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Bioefficacy of bioagents against *Fusarium oxysporum* f. sp. *capsici*

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Abstract
Chilli (*Capsicum annum* L.) is associated with various diseases, which can lead to substantial yield reductions. Timely detection of causal pathogens is essential for the implementation of effective management strategies, thereby preventing epidemic development and minimizing losses. accordingly, the present investigation designed to evaluate the performance of selected bio-agents against *Fusarium oxysporum* f. sp. *capsici*. *In-vitro* evaluation of four biocontrol agents *Trichoderma asperellum*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* against *F. oxysporum* f. sp. *capsici* were assessed in dual culture technique. The highest growth inhibition percentage was also recorded in *Trichoderma harzianum* (82.08%), followed by *Trichoderma asperellum* (80.63%), *Pseudomonas fluorescens* (76.01%) and *Bacillus subtilis* (73.12%).

Keywords: *Fusarium*, *In-vitro*, bioagents, chilli, *Trichoderma spp*.

1. Introduction
Chilli (*Capsicum annuum* L.), a member of the *Solanaceae* family, holds significant agronomic and economic importance in India, serving both as a vegetable and a spice crop. It is among the earliest domesticated and cultivated crops and is predominantly grown in tropical and subtropical regions for its pungent fruits, which are consumed in both green and mature forms. India has established a prominent position in the global chilli market as the leading producer, consumer and exporter. Globally, India ranks first in chilli production, followed by China, Thailand, Indonesia and Ethiopia. Chilli contributes approximately 42% of India's total spice export volume, with major export destinations including China, Sri Lanka, Malaysia, Bangladesh, Singapore, Thailand, and the United Arab Emirates.
During the 2023-24 period, chilli was cultivated on 8.09 lakh hectares in India, yielding an estimated 29.13 lakh tonnes, with an average productivity of 3273 kg/ha. Andhra Pradesh is the leading state in chilli production, accounting for 14.44 lakh tonnes (49.57%) from 2.47 lakh hectares (27.75%), followed by Telangana, Madhya Pradesh, Karnataka and West Bengal.
However, chilli cultivation is adversely affected by a range of phytopathogens, including fungal, bacterial viral and nematode agents at various growth stages. Among these, *F. oxysporum* f. sp. *capsici* has emerged as a significant constraint across chilli growing regions in India, with yield losses ranging from 10% to 50% (Bai *et al.*, 2018) [2]. The disease manifests at both seedling and later growth stages, with peak mortality observed during flowering and fruiting, often resulting in total crop failure. Typical symptoms include vascular browning, upward and inward curling of the upper leaves and eventual wilting of the entire plant (Priya and Mesta, 2018) [2]. Initially localized, the disease can spread throughout the field under monoculture conditions. The pathogen's broad host range and capacity to form long-lasting chlamydospores enable its survival in soil for extended periods. Furthermore, dry weather conditions combined with excessive soil moisture have been found to facilitate disease proliferation (Khan *et al.*, 2018) [3].

2. Materials and Methods
The present study entitled "Bioefficacy of bioagents against *Fusarium oxysporum* f. sp.

capsici" was conducted in Plant Pathology Laboratory, College of Agriculture, Nagpur during the year 2024-2025.

2.1 Isolation of *Fusarium oxysporum* f. sp. *capsici*

Plant samples exhibiting characteristic symptoms of *Fusarium* wilt were collected for pathogen isolation. The standard tissue isolation method was employed to recover the causal organism. Infected tissues, including adjoining healthy sections, were surface sterilized using a 0.1% aqueous solution of sodium hypochlorite (NaOCl) for two minutes. These tissue segments were then rinsed sequentially three times in sterile distilled water to remove residual disinfectant. Subsequently, the segments were aseptically transferred to Petri dishes and incubated at 28 ± 1 °C for seven days in a BOD incubator.

After the incubation period, uncontaminated and well-developed mycelial growth was observed. The fungus was then isolated using the single hyphal tip technique and aseptically transferred to potato dextrose agar (PDA) slants in sterile test tubes. The isolate was further purified through repeated sub-culturing and a pure culture was maintained on PDA slants under refrigerated conditions for subsequent experimentation.

2.2 Evaluation of antagonistic potential of bioagents

The antagonistic activity of *Trichoderma asperellum*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* against *Fusarium oxysporum* f. sp. *capsici* was assessed using the dual culture technique. Sterile Petri plates were prepared by pouring 15 ml of autoclaved potato dextrose agar (PDA) medium and allowing it to solidify under aseptic conditions. Seven-day-old actively growing cultures of both the pathogen and each bioagent were employed in the assay. Agar discs, 5 mm in diameter, were aseptically excised from the advancing margins of the fungal and bacterial cultures using a sterile cork borer.

One disc of the pathogen and one of the respective antagonist were placed on opposite sides of each PDA plate, equidistant from the center. Control plates, inoculated only with the pathogen, were maintained under identical conditions. All plates were incubated at 27 ± 2 °C for a period of seven days. Each treatment was replicated four times. The antagonistic effect was evaluated by comparing the radial growth of the pathogen in the dual culture plates with that in the control plates.

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

Where,

I = Percent inhibition

C = Mycelial growth of *F. oxysporum f. sp. capsici* in control (mm)

T = Mycelial growth of *F. oxysporum f. sp. capsici* in treatment (mm)

2.3 Details of experiment

Design: CRD (Completely Randomized Design)

Treatments: 5

Replications: 4

Details of treatment

Treatment No.	Treatment details
T_1	*Trichoderma asperellum*
T_2	*Trichoderma harzianum*
T_3	*Pseudomonas fluorescens*
T_4	*Bacillus subtilis*
T_5	Control

3. Results

The *in-vitro* efficacy of four bioagents *viz.*, *Trichoderma asperellum*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were assessed against *Fusarium oxysporum* f. sp. *capsici* using the dual culture technique. The results, summarized in Table 1, revealed that all tested antagonists significantly inhibited the radial growth of the pathogen.

