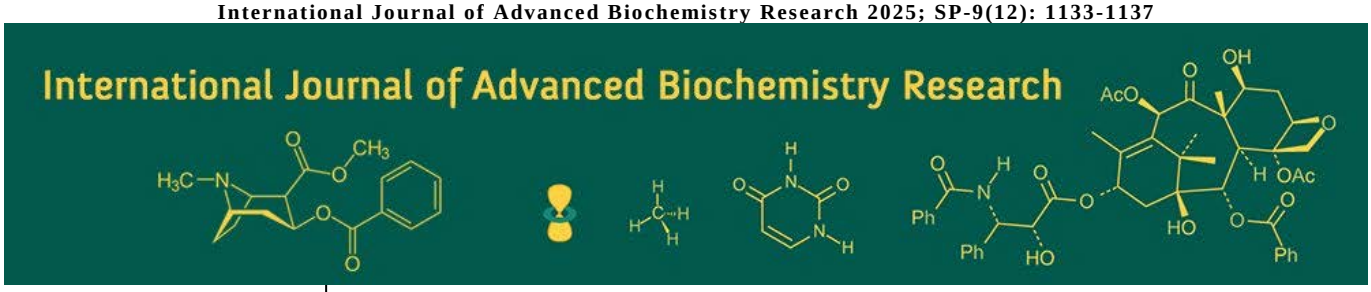

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; SP-9(12): 1133-1137
www.biochemjournal.com
Received: 15-10-2025
Accepted: 10-11-2025

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In vitro antagonistic activity of bioagents against *Fusarium oxysporum* F. sp. *capsici* in Chilli

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DOI: https://www.doi.org/10.33545/26174693.2025.v9.i12Sn.6676

Abstract
Chilli wilt, caused by *Fusarium oxysporum* F. sp. *capsici*, is a major disease that significantly reduces chilli yield and quality. The present study evaluated the *in vitro* antagonistic activity of selected fungal and bacterial bioagents against this pathogen. Seven bioagents, including *Trichoderma viride*, *T. harzianum*, *T. hamatum*, *T. virens*, *Bacillus subtilis* and *Pseudomonas fluorescens*, were tested using dual culture and streak plate techniques on Potato Dextrose Agar (PDA). The experiment was conducted in a Completely Randomized Design (CRD) with four replications at the Department of Plant Pathology, Dr. Sharadchandra Pawar College of Agriculture, Baramati, during the academic year 2023-24.

Results revealed significant variation in antagonistic potential among the bioagents. *Trichoderma harzianum* exhibited the highest mycelial growth inhibition (74.17%) with the lowest colony diameter (23.25 mm), followed by *T. viride* (69.30%), *T. hamatum* (65.13%), and *T. virens* (62.63%). The bacterial bioagents, *Bacillus subtilis* and *Pseudomonas fluorescens*, showed lower efficacy, inhibiting pathogen growth by 40.55% and 36.52%, respectively.

The findings indicate that *T. harzianum* is the most effective bioagent for controlling chilli wilt under laboratory conditions. The study highlights the potential application of selected bioagents as eco-friendly and sustainable alternatives to chemical fungicides for managing *F. oxysporum* F. sp. *capsici* in chilli.

Keywords: Chilli wilt, *Fusarium oxysporum* F. sp. *capsici*, bioagents, *in vitro* antagonism, biological control

1. Introduction

Chilli (*Capsicum annuum* L.) is a highly valued vegetable and spice crop, widely cultivated for its pungency, flavor and nutritional benefits. It belongs to the Solanaceae family and has a chromosome number of 2n=24. Commonly referred to as chilli pepper, hot pepper or red pepper, it has been cultivated since ancient times in the Americas and is considered among the earliest domesticated crops (Chiou *et al*., 2014) [8]. Archaeological studies indicate that chilli peppers existed in Mexico and Peru as early as 7000 BC, with Mesoamerica, particularly Mexico's Tehuacan Valley, identified as the primary center of domestication (Ettenberg, 2019) [13]. The wild ancestor, *C. annuum var. glabriusculum*, also called the "Mother Chilli," bears small fruits that are dispersed naturally by birds, aiding its widespread distribution (Basu & De, 2003) [2]. Chilli was introduced to Europe in 1493 by Columbus and later reached India through Portuguese traders in the seventeenth century (Pandit *et al.*, 2020) [18].

Chilli fruits are rich in essential nutrients, including vitamins C, A and E, which function as antioxidants, strengthen immunity and support vision and skin health. Capsaicin, the compound responsible for the crop's characteristic pungency, exhibits diverse pharmacological properties such as anti-inflammatory, analgesic, antibacterial and anticancer effects. Additionally, capsaicin contributes to the management of obesity, type-2 diabetes, and cardiovascular disorders (Diaz-Laviada, 2010) [12]. The red coloration of the fruit is primarily attributed to capsanthin, while oleoresins extracted from chilli have extensive applications in food, pharmaceutical, and cosmetic industries (Sambamurty & Subrahmanyam, 1989) [22].

