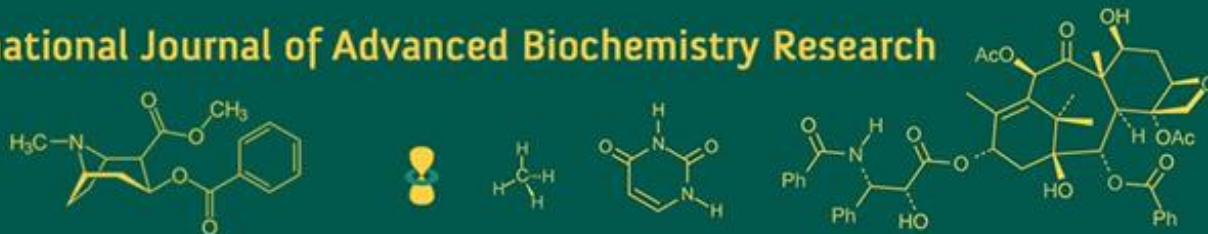


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; 10(7): 1010-1014
www.biochemjournal.com
 Received: 25-04-2026
 Accepted: 29-05-2026

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Eco-Friendly Management of Chickpea Wilt: *In vitro* Evaluation of Bioagents and Botanicals against *Fusarium oxysporum* f. sp. ciceri

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i7m.9302>

Abstract

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. ciceri, is one of the most serious constraints to sustainable chickpea production worldwide. It is an ubiquitous pathogen both soil- and seed-borne, ranks 5th among the 10 most economically important fungal phytopathogens. The pathogen invades the root system and vascular tissues, disrupting water transport and causing progressive wilting, yellowing, and ultimately plant death. The disease is difficult to manage due to its ability to survive in soil for several years. Biological control agents and plant-derived botanicals offer the potential to suppress FOC while minimizing adverse effects on agroecosystems. The present study investigated the *in vitro* antifungal activity of five bioagents and six botanical extracts against FOC isolated from naturally infected chickpea plants. Among the tested bioagents, *Chaetomium globosum* exhibited the highest inhibition of mycelial growth (83.33%), followed by *Pseudomonas fluorescens* (78.89%) and *Trichoderma harzianum* (77.78%). Among the botanicals, garlic clove extract at 10% concentration was the most effective, producing 82.22% inhibition, while ajwain seed and neem leaf extracts also demonstrated appreciable antifungal activity.

Keywords: Chickpea, *Fusarium oxysporum* f. sp. ciceri, sustainable agriculture, biological control, botanicals, aqueous plant extracts, organic disease management

Introduction

Chickpea (*Cicer arietinum* L.) is one of the world's most important pulse crops, serving as a major source of plant protein, dietary fibre, and essential micronutrients for millions of people. In addition to its nutritional significance, chickpea contributes to sustainable agriculture through biological nitrogen fixation, improving soil fertility and reducing dependence on synthetic fertilizers (Zhang *et al.*, 2023) [1]. These characteristics make the crop particularly valuable in low-input farming systems where maintaining soil health and environmental quality is increasingly recognized as a priority.

Despite its agronomic importance, chickpea production is severely constrained by *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. ciceri (FOC). It is an ubiquitous pathogen both soil- and seed-borne, ranks 5th among the 10 most economically important fungal phytopathogens (Sharma *et al.*, 2016) [2]. The pathogen invades the root system and vascular tissues, disrupting water transport and causing progressive wilting, yellowing, and ultimately plant death. Its ability to survive in soil for several years through resilient chlamydospores makes the disease difficult to manage (Chaudhary *et al.*, 2025) [3]. Consequently, the disease causes substantial yield losses in many chickpea-growing regions and poses a persistent challenge to sustainable crop production. Sustainable agriculture emphasizes maintaining crop productivity while preserving ecological integrity. Within this framework, biological control agents have emerged promising because they suppress pathogens through naturally occurring mechanisms such as competition, antibiosis, mycoparasitism, and the induction of plant defense responses. Organisms including *Trichoderma* spp., *Chaetomium globosum*, *Pseudomonas fluorescens*, and *Bacillus subtilis* have demonstrated considerable potential, many of these microorganisms also improve rhizosphere health and support plant growth (Singh *et al.*, 2016) [4], making them attractive components of integrated crop management rather than simple replacements for chemical pesticides.