Among the treatments, *Trichoderma harzianum* demonstrated the highest antagonistic effect, restricting the mycelial growth of *F. oxysporum* f. sp. *capsici* to 15.50 mm. This was followed by *Trichoderma asperellum* (16.75 mm), *Pseudomonas fluorescens* (20.75 mm) and *Bacillus subtilis* (23.25 mm). In terms of percentage growth inhibition, *T. harzianum* recorded the maximum reduction (82.08%), followed by *T. asperellum* (80.63%), *P. fluorescens* (76.01%) and *B. subtilis* (73.12%).

These findings align with those of Rini and Sulochana (2007) [11], who reported the superior efficacy of *Trichoderma harzianum* over *Pseudomonas fluorescens* in suppressing *Fusarium oxysporum* f. sp. *capsici*. Additionally, the antagonistic efficacy of *Trichoderma spp.* against various *Fusarium* species has been consistently reported in previous studies, including those by Wani *et al.* (2014) [5] and Srideepthi and Krishna (2015) [4].

Table 1: *in vitro* efficacy of bio-agents against *F. oxysporum* f. sp. *capsici* on 3rd DAI, 5th DAI and 7th DAI.

Treatment		*Fusarium oxysporum* f. sp. *capsici*					
		Mean Colony diameter (mm)			Per cent growth inhibition		
		3 DAI	5 DAI	7 DAI	3 DAI	5 DAI	7 DAI
T_1	*Trichoderma asperellum*	11.25	14.25	16.75	67.62	76.05	80.63
T_2	*Trichoderma harzianum*	10.50	13.50	15.50	69.78	77.31	82.08
T_3	*Pseudomonas fluorescens*	16.25	20.00	20.75	53.23	66.38	76.01
T_4	*Bacillus subtilis*	19.25	22.50	23.25	44.60	62.18	73.12
T_5	Control	34.75	59.50	86.50	-	-	-
	F test	Sig	Sig	Sig			
	S.E (±m)	0.40	0.60	0.63			
	C.D (P = 0.01)	1.70	2.50	2.64			

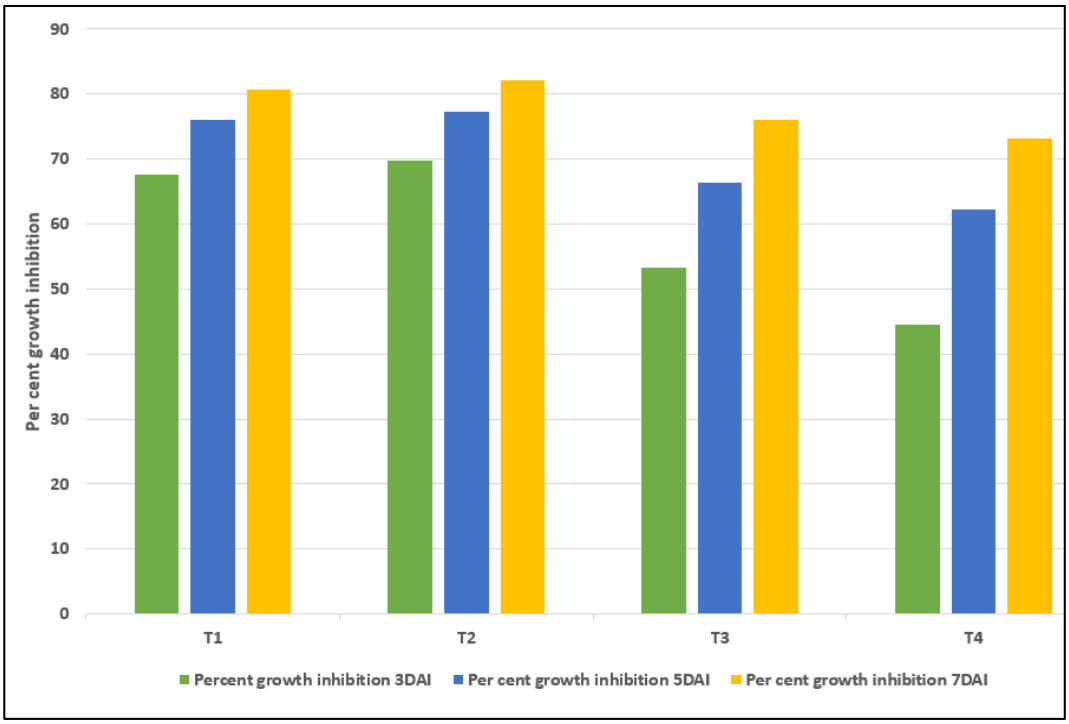

Fig 1: Effect of bio-agents on percent growth inhibition of *F. oxysporum* f. sp. *capsici*

4. Conclusion

The *in-vitro* assessment of four biocontrol agents *Trichoderma asperellum*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* against *F. oxysporum* f. sp. *capsici* using the dual culture method demonstrated significant antagonistic activity by all tested bioagents. Among them, *Trichoderma harzianum* proved to be the most effective, achieving the highest mycelial growth inhibition of 82.08%. These findings highlight the potential of *T. harzianum* as a promising antagonist for the biological management of *Fusarium* wilt in chilli cultivation.

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