Being a day-neutral crop, chilli can be grown throughout the year in tropical and subtropical climates, with optimal growth occurring between 15 and 35 °C.

Among the five cultivated species of *Capsicum*, *C. annuum* dominates Indian agriculture, cultivated both for pungent and non-pungent varieties. Other species like *C. frutescens*, *C. chinense* and *C. baccatum* are grown mainly in home gardens or localized areas (Popelka *et al.,* 2017) [20]. India leads global chilli production, consumption, and export, with Andhra Pradesh, Telangana, Madhya Pradesh and Karnataka being the main producing states (National Horticulture Board, 2021-22).

Chilli cultivation faces serious challenges from biotic stresses, particularly fungal pathogens. Among these, *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *capsici* is one of the most destructive diseases affecting yield and quality. This soil-borne fungus can persist in the soil for several years, infects the root system and colonizes the xylem vessels, leading to water transport disruption, leaf curling, wilting, vascular browning and plant death (Booth, 1971; Di Primo *et al*., 2001; Agrios, 2005) [4, 11, 1]. In India, *Fusarium* wilt can cause up to 80% yield losses and in the United States, disease incidence has been reported up to 35% (Najar *et al.,* 2006; Roberts *et al.,* 2004) [16, 21].

Management of *Fusarium* wilt traditionally relies on chemical fungicides, which, although effective, can be costly and environmentally harmful. Biological control using antagonistic microorganisms provides an eco-friendly alternative. Bioagents can suppress the pathogen through mechanisms such as competition, antibiosis and induction of host resistance. *In vitro* evaluation of these bioagents is essential to identify effective strains that can be incorporated into integrated disease management strategies, offering sustainable solutions for *Fusarium* wilt control in chilli (Joshi *et al.,* 2013; Thoyajakshi *et al.,* 2018) [14, 24].

2. Materials and Methods

2.1 Isolation and Identification of *Fusarium oxysporum* F. sp. *Capsici*

Wilt-affected chilli plants were collected from infected fields located in Malegaon, Baramati. The diseased plants displayed typical wilt symptoms, such as yellowing and desiccation of leaves, along with browning of the vascular tissues. The pathogen was isolated from infected roots and stem portions of chilli using the tissue isolation technique on Potato Dextrose Agar (PDA). After an incubation period of 3-4 days, fungal growth was observed in the Petri plates. A pure culture was obtained through the hyphal-tip technique under aseptic conditions and subsequently preserved on PDA slants in test tubes.

Identification of the pathogen was carried out based on its distinct cultural and morphological attributes. After 7 days of incubation at 27±1 °C, the culture attained maximum growth, showing circular, fluffy mycelial growth with whitish to pinkish colonies. The colony pigmentation varied from pink to brown. Microscopic examinations were performed to study mycelial characteristics, conidial morphology (shape and size), and the presence of chlamydospores. The observed features were compared and confirmed using the standard descriptions of *Fusarium* species given by Booth (1971) [4].

2.2 Pathogenicity of *Fusarium oxysporum* F. sp. *capsici*

Pathogenicity of *Fusarium oxysporum* F. sp. *capsici* was established in accordance with Koch's postulates using the sick soil method. The mass-cultured pathogen was thoroughly mixed into sterilized soil filled in pots, and chilli seedlings of the cultivar Pusa Jwala were transplanted into the infested soil. Observations were recorded 10-12 days after transplanting. The inoculated seedlings exhibited characteristic wilt symptoms, including yellowing of leaves, browning of vascular tissues, basal stem discoloration, and subsequent wilting. In contrast, the seedlings in uninoculated (control) soil remained healthy and symptom-free. The fungus was reisolated from symptomatic plants and re-cultured, and the morphological characteristics of the reisolated culture were identical to those of the original isolate obtained from naturally infected plants. These results confirmed *Fusarium oxysporum* F. sp. *capsici* as the causal organism of chilli wilt, thus fulfilling Koch's postulates.

2.3 Evaluation of biocontrol agents against test pathogen

2.3.1 Antagonistic activity of bioagents against chilli wilt pathogen

The antagonistic potential of different biocontrol agents-*Trichoderma viride*, *T. harzianum*, *T. hamatum*, *T. virens*, *Bacillus subtilis* and *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *capsici* was assessed using the dual culture assay on PDA, following the method described by Dennis and Webster (1971) [10].