Similarly, botanical extracts have gained renewed interest as biodegradable and renewable sources of antimicrobial compounds. Many medicinal and aromatic plants synthesize secondary metabolites capable of inhibiting fungal growth and possess no environmental risks.

(Shukla and Dwivedi, 2012) [5] Extracts derived from garlic, neem, tulsi, ajwain, bael, and other plants have demonstrated antifungal activity against diverse phytopathogens (Cowan, 1999; Khanna *et al.*, 2024) [6, 7].

Against this background, the present study evaluated the antagonistic potential of selected microbial bioagents and botanical extracts against *FOC* under controlled laboratory conditions. The study seeks to contribute to the development of environmentally sustainable and integrated disease management strategies. The approach aligns with the broader goal of reducing dependence on synthetic fungicides while supporting productive, resilient, and ecologically balanced chickpea production systems.

Materials and Methods

Isolation and purification of the pathogen

Diseased plants with typical wilt symptoms were taken for isolation. Sample were washed thoroughly in distilled water to remove adhering dirt particles and surface contaminants and thereafter, placed in between blotting paper to remove excess moisture, if any. The infected parts were split open into half and aseptically cut into small pieces. Surface sterilized with Sodium hypochlorite (1%) solution for 30 seconds. Later on, these pieces were taken out from solution and subsequently washed three-time with sterilized distilled water to remove the traces of Sodium hypochlorite solution, if any. To remove the excess moisture the cut tissues were pressed between two folds of sterilized blotting paper under aseptic condition. Prepared sterilized cut pieces were placed at equal distance in petri dishes containing potato dextrose agar medium with the help of sterilized forceps under aseptic conditions in laminar air flow. The inoculated petri plates were incubated at 26±1 °C for five days in the B.O.D. Incubator. The petri dishes were observed regularly for mycelial growth of the fungus. Purification of the fungus was done by taking freshly grown mycelial tips. Sub culture was done on petri plates and subsequently, pathogen was transferred into culture tubes to keep it viable for further use.

In vitro evaluation of bio control agent against FOC

The effect of five bio-agents were tested for their efficacy against *FOC* in three replications in completely randomized design under *in vitro* condition in Post graduate laboratory, Department of Plant Pathology, SVPUAT, Meerut. The bio-agents were isolated from soil using serial dilution method. Chemicals were procured from store room, in the Department of Plant Pathology of S.V.P University of Agriculture & Technology Meerut (U.P.). For culture multiplication PDA was used. Dual culture techniques as given by Morton and Stroube (1955) was used for testing antagonistic activities of bio-agents against *FOC* on PDA.

Dual culture technique

A mycelial disc of 5 mm diameter, obtained from the peripheral region of 5 to 7-day old culture of *FOC* on PDA, was placed on fresh PDA plate (3 cm from the center) and incubated at 28 °C for 48 hr to initiate growth. Then a mycelial disc (5 mm), obtained from the periphery of a 5 to 7-day old culture of *Trichoderma viride*, *Trichoderma harzianum*, *Chaetomium globosum*, *Bacillus subtilis* and *Pseudomonas fluorescense*, was placed 3 cm away from the inoculum of the pathogen, the plates were incubated at 26±1 °C, the measurements were taken

after 5 days. In control experiment a sterile agar disc (5 mm) was placed in the dish instead of bio-agents. At the end of incubation period, radial growth was measured. Radial growth reduction was calculated in relation to growth of the control as follows:

Percent inhibition over control was worked out using the formula given by Vincent (1947).

$$\% \text{ Inhibition of mycelial growth} = \frac{C - T}{C} \times 100$$

Where,

C = Growth of pathogen mycelium in control

T = Growth of pathogen mycelium in treatment

Table 1: List of bio agents used *in vitro* against FOC

S. No.	Treatments	Bio agents
1	T1	<i>Pseudomonas fluorescense</i>
2	T2	<i>Bacillus subtilis</i>
3	T3	<i>Trichoderma harzianum</i>
4	T4	<i>Trichoderma viride</i>
5	T5	<i>Chaetomium globosum</i>
6	T6	Control

Observation recorded

- Radial growth of the pathogen.
- Percentage (%) growth inhibition.

