar without supplementary carbon or nitrogen sources. On all five nutrient-supplemented media, the initial growth of the fungus consisted of white, fluffy aerial mycelium with an irregular margin; these characteristics were in accordance with (Chopada *et al.,* 2015). As the colonies matured, a characteristic purple pigmentation developed and diffused into the agar on PDA, SDA, and MYA. This pigmentation, associated with the production of secondary metabolites as reported by (Lebeau *et al.,* 2019; Medentsev *et al.,* 2005), was absent in the colonies grown on RBA and CDA.

Effect of various solid media on growth

Statistical analysis

The radial growth of FOL was evaluated across six culture media. Quantitative analysis indicated that Sabouraud Dextrose Agar (SDA) supported slightly higher radial growth compared with other nutrient-rich media. However, as presented in Table 2, statistical analysis indicated no significant difference ($p>0.05$) in radial growth among the five nutrient-supplemented media. PDA is a well-established maintenance medium for both *Fusarium* species (Sumana *et al.,* 2014) and was the native medium utilized for the initial isolation of the UBN biocontrol agents. Because PDA inherently supports the vigorous growth of the antagonists and was experimentally proven to support statistically optimal growth of the FOL pathogen, it was selected as the common substrate for all subsequent interaction studies. This ensures that any observed inhibition during the dual-culture assay is strictly a biological response

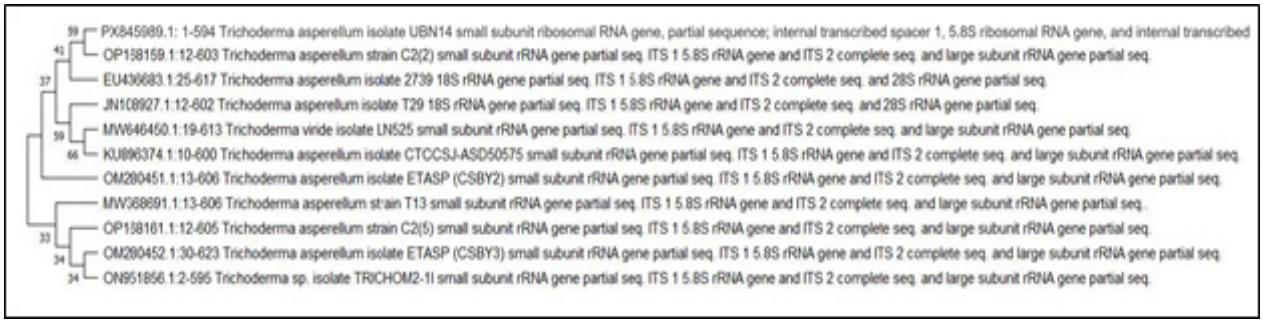


Figure 1. Molecular phylogenetic analysis by the N-J method showing UBN14 at the top.

Table 1. Sequence similarity of the isolate with reference database strains

Sequences producing significant alignments:
(Top 10 Matches)

Description	Max Score	Total Score	Query Cover	E value	Per. ident	Accession
Trichoderma asperellum isolate ETASP_(CSBY2) small subunit rRNA gene, partial sequence; ITS 1, 5.8S rRNA gene, and ITS 2, complete sequence; and large subunit rRNA gene, partial sequence	1098	1098	100%	0	100	OM280451.1
Trichoderma asperellum strain T13 small subunit rRNA gene, partial sequence; ITS 1, 5.8S rRNA gene, and ITS 2, complete sequence; and large subunit rRNA gene, partial sequence	1098	1098	100%	0	100	MW368691.1
Trichoderma asperellum strain C2(5) small subunit rRNA gene, partial sequence; ITS 1, 5.8S rRNA gene, and ITS 2, complete sequence; and large subunit rRNA gene, partial sequence	1098	1098	100%	0	100	OP168161.1
Trichoderma asperellum isolate ETASP_(CSBY3) small subunit rRNA gene, partial sequence; ITS 1, 5.8S rRNA gene, and ITS 2, complete sequence; and large subunit rRNA gene, partial sequence	1098	1098	100%	0	100	OM280452.1
Trichoderma sp. isolate TRICHOM2-1I small subunit rRNA gene, partial sequence; ITS 1, 5.8S rRNA gene, and ITS 2, complete sequence; and large subunit rRNA gene, partial sequence	1098	1098	100%	0	100	ON951856.1
Trichoderma asperellum isolate 2739 18S rRNA gene, partial sequence; ITS 1, 5.8S rRNA gene, and ITS 2, complete sequence; and 28S rRNA gene, partial sequence	1096	1096	100%	0	100	EU436683.1
Trichoderma asperellum strain C2(2) small subunit rRNA gene, partial sequence; ITS 1, 5.8S rRNA gene, and ITS 2, complete sequence; and large subunit rRNA gene, partial sequence	1094	1094	100%	0	100	OP168159.1
Trichoderma asperellum isolate T29 18S rRNA gene, partial sequence; ITS 1, 5.8S rRNA gene, and ITS 2, complete sequence; and 28S rRNA gene, partial sequence	1092	1092	99%	0	100	JN108927.1
Trichoderma viride isolate LN525 small subunit rRNA gene, partial sequence; ITS 1, 5.8S rRNA gene, and ITS 2, complete sequence; and large subunit rRNA gene, partial sequence	1092	1092	100%	0	99.83	MW646450.1