2.3.2 Dual Culture Technique

The antagonistic interaction between bioagents and *Fusarium oxysporum* F. sp. *capsici* was evaluated using the dual culture assay. Sterilized molten Potato Dextrose Agar (20 mL) was dispensed into 90 mm Petri plates and allowed to solidify. After solidification, 5 mm mycelial discs of the pathogen and the fungal antagonists were excised from the margins of 7-day-old cultures using a sterile cork borer and placed on opposite sides of the plate simultaneously at equal distance from the center.

In the case of bacterial antagonists, a 5 mm disc of the pathogen was positioned on one side of the PDA plate, while the bacterial bioagent was streaked on the opposite side at the same time. Plates containing only the pathogen, without any antagonist, served as the untreated controls. Each treatment consisted of four replications, and all plates were incubated at 27±2 °C under a completely randomized design.

The percentage inhibition of mycelial growth of the pathogen, relative to the control, was computed using Vincent's formula (1927) [25].

$$I = \frac{C-T}{C} \times 100$$

Where,

I= Per cent Inhibition of fungal growth

C= Colony growth diameter (mm) in control plate

T= Colony growth diameter (mm) in treatment plate

2.3.3 Experimental Design and Statistical Analyses

The experiment was laid out in a Completely Randomized Design (CRD) comprising seven treatments with four replications each. The dual culture technique described by Dennis and Webster (1971) [10] was employed for evaluating the antagonists. The recorded data were subjected to statistical analysis following the procedures outlined by Panse and Sukhatme (1967) [19]. Standard error (SE) and Critical Difference (CD) at the 1% probability level

(P=0.01) were calculated to determine the significance of differences among treatment means.

3. Results and Discussion

3.1 Antifungal Efficacy of bioagents against *Fusarium oxysporum* F. sp. *Capsici*

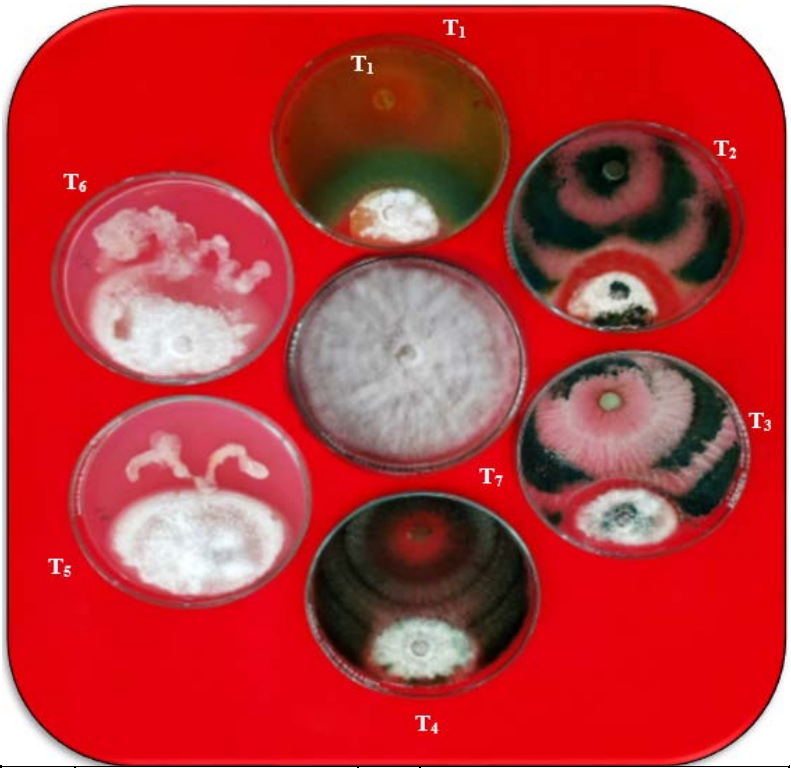
The antagonistic potential of seven bioagents comprising fungal species (*Trichoderma* spp.) and bacterial species (*Bacillus subtilis* and *Pseudomonas fluorescens*) was assessed against *Fusarium oxysporum* F. sp. *capsici*. The evaluation was performed using the dual culture and streak plate techniques on PDA as the basal medium. The experimental outcomes are presented in Table 1, Figure 1, and Plate 1. A marked variation in the antagonistic response of the bioagents was recorded. Among all treatments, *Trichoderma harzianum* proved to be the most effective and produced the smallest pathogen colony diameter (23.25 mm), corresponding to the highest mycelial growth inhibition of 74.17%. The next best performer was *Trichoderma viride*, which exhibited a colony diameter of 27.63 mm and 69.30% growth inhibition. *Trichoderma hamatum* and *T. virens* showed moderate suppression of the pathogen, with colony diameters of 31.38 mm and 33.68 mm and inhibition rates of 65.13% and 62.63%, respectively. In contrast, the bacterial antagonists were comparatively less effective; *B. subtilis* and *P. fluorescens* recorded colony diameters of 53.50 mm and 57.13 mm and inhibition percentages of 40.55% and 36.52%, respectively.