In vitro screening of different botanicals against FOC

6 different plants were selected for the study. The study was conducted in three replications in completely randomized design *in vitro* in the Department of Plant Pathology, SVPUAT, Meerut. Mature leaves were collected from at least five different individual plants from campus of SVPUAT, Meerut. The leaves (200g) were properly washed with distilled water to remove any dirt adhering to it followed by surface sterilization of leaves in 0.1% sodium hypochlorite solution and washing thrice with distilled water. Then the plant part along with equal amount of water were macerated in sterilized mortar and pestel. The macerated extract was filtered through three-folded muslin cloth to remove fibrous and suspended material, this extract was subjected to centrifugation at 12,000 rpm for 10 minutes. The pellets were discarded and the required amount of aqueous extract were mixed with sterilized PDA. 20 ml of poisoned media was poured into 90 mm sterilized petri plates and allowed to solidify in aseptic condition. All plates were inoculated with actively growing 5 mm mycelial disc of *FOC*. Three replications were maintained for each treatment. These plates were incubated at

26±1 °C for five days in a B.O.D. Incubator and colony diameter was recorded. Percent inhibition of mycelial growth over control was calculated by using the formula given by Vincent (1947).

$$\% \text{ Inhibition of mycelial growth} = \frac{C - T}{C} \times 100$$

Where,

C = Growth of mycelium in control

T = Growth of mycelium in treatment

Table 2: Details of botanicals

S. No	Common Name	Scientific Name	Family	Plant part used	Major antifungal compounds	Concentration
1	Ajwain	Trachyspermum ammi (L.) Sprague	Apiaceae	seed	Thymol (major), γ -terpinene, p-cymene	5%, 10%
2	Garlic	Allium sativum L.	Amaryllidaceae	clove	Allicin (major), ajoene, diallyl sulfides	5%, 10%
3	Neem	Azadirachta indica A. Juss.	Meliaceae	Leaf	Azadirachtin, nimbin, salannin, gedunin, nimbolide	5%, 10%
4	Tulsi	Ocimum tenuiflorum L.	Lamiaceae	Leaf	Eugenol (major), methyl eugenol, linalool, ursolic acid	5%, 10%
5	Bael	marmelos (L.) Corrêa	Rutaceae	Leaf	Marmelosin, aegeline, skimmianine, tannins	5%, 10%
6	Bhang	Cannabis sativa L.	Cannabaceae	leaf	Cannabidiol (CBD), cannabigerol (CBG), terpenes (β -caryophyllene, limonene), flavonoids	5%, 10%

Observation recorded

- Radial growth of the pathogen.
- Percentage (%) growth inhibition.

Result and Discussion**Isolation, purification and identification of FOC**

Infected chickpea plants showing typical wilt symptoms were brought to the laboratory and isolation was done from the collar region on potato dextrose agar (PDA) medium. FOC was found to be associated with plant / root of the collected samples from Crop Research Centre and farmers' fields. The isolated fungus was identified on the basis of cultural and morphological characters with the help of microscope at 10x and 40x magnification. The isolated fungus produced pinkish-white fluffy colony with distinct margin on PDA showing moderate aerial mycelial growth. The pinkish white mycelium was