Table 2. Comparative results of mycelial growth on various medium - FOL

S. No.	Media	RG (mm)* (1 DAI)	RG (mm)* (2 DAI)	RG (mm)* (3 DAI)	RG (mm)* (4 DAI)	RG (mm)* (5 DAI)	RG (mm)* (6 DAI)	RG (mm)* (7 DAI)
1	PDA	4.01±0.24^{b}	10.63±0.32^{b}	17.01±0.24^{b}	24.01±0.24^{b}	30.51±0.24^{b}	36.38±0.32^{b}	39.63±0.54^{b}
2	RBA	4.38±0.54^{b}	10.50±0.66^{b}	16.27±0.65^{b}	22.88±1.02^{b}	29.50±1.32^{b}	36.75±0.66^{b}	40.48±0.45^{b}
3	SDA	5.63±0.54^{b}	13.50±0.90^{b}	19.38±1.27^{b}	26.13±1.76^{b}	33.13±1.76^{b}	39.50±0.66^{b}	41.00±0.43^{b}
4	CDA	4.75±0.25^{b}	11.25±1.15^{b}	18.13±0.78^{b}	25.52±0.42^{b}	33.25±0.43^{b}	39.40±0.30^{b}	39.40±0.30^{b}
5	MYA	5.50±0.66^{b}	12.00±0.25^{b}	19.38±0.32^{b}	26.63±0.54^{b}	33.77±0.42^{b}	40.02±0.42^{b}	40.75±0.25^{b}
6	WA	4.63±0.32^{a}	10.25±0.43^{a}	14.63±0.32^{a}	20.25±0.90^{a}	25.25±0.43^{a}	31.00±0.90^{a}	34.75±0.25^{a}

Note: RG = Radial Growth; * = mean of triplicate; DAI = Days After Inoculation.
Values with ^{a} are significant at p = 0.05, and values with ^{b} are non-significant.

and not an artifact of nutrient stress on the pathogen. The data is given in Table 3.

Confirmation of pathogenicity of FOL on tomato

The impact of FOL was studied on tomato plants across different developmental phases. At the early seedling stage, signs of infection became more distinct. As the infection intensified, the lower leaves yellowed and wilted irreversibly, as reported by (Yang *et al.,* 2024). In both methods, FOL caused leaf wilting. The seed dipping method was more deleterious compared to the foliar spray method. The phytopathogen was successfully re-isolated from the

Table 3. Statistical analysis of mycelial growth on various solid media

Tests of Within-Subjects Effects									
Measure:	**MEASURE_1**								
Source		**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**	**Partial Eta Squared**	**Noncent. Parameter**	**Observed Power[a]**
Day	**Sphericity Assumed**	18466.549	6	3077.758	15657.221	0.000	0.999	93943.324	1.000
	Greenhouse-Geisser	18466.549	2.031	9093.722	15657.221	0.000	0.999	31794.991	1.000
	Huynh-Feldt	18466.549	3.466	5328.094	15657.221	0.000	0.999	54266.094	1.000
	Lower-bound	18466.549	1.000	18466.549	15657.221	0.000	0.999	15657.221	1.000
Day * Medium	**Sphericity Assumed**	148.860	30	4.962	25.243	0.000	0.913	757.284	1.000
	Greenhouse-Geisser	148.860	10.153	14.661	25.243	0.000	0.913	256.302	1.000
	Huynh-Feldt	148.860	17.329	8.590	25.243	0.000	0.913	437.443	1.000
	Lower-bound	148.860	5.000	29.772	25.243	0.000	0.913	126.214	1.000
Error (Day)	**Sphericity Assumed**	14.153	72	0.197					
	Greenhouse-Geisser	14.153	24.368	0.581					
	Huynh-Feldt	14.153	41.591	0.340					
	Lower-bound	14.153	12.000	1.179					
a. Computed using alpha = .05									

surface-sterilized infected leaf of the tomato. Re-isolation of the pathogen from infected tissues confirmed Koch's postulates. Typical purple coloured hue on PDA, mycelium structure, and structure of conidia, all characteristics were confirmed by morphological and microscopic observations. It is illustrated in the Figure 2.