These results are consistent with earlier findings. Singh *et al*. (2018) reported that *T. harzianum* showed maximum inhibition of *F. oxysporum* (75.7%), followed by *T. viride* under *in vitro* conditions. Dar *et al.* (2013) also observed similar trends, with *T. harzianum* and *T. viride* recording growth inhibition of 92.5% and 86.2%, respectively. Comparable reports by Chakraborty and Chatterjee (2008) [5], Bhat *et al.* (2016) [3], Mishra *et al.* (2017) [15], Cherkupally *et al*. (2017) [7], and Coral *et al*. (2017) [6] further confirmed that *T. harzianum* consistently displayed the greatest antagonistic effect against the wilt pathogen through its mycoparasitic ability.

Table 1 Efficacy of various bioagents against *F. oxysporum* F. sp. *capsici* under *in vitro* conditions

Tr. No.	Treatments	Colony Diameter of the Pathogen* (mm)	% Growth Inhibition
T_1	*Trichoderma viride*	27.63	69.30
T_2	*Trichoderma harzianum*	23.25	74.17
T_3	*Trichoderma hamatum*	31.38	65.13
T_4	*Trichoderma virens*	33.63	62.63
T_5	*Bacillus subtilis*	53.50	40.55
T_6	*Pseudomonas fluorescens*	57.13	36.52
T_7	Control	90.00	
	S.E.(m)±	0.42	
	C.D. (0.01)	1.66	

*Mean of four replications



T_1	*Trichoderma viride*	T_5	*Bacillus subtilis*
T_2	*Trichoderma harzianum*	T_6	*Pseudomonas fluorescens*
T_3	*Trichoderma hamatum*	T_7	Control
T_4	*Trichoderma virens*		

Plate 1: Efficacy of various bioagents against *Fusarium oxysporum* F. sp. *capsici*

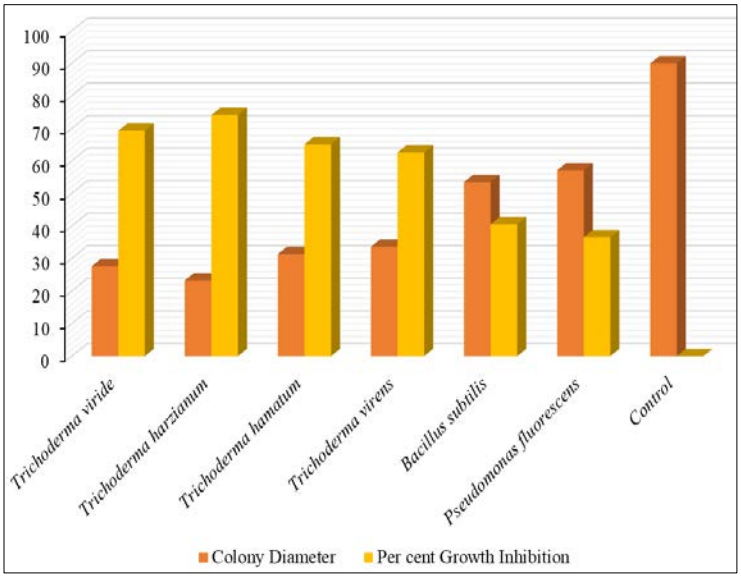


Fig 1: Efficacy of various bioagents against *Fusarium oxysporum* F. sp. *capsici*

4. Conclusion

Under *in vitro* conditions, all the bioagents evaluated exhibited notable antagonistic effects against *Fusarium oxysporum* F. sp. *capsici*. Among them, *Trichoderma harzianum* proved to be the most effective, recording the highest mycelial growth inhibition (74.17%) with the minimum colony diameter (23.25 mm). This was closely followed by *T. viride*, *T. hamatum* and *T. virens*, which also displayed substantial suppressive activity against the pathogen. In contrast, the bacterial bioagents, *Bacillus subtilis* and *Pseudomonas fluorescens*, were comparatively less effective, inhibiting only 40.55% and 36.52% of pathogen growth, respectively. Overall, the results indicate that *T. harzianum* holds the greatest potential for the biological management of chilli wilt under laboratory conditions. The results emphasize the potential use of compatible biocontrol agents as an eco-friendly and sustainable alternative to chemical control strategies for managing wilt disease in chilli.

5. Acknowledgments

I express my sincere gratitude to Dr. Sharadchandra Pawar College of Agriculture, Baramati, for providing the necessary facilities and support that facilitated the successful completion of this research.

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