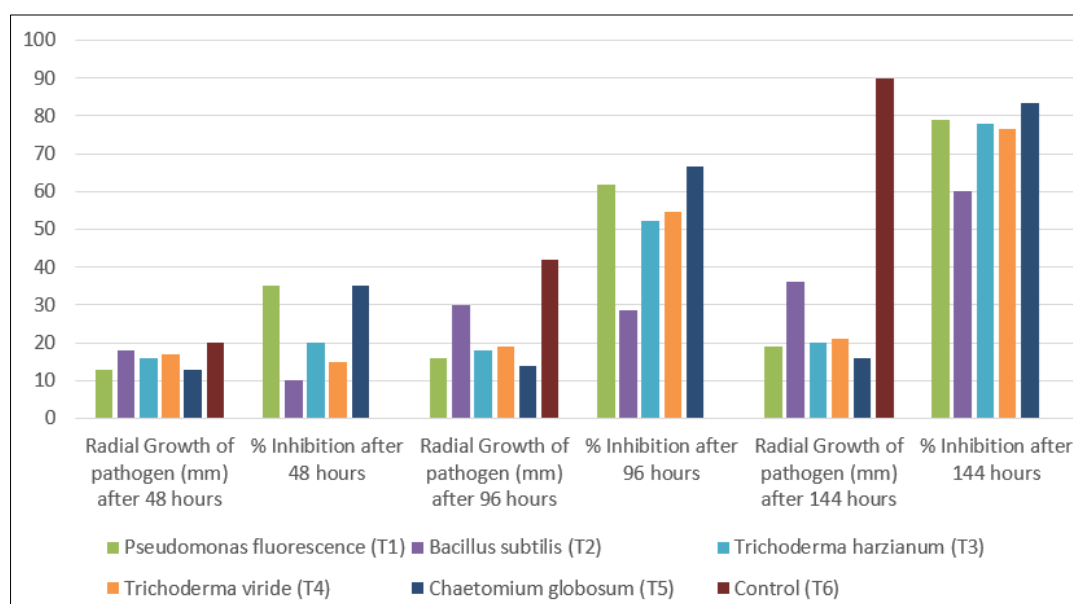
distinctly visible with clear colony margin on turning the petri plate to bottom side. The colony diameter ranged from 75 to 90 mm on PDA at 7 DAI at $26 \pm 1^\circ\text{C}$ in 90 mm petri plate. The fungus produces three types of spores viz., microconidia, macroconidia and chlamydospore.

In vitro effect of biocontrol agents on the growth of FOC

Biocontrol agents are the nature's way of controlling the pathogen by antagonism. 5 antagonists were evaluated for their efficacy against FOC through dual culture technique as explained in materials and methods. All five bioagents reduced the growth of FOC significantly as compared to control. *Chaetomium globosum* (83.33%) followed by *Pseudomonas fluorescens* (78.89%) and *Trichoderma harzianum* (76.67%) showed maximum inhibition.

Table 3: Effect of different bioagents on the radial growth of FOC *in vitro*

Treatments	Inhibition after 48 hours		Inhibition after 96 hours		Inhibition after 144 hours	
	Radial Growth of pathogen (mm)	% Inhibition	Radial Growth of pathogen (mm)	% Inhibition	Radial Growth of pathogen (mm)	% Inhibition
<i>Pseudomonas fluorescens</i> (T1)	13.00	35	16.00	61.90	19.00	78.89
<i>Bacillus subtilis</i> (T2)	18.00	10	30.00	28.57	36.00	60.00
<i>Trichoderma harzianum</i> (T3)	16.00	20	18.00	52.38	20.00	77.78
<i>Trichoderma viride</i> (T4)	17.00	15	19.00	54.76	21.00	76.67
<i>Chaetomium globosum</i> (T5)	13	35	14.00	66.67	16.00	83.33
Control (T9)	20.00		42.00		90.00	
SE(m)	1.000		0.092		0.833	
CD	1.779		1.669		1.624	

**Fig 1:** Effect of biocontrol agents on the growth of FOC

Effect of different biocontrol agents against FOC

The inhibition of mycelial growth of pathogen by bio-agents was recorded after 48, 96, and 144 hrs. The result revealed that there was significant mycelial growth inhibition of *FOC* by all the tested biocontrol agents. The maximum mycelial growth inhibition was recorded by *Chaetomium globosum* (83.33%) which was significantly superior to all the tested bioagents, followed by and *Pseudomonas fluorescens* (78.89%) and *Trichoderma harzianum* (77.78%).

