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Bio-efficacy of microbial antagonists against soil borne pathogens under *in vitro* conditions in tomato

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Abstract
Tomato is one of the important vegetable crops grown worldwide, valued both for its nutritional content and economic significance. However, its productivity is frequently constrained by soilborne pathogens. Therefore, present study aimed to isolate indigenous antagonistic microorganisms from tomato-growing soils of Karnataka and evaluate their efficacy against key soil-borne pathogens. Ten *Trichoderma* isolates were recovered from eight tomato growing areas of Karnataka and investigated for their biocontrol activity against fungal pathogens (*Fusarium oxysporum* f. sp. *lycopersici, Sclerotium rolfsii* and *Rhizoctonia solani*) and the bacterial wilt pathogen (*Ralstonia solanacearum*). Dual culture assays revealed significant inhibition of fungal mycelial growth by all *Trichoderma* isolates, with inhibition ranging from 45–88% *against F. oxysporum*, 48–73% against *S. rolfsii,* and 34–64% against *R. solani.* Among the isolates, UHSBF1 exhibited the highest inhibition of *F. oxysporum* (88.23%) and *S. rolfsii* (73.56%), while UHSCF2 demonstrated maximum suppression of *R. solani* (63.98%). In addition, agar well diffusion test against *R. solanacearum* showed that UHSBF1 produced the largest inhibition zone (22.12 mm), followed by UHSCF2 (19.78 mm) and UHSTF1 (18.48 mm). These findings demonstrate the strong biocontrol potential of indigenous *Trichoderma* isolates, particularly UHSBF1, UHSCF2, and UHSTF1, against multiple soil-borne fungal and bacterial pathogens of tomato. The results highlight the scope for exploiting these isolates in sustainable agriculture to reduce dependence on chemical pesticides and promote sustainable tomato production.

Keywords: Tomato, biocontrol, Trichoderma, soil borne pathogens, per cent inhibition

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops cultivated globally, valued for nutritivity and antioxidants, but also for its substantial economic contribution to agricultural markets (Sehim *et al*., 2023) [1]. But, tomato production is frequently threatened by a plethora of soil-borne pathogens, among which *Fusarium oxysporum* f. sp. *lycopersici*, *Sclerotium rolfsii*, *Rhizoctonia solani*, and the bacterial wilt pathogen *Ralstonia solanacearum* are particularly destructive. These pathogens cause severe diseases such as wilt and root rot, which often result in considerable yield losses and pose a major challenge to tomato cultivation worldwide. Conventional management practices largely depend on the application of synthetic chemical pesticides. While effective to some extent, their continuous use has raised multiple concerns, including the accumulation of toxic residues in food and soil, the emergence of pesticide-resistant pathogen strains, and long-term negative impacts on environmental and human health. Furthermore, host resistance is either absent or limited, as durable resistant tomato varieties against these pathogens are not yet widely available. Given these constraints, there is an urgent need to develop and adopt sustainable, eco-friendly alternatives that not only manage these devastating pathogens effectively but also align with environmentally safe agricultural practices.

Growth inhibition of phytopathogens is achieved through biological control agents, and microbial priming promotes prior activation of host immunity against virulent infection (Singh *et al*., 2020) [2]. Beneficial microorganisms be instrumental in sustainable agriculture, and among them, *Trichoderma* spp. have attracted significant interest as promising biocontrol agents owing to their broad-spectrum antagonistic attribute (Chen *et al*., 2021) [3]. These fungi utilize a range of direct mechanisms such as mycoparasitism, in which they parasitize pathogenic fungi, and antibiosis, where they secrete metabolites that inhibit pathogen growth. They also release hydrolytic enzymes, including chitinases, cellulases, and glucanases, that degrade the cell walls of phytopathogens, thereby suppressing their proliferation. Moreover, *Trichoderma* spp. adopt indirect mechanisms such as competing for nutrients and space, which reduces the chances of pathogen establishment in the rhizosphere. Beyond their role in disease suppression, these fungi are well-documented for their plant growth–promoting attributes, including enhanced nutrient uptake, phytohormone production, and improvement of root architecture. Additionally, they are capable of inducing systemic resistance in host plants, thereby priming the plant immune system for faster and greater defense reaction against pathogens (Husaini *et al*., 2018) [4]. Owing to this multifaceted nature, *Trichoderma* spp. are increasingly recognized as key components of integrated disease management (IDM) programs. In light of this, the present study was done to identify the antimicrobial attribute of newly isolated indigenous *Trichoderma* spp. recovered from tomato rhizosphere soils, with an emphasis on their efficacy against major soil-borne pathogens affecting tomato cultivation.