Antagonistic activity of fungal isolates

Dual culture assay results

Seven isolates from ten grew radially, and the remaining three isolates were unable to grow radially; therefore, they were eliminated from this assay. For the first three (3) Days After Inoculation (DAI), there was no physical contact

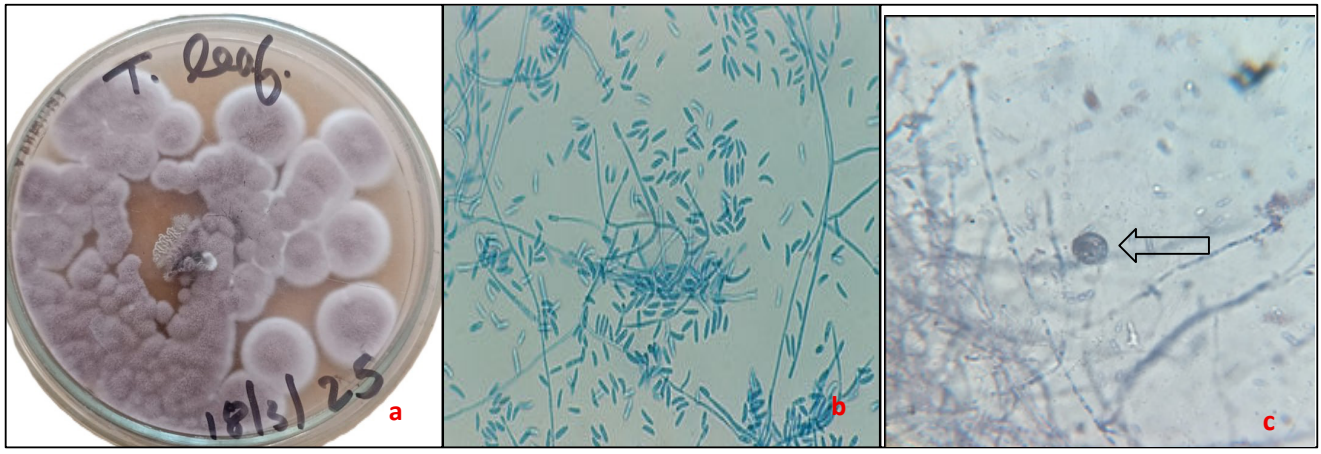


Figure 2. (a). Re-isolated FOL from Infected Leaf, **(b).** Microconidia of FOL, **(c).** Chlamydospores of FOL.



Figure 3. All UBN cultures against FOL and a control FOL all @7DAI.

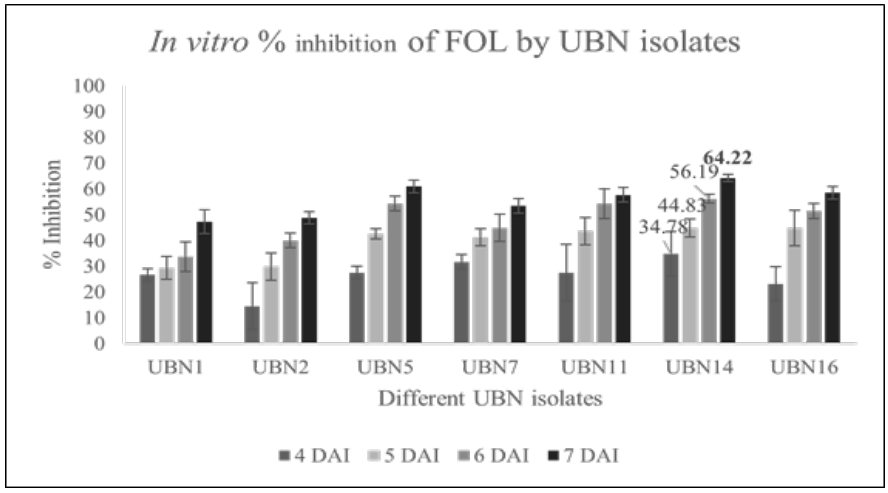


Figure 4. In vitro inhibition (%) of FOL by UBN isolates across 4-7 DAI.

between UBN and FOL, so no effect was assessed. Fourth (4th) day onwards, the growth of radial diameter in mm was recorded for both test and control plates. All cultures were able to inhibit the mycelial growth of FOL compared to the control plate. For 4th DAI, UBN2 showed minimum inhibition, and then UBN1 was least effective from 5th to 7th DAI. UBN14 outcompeted all other isolates throughout all days. % Inhibition @ 7DAI is given in Figure 3. Maximum inhibition was 64.22% on 7 DAI, which is in accordance with many previous studies (Haque *et al.,* 2023; Lakhdari *et al.,* 2018). Comparative % inhibition of all isolates along the incubation time is depicted in Figure 4.