The minimum mycelial growth inhibition was recorded by *Bacillus subtilis* (60.00%) followed by *Trichoderma viride* (76.67%) at 144 hours.

In vitro screening of different botanicals against FOC

An *in vitro* experiment was conducted to find out efficacy of 6

different botanical extract at two different concentrations *i.e.*, 5% and 10% concentration. Aqueous extract of following plants- ajwain seeds, garlic clove, neem leaf, tulsi leaf, bael leaf, bharg leaf was prepared as mentioned in materials and methods and their efficacy was evaluated. Garlic clove extract was found to be most efficient against *FOC*. 82.22%, 75.56% growth inhibition was recorded by garlic clove extract at 10% and 5% w/v concentration respectively followed by 10% Ajwain seed extract (67.78%) and 10% neem leaf extract (62.22%). The results revealed that there is significant difference in percent inhibition of mycelial growth of *FOC* with tested botanicals. Result in tabulated form have been presented in Table 4 and Fig. 2 Bael leaf extract was found to be least effective with 46.67% and 51.11% inhibition of mycelial growth at both 5% and 10% concentrations respectively.

Table 4: Effect of botanicals on mycelial growth of *FOC* @ 5% and 10% *in vitro*

Treatments	Inhibition after 48 hours		Inhibition after 96 hours		Inhibition after 144 hours	
	Colony diameter (mm)	% Inhibition	Colony diameter (mm)	% Inhibition	Colony diameter (mm)	% Inhibition
T ₁ - Ajwain seed extract @ 5% concentration	18	40.00	22	51.11	42	53.33
T ₂ - Ajwain seed extract @ 10% concentration	16	46.67	20	55.56	29	67.78
T ₃ - Garlic clove extract @ 5% concentration	13	56.67	17	62.22	22	75.56
T ₄ - Garlic clove extract @ 10% concentration	11	63.33	14	68.89	16	82.22
T ₅ - Neem leaf extract @ 5% concentration	20	33.33	26	42.22	36	60.00
T ₆ - Neem leaf extract @ 10% concentration	17	43.33	24	46.67	34	62.22
T ₇ - Tulsi leaf extract @ 5% concentration	22	26.67	32	28.89	40	55.56
T ₈ - Tulsi leaf extract @ 10% concentration	20	33.33	30	33.33	37	58.89
T ₉ - Bael leaf extract @ 5% concentration	24	20.00	30	33.33	48	46.67
T ₁₀ - Bael leaf extract @ 10% concentration	22	26.67	36	20.00	44	51.11
T ₁₁ - Bharg leaf extract @ 5% concentration	20	33.33	32	28.89	40	55.56
T ₁₂ - Bharg leaf extract @ 10% concentration	18	40.00	23	48.89	38	57.78
T ₁₃ - Control	30		45		90	
SE(m)	0.923		0.899		0.900	
CD (5%)	1.613		1.573		1.598	