2. Materials and Methods

a. Collection of soil samples for the isolation of indigenous microorganisms

Eight soil samples, each weighing approximately 20 g, were extracted from six main tomato-growing regions of Karnataka (Plate 1). The selected sites represented diverse tomato cultivation zones, thereby providing variability in soil microrganisms associated with different agro-ecological conditions. All samples were placed in sterile polythene bags and properly labeled. The geographical coordinates and other relevant details of the sampling sites are summarized in Table 1.

Table 1: Geographical details of soil sample collected sites

Soil samples	Location	GPS reading
01	COH, GKVK campus, Bengaluru - 560 065	Latitude- 13.093256º Longitude- 77.566148º
02	Bommachihalli, Hassan Karnataka -573 119	Latitude- 13.145668º Longitude- 76.280767º
03	Hassan, Karnataka -573 162	Latitude- 13.146069º Longitude- 76.251556º
04	Devarahalli, Chikmagalur, Karnataka -577 168	Latitude- 13.342392º Longitude- 75.849347º
05	Arabhavi, Belagavi, Karnataka -577 168	Latitude- 13.339747º Longitude- 75.837083º
06	Tumkur, Karnataka -572102	Latitude- 13.338263º Longitude- 77.101410º
07	Kolar, Karnataka -563102	Latitude- 13.074800º Longitude-78.074800º
08	Bagalkot, Karnataka- 587101	Latitude- 16. 10541200º Longitude- 75.41448836º

Plate 1: Tomato rhizospheric soil samples collected from different places of Karnataka

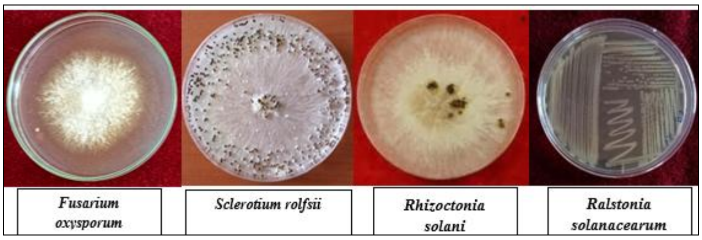
b. Isolation of *Trichoderma* spp. from soil samples

For the isolation of *Trichoderma* spp., soil samples were processed using a five-fold serial dilution technique in sterile distilled water. From each dilution, 1 ml aliquots were aseptically plated onto Trichoderma Selective Medium (TSM). To inhibit bacterial contamination, 0.5 ml of chloramphenicol (5 mg ml^{-1}) was added to the medium prior to inoculation. The inoculated plates were incubated at 28 ± 2 °C under dark conditions, and by the fourth day of incubation, colonies with typical Trichoderma morphology became visible and were selected for further purification.

c. Isolation of soil borne phytopathogens

Fungal pathogens *viz*., *Fusarium oxysporum f. sp. lycopersici*, *Sclerotium rolfsii*, *Rhizoctonia solani* and bacterial pathogen-*Ralstonia solanacearum* are the important soil borne pathogens in the tomato plants used for the bio-efficacy studies. *Ralstonia solanacearum* was isolated from wilted tomato stem tissues on Casamino Acid Peptone Glucose (CPG) agar and incubated at 28 ± 2 °C for 48 h. Colonies appearing large, irregular, smooth, and cream-white were identified as presumptive isolates. Pure cultures were obtained by repeated streaking on agar plates based on colony characteristics (Plate 2).