Statistical analysis

The two-way ANOVA revealed highly significant main effects for both independent variables, *viz*. UBN isolates and incubation time ($p<0.001$) on the percentage of inhibition, while the interaction effect remained non-significant ($p>0.05$). Tukey's HSD as a post-hoc analysis

Table 4. Effect of different UBN isolates and incubation time on the percentage inhibition of FOL

S. No.	Type of UBN	% Inhibition 4 DAI *	% Inhibition 5 DAI *	% Inhibition 6 DAI *	% Inhibition 7 DAI *
1	UBN1	26.67 ± 2.44^{aA}	29.41 ± 4.44^{aB}	33.73 ± 5.72^{aC}	47.3 ± 4.64^{aD}
2	UBN2	14.49 ± 9.05^{aA}	29.88 ± 5.26^{aB}	40.00 ± 2.86^{aC}	48.78 ± 2.44^{aD}
3	UBN5	27.54 ± 2.51^{bA}	42.53 ± 1.99^{bB}	54.29 ± 2.86^{bC}	60.98 ± 2.44^{bD}
4	UBN7	31.67 ± 2.86^{bA}	41.18 ± 3.27^{bB}	44.86 ± 5.27^{bC}	53.46 ± 2.86^{bD}
5	UBN11	27.54 ± 10.94^{bA}	43.68 ± 5.27^{bB}	54.29 ± 5.72^{bC}	57.73 ± 2.82^{bD}
6	UBN14	34.78 ± 8.7^{bA}	44.83 ± 3.45^{bB}	56.19 ± 1.65^{bC}	64.22 ± 1.41^{bD}
7	UBN16	23.19 ± 6.64^{bA}	44.83 ± 6.9^{bB}	51.43 ± 2.86^{bC}	58.54 ± 2.44^{bD}

Note: Values represent Mean ± S. D. (n=3). Values sharing the same lowercase letter in the column are not significantly different. Means sharing the same uppercase letter in a row are not significantly different.

Table 5. Statistical variance among the tested UBN isolates

Pairwise Comparisons						
Dependent Variable:	**Inhibition**					
(I) UBN	**(J) UBN**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval for Difference**	
					Lower Bound	**Upper Bound**
UBN2	UBN11	-12.518*	2.110	0.000	-18.787	-6.250
	UBN14	-16.717*	2.110	0.000	-22.986	-10.449
	UBN16	-11.207*	2.110	0.000	-17.475	-4.938
	UBN5	-13.043*	2.110	0.000	-19.312	-6.775
Based on estimated marginal means						
* The mean difference is significant at the .05 level.						

divided isolates into two groups. Low-performing (Group A) included UBN1, and 2 demonstrated 34.27% and 33.28% overall mean inhibition, respectively, while high-performing (Group B) included UBN5, 7, 11, 14, and 16. Among these, UBN14 demonstrated the highest inhibition on 7DAI with 64.22% and showed 50.01% of overall mean inhibition. Simultaneously, the antagonistic activity showed a progressive and significant increase with the duration of incubation, rising from a mean of 26.55% at 4 DAI to a maximum of 55.86% at 7 DAI. As indicated by the uppercase superscripts (A-D) in Table 4, significant differences were observed between all-time points, confirming that inhibition potential improved consistently over time regardless of the isolate tested. Data is depicted in Table 5.

CONCLUSION

The present study evaluated the antagonistic ability of indigenous fungal isolates against the standard known phytopathogen of tomato, *i.e.*, *Fusarium oxysporum* f. sp. *lycopersici*. A primary study on the cultural characteristics of FOL on various media indicated the selection of PDA for the subsequent study of biocontrol activity assessment through dual culture plate assay. From the total isolates, ten

unique isolates were subjected to antagonistic behaviour, which demonstrated that specific indigenous strains possess significant biocontrol activity, effectively inhibiting the radial growth of the procured FOL culture. Molecular analysis subsequently identified the most potent isolate as *Trichoderma asperellum* accession no. PX845989. Among the tested isolates, *Trichoderma asperellum* isolate UBN14 recorded the highest %inhibition, suggesting a vigorous antagonistic ability. These findings suggested that indigenous fungal strains can be tagged as potential biocontrol agents. Future studies will focus on the development of an effective formulation for the practical application of this isolate, establishing it as a sustainable and eco-friendly alternative for managing *Fusarium* wilt in tomato.

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AUTHOR CONTRIBUTION

Udaykumar Bhanushali: Methodology, investigation, formal analysis, data curation, visualisation, writing original draft. Dr. Nikhil S. Bhatt: Conceptualisation, resources, supervision, validation, review and editing, project administration. All authors have read and agreed to the published version of the manuscript.