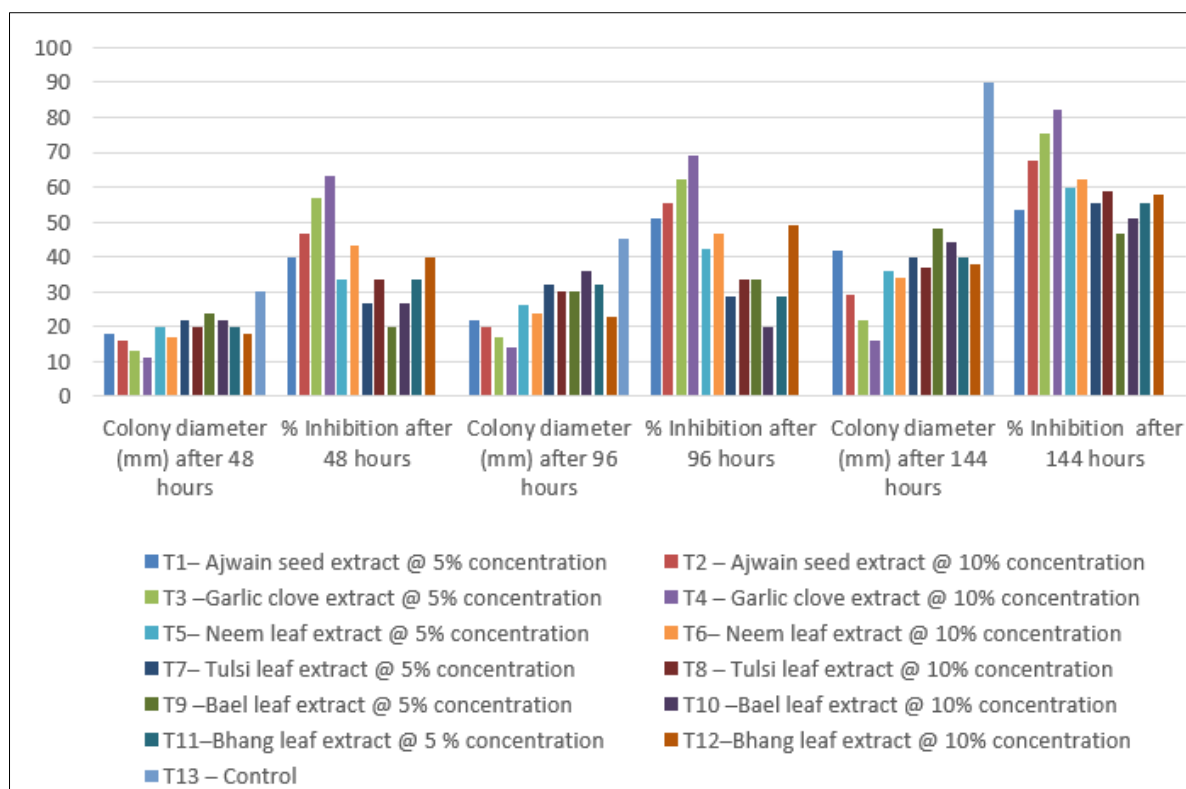


Fig 2: Effect of different botanicals on mycelial growth of *FOC* @ 5% and 10% *in vitro*

Conclusion

The result revealed that there was significant mycelia growth inhibition of FOC by all the tested bio-agents. The maximum mycelial growth inhibition was recorded by *Chaetomium globosum* (83.33%) which was superior to all the tested bioagents, followed by *Pseudomonas fluorescens* (78.89%) and *Trichoderma harzianum* (77.78%). The minimum mycelial growth inhibition was recorded by *Bacillus subtilis* (60.00%) followed by *Trichoderma viride* (76.67%) at 144 hours.

Garlic clove extract found to be most efficient against FOC 82.22%, 75.56% growth inhibition was recorded by at 10% and 5% w/w concentration respectively followed by 10% Ajwain seed extract (67.78%) and 10% neem leaf extract (62.22%). The results revealed that there is significant difference in per cent inhibition of mycelial growth of FOC with tested plant extracts. Minimum inhibition was found in bael leaf extract at 46.67% and 51.11% at both 5% and 10% concentrations respectively.

Although all treatments significantly reduced fungal growth compared to control, differences in their effectiveness highlighted the importance of selecting suitable biological inputs for disease management. These findings suggest that selected microbial antagonists and botanical extracts possess considerable potential as environmentally compatible tools for suppressing Fusarium wilt. While laboratory results cannot be directly extrapolated to field conditions, they provide a strong foundation for developing sustainable, integrated disease management strategies that reduce reliance on synthetic fungicides and promote resilient chickpea production systems.

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