Fungal isolates were obtained by sub-culturing from the mother culture under aseptic conditions. The inoculated plates were incubated at a temperature range of 25–27 °C for 5–7 days to allow proper growth and establishment of pure cultures. Once purified, the fungal isolates were carefully maintained by storing at 4 °C to ensure their long-term preservation and availability for subsequent experimental studies. (Zehra *et al*., 2017) [6].



d. Antagonistic assay *in vitro*

- **Determination of antagonistic activity of *Trichoderma* isolates against soil borne fungal pathogens**

Actively growing mycelial plugs (5 mm) were excised from the margins of colonies and inoculated onto fresh PDA plates. Each plate received two discs of *Trichoderma* placed opposite to the pathogen disc near the periphery, followed by incubation at ambient temperature for 7 days. After the incubation period, radial growth of the pathogen was measured by the cross-method, and the percentage of growth inhibition was determined.

Percentage inhibition, IR (%) = (C − T)/C × 100

IR: Inhibition rate;
C: Diameter of the fungal colony in the control plates;
T: Diameter of the fungal colony in the presence of the antagonist

- **Determination of antagonistic activity of *Tricoderma* spp. against soil borne bacterial pathogen, *R. solanacerum***

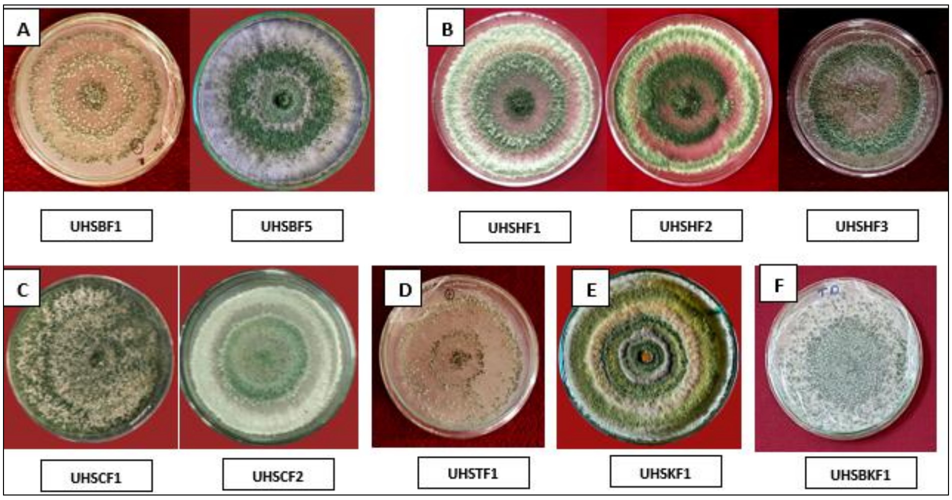
The agar surface was inoculated with the pathogen using the cell-free supernatant to ensure uniform distribution. Wells measuring 6–8 mm in diameter were aseptically prepared with the help of a sterile cork borer, and each well was loaded with 50 µl of the *Trichoderma* culture filtrate. The Plates were then kept at 37 °C to allow observation of inhibition zones.

3. Results and Discussion

Trichoderma spp. demonstrated strong biocontrol potential against soil phytopathogens through direct mechanisms such as mycoparasitism and antibiosis, as well as indirect strategies including nutrient competition, stimulation of plant defense, and promotion of plant growth (Bhardwaj *et al.*, 2017) [7]. In this regard, ten *Trichoderma* isolates naturally associated with tomato rhizosphere soils of different regions of Karnataka were isolated (Plate 3) and evaluated for their antagonistic potential against major soil-borne fungal and bacterial pathogens of tomato.

a. Antagonistic activity against fungal pathogens

All isolates exhibited strong inhibitory effects on the radial growth of fungal pathogens in dual culture, with growth suppression being significant relative to the control (Table 2). However, the degree of inhibition varied among isolates. Per cent mycelial inhibition of *F. oxysporum* by *Trichoderma* isolates ranged from 45.98% (UHSBF5) to 88.23% (UHSBF1). Isolates UHSBF1 and UHSTF1 (82.35%) were most effective, while UHSBF5 was least effective. Similarly, *Trichoderma* spp. exhibited strong mycoparasitic and competitive activities against *F. oxysporum* (Singh *et al.*, 2018; Chen *et al.*, 2021) [8, 3].



A. Bengaluru, Karnataka B. Hassan, Karnataka C. Chikkamagalur, Karnataka
D. Tumkur, Karnataka E. Kolar, Karnataka F. Bagalkot, Karnataka

Plate 3: *Trichoderma* spp. isolated from soil samples from different places of Karnataka

For *S. rolfsii*, maximum inhibition was recorded with UHSBF1 (73.56%), followed by UHSCF2 (69.09%), while UHSBF5 showed the lowest mycelial inhibition (48.98%). The percent reduction in *S. rolfsii* mycelial growth by all the tested *Trichoderma* isolates ranges between 73 and 48%. Studies reported suppression of *S. rolfsii* by *Trichoderma* spp. through secretion of hydrolytic enzymes such as chitinases and glucanases, as well as antifungal metabolites (Sharma *et al.*, 2012) [9]. In *Rhizactonia solani*, inhibition ranged from 33.89% (UHSHF2) to 63.98% (UHSCF2). The *Trichoderma* isolate UHSCF2 was rendered to maximum growth inhibition of *Rhizoctonia solani* (63.98%). These findings are in agreement with reports by Dubey *et al.* (2007), who observed that native *Trichoderma harzianum* isolates suppressed *R. solani* by more than 60% *in vitro*. Similarly, Sharma *et al.* (2012) [9] documented significant inhibition of *R. solani* by *T. viride* through the production of extracellular enzymes and antifungal compounds. Although all isolates suppressed *Rhizactonia* pathogen growth, the efficacy was comparatively lower than that observed against *F. oxysporum* and *S. rolfsii*. This variation may be attributed to differences in pathogen susceptibility and the mode of antagonism exhibited by *Trichoderma* isolates (Kumar *et al.*, 2018) [10].

Table 2: Antagonistic activity of *Trichoderma* spp. against *Fusarium oxysporum, Sclerotium rolfsii* and *Rhizoctonia solani* evaluated by dual culture interaction

Isolates	Per cent inhibition		
	Fusarium oxysporum	*Sclerotium rolfsii*	*Rhizoctonia solani*
UHSBF1	88.23	73.56	52.78
UHSBF5	45.98	48.98	48.67
UHSHF1	59.45	57.45	48.79
UHSHF2	63.25	62.98	33.89
UHSHF3	69.65	63.56	57.09
UHSCF1	72.98	56.65	43.98
UHSCF2	71.09	69.09	63.98
UHSTF1	82.35	57.45	41.26
UHSKF1	71.92	55.36	48.87
UHSBKF1	68.55	61.87	59.78
C.D.	3.53	3.58	3.16
SE(m)	1.23	0.87	0.77

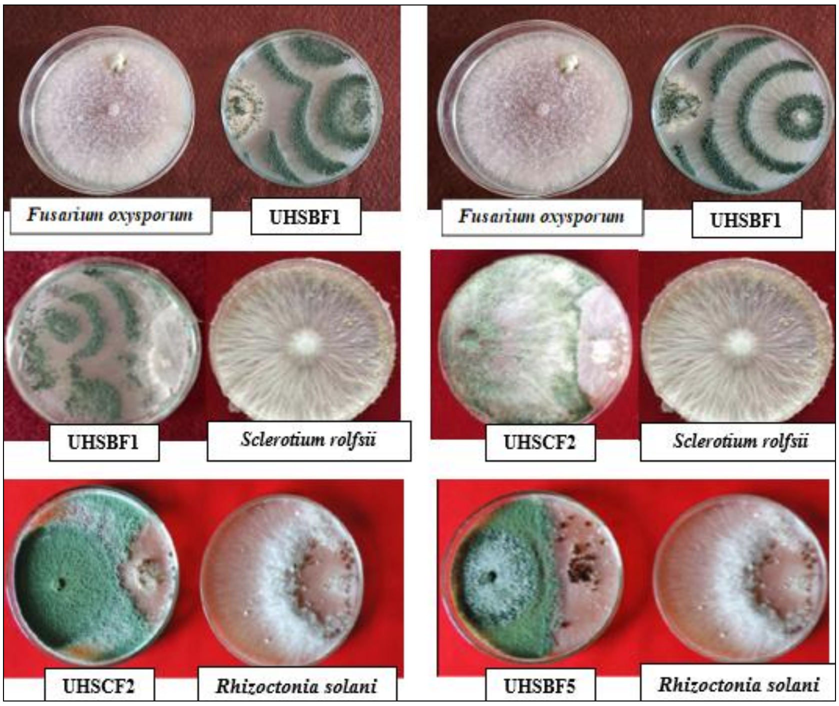


Plate 4: Dual culture assay of *Trichoderma* species against soil borne fungal pathogens

b. Antagonistic activity against *Ralstonia solanacearum*

The antagonistic potential of *Trichoderma* isolates against the bacterial wilt pathogen *R. solanacearum* was assessed through the agar well diffusion method. All isolates exhibited measurable zones of inhibition, though with considerable variation in activity (Table 3). The inhibition zones ranged from 6.73 mm (UHSKF1) to 22.12 mm (UHSBF1). The isolates UHSBF1 (22.12 mm), UHSCF2 (19.78 mm), and UHSTF1 (18.48 mm) recorded significantly higher antibacterial activity compared to the others. The ability of *Trichoderma* spp. to suppress bacterial pathogens has been attributed to the production of antibiotics, volatile organic compounds, and secondary metabolites with antimicrobial properties (Hermosa *et al*., 2012) [11]. The observed inhibition zones indicate extracellular secretion of diffusible compounds, suggesting that metabolites rather than physical interactions are responsible for suppression *in vitro*. These findings are in line with previous studies where *Trichoderma* isolates were shown to inhibit *R. solanacearum* both *in vitro* and in planta. For instance, Singh *et al*. (2014) [12] reported that *T. harzianum* reduced wilt incidence in tomato by producing antibiotics and enhancing host defense responses. The pronounced antagonism observed in UHSBF1 and UHSCF2 suggests that these isolates may produce higher levels of such bioactive compounds.

Table 3: Per cent inhibition of *R. solanacerum* by *Trichoderma* isolates

Isolates	Inhibition zone (mm)
UHSBF1	22.12
UHSBF5	18.76
UHSHF1	8.91
UHSHF2	12.58
UHSHF3	12.98
UHSCF1	6.87
UHSCF2	19.78
UHSTF1	18.48
UHSKF1	6.73
UHSBKF1	11.29
C.D.	1.13
SE(m)	0.27

4. Conclusion

The present study demonstrated that native *Trichoderma* isolates from tomato-growing soils of Karnataka exhibit strong antagonistic activity against major soil-borne pathogens. All isolates inhibited the growth of fungi in dual culture assays, with UHSBF1 and UHSCF2 showing consistently high inhibition levels. In addition to antifungal efficacy, several isolates exhibited antibacterial activity against *R. solanacearum*. Overall, the study highlights the broad-spectrum antagonistic potential of indigenous *Trichoderma* strains, with UHSBF1, UHSCF2, and UHSTF1 emerging as promising antagonistic candidates. Their dual efficacy against both fungal and bacterial pathogens underscores their potential role in integrated disease management (IDM) strategies, reducing dependency on chemical pesticides and promoting sustainable tomato cultivation.

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