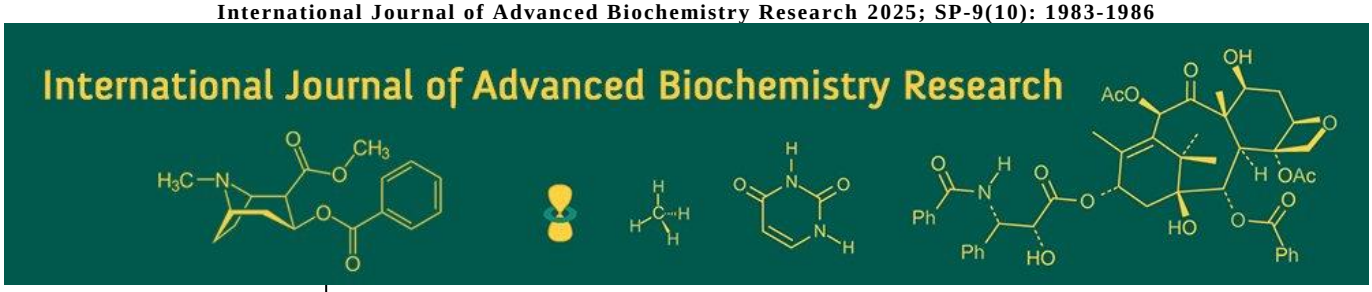

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In vitro efficacy of bioagents and fungicides against chickpea wilt caused by *Fusarium oxysporium* f. sp. *ciceri*

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Abstract
Chickpea (*Cicer arietinum* L.) is one of the most important legume crops belonging to the family Fabaceae (Leguminosae). Among various fungal diseases, *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceri* has emerged as a major constraint to chickpea production in Maharashtra as well as across India. The disease has been reported to cause yield losses of more than 60-70%. Considering this the present study was conducted to evaluate the efficacy of bioagents and fungicides against *Fusarium* wilt of chickpea. Among all seven bioagents tested *in vitro* were found antagonistic to *F. oxysporum* f. sp. *ciceri*. *Trichoderma harzianum* resulted with highest mycelial growth inhibition, followed by *Aspergillus niger*. The most effective fungicide evaluated *in vitro* (1500, 1750, 2000 ppm), was Carbendazim 12% + Mancozeb 63% WP (100% mycelial growth inhibition), followed by Carboxin 37.5% + Thiram 37.5% DS and Thiophanate Methyl 45% + Pyraclostrobin 5% FS.

Keywords: Chickpea wilt, *Fusarium oxysporum* f. sp. *ciceri*, bioagents, fungicides, *Trichoderma harzianum*, Carbendazim 12% + Mancozeb 63% WP

Introduction
Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops grown and consumed worldwide, particularly in Afro-Asian countries. In India, it is a major *rabi* pulse cultivated under both rainfed and irrigated conditions. Chickpea grains contain about 21.1% protein, 61.5% carbohydrates and 4.55% fat, with protein quality superior to many other pulses. During 2019-2020, chickpea occupied 9.85 million ha in India with a total production of 10.90 million tonnes and an average productivity of 1142 kg ha^{-1}. It is cultivated in 16 states, with Madhya Pradesh, Rajasthan, Uttar Pradesh, Maharashtra and Haryana as the leading producers. Madhya Pradesh tops the list with 26.8 lakh ha, whereas Maharashtra ranks second with 25.94 lakh ha and a production of 28.46 lakh tonnes at 1097 kg ha^{-1} productivity (Chief Statistician, Maharashtra, 2021). Due to its nitrogen-fixing ability, chickpea improves soil fertility and can restore up to 140 kg N ha^{-1} during its growth period (Poonia and Pithia, 2013) [12].

Despite its importance, chickpea is susceptible to more than 50 pathogens (Nene *et al*., 1981) [10]. Wilt (*Fusarium oxysporum* f. sp. *ciceri*), root rot (*Rhizoctonia bataticola*), and collar rot (*Sclerotium rolfsii*) are major constraints in Marathwada (Maharashtra) and many other chickpea-growing regions. Wilt disease occurs widely across India, Pakistan, Iran, Nepal, Myanmar, Mexico, Spain, Peru, Syria and the USA, causing regular yield losses of 5-10%, which may reach 60-70% during severe epidemics (Singh and Dahiya, 1973) [15]. The pathogen *F. oxysporum* f. sp. *ciceri* was first reported in India by Butler (1918) [4]. It is a facultative, soil-borne saprophyte that can persist as chlamydospores or mycelium for up to six years even in the absence of a susceptible host (Haware *et al*., 1986a) [7]. The fungus colonizes the xylem vessels, blocks water translocation, and leads to rapid wilting and plant death (Padwick, 1948; De *et al*., 1996) [11, 5].

Several approaches have been investigated to manage wilt, including resistant varieties (Brisa *et al*., 2007) [3], chemical control, plant extracts (Kumar and Tripathi, 1991) [8] and biological control agents (Rojo *et al*., 2007; Dubey *et al*., 2007) [14, 6]. Given the seriousness of the disease and the significant yield losses it causes, the present investigation was undertaken to develop an effective integrated management strategy for chickpea wilt.

Materials and Methods

An experiment for the management of wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* using different fungicides and bioagents was carried out at Department of Plant Pathology, College of Agriculture, Badnapur, during 2024-25.

In vitro efficacy of bioagents

A total of seven fungal bioagents as detailed under treatments were evaluated in *in vitro* against *Fusarium oxysporum* f. sp. *ciceri*, by applying Dual culture technique (Arora and Upadhay, 1978) [1]. Seven days old pure cultures of the test pathogen and test bioagents grown on PDA medium were used for the study. Two 5 mm culture discs, one each of the test pathogen and the test bioagent were cut out with sterilized cork borer and placed at equidistance and exactly opposite to each other on autoclaved and solidified PDA medium in Petri plates and were incubated at 25±1°C. PDA plates inoculated alone with pure culture disc (5 mm) of the test pathogen were maintained as control.

Observations on radial mycelial growth and colony diameter of *Fusarium oxysporum* f. sp. *ciceri* was recorded when untreated control plates were fully covered with mycelial growth. Per cent mycelial growth inhibition of the pathogen with the bioagents, over untreated control was calculated by using formula suggested by (Arora and Upadhyay, 1978) [1].

$$\text{Percent growth inhibition (\%)} = \frac{C - T}{C} X\ 100$$

Where,
C = Growth of test pathogen in control
T = Growth of test pathogen in treated plates

Detals of the experiment
Design: CRD
Treatments: Eight
Replication: Three

Treatment details

Tr. No.	Treatments
T_1	*Trichoderma asperellum*
T_2	*T. harzianum*
T_3	*Verticillium lecanii*
T_4	*Bacillus subtilis*
T_5	Pink Pigmented Facultative Methylotrophs (PPFMs)
T_6	*Aspergillus niger*
T_7	*Pseudomonas fluorescens*
T_8	Control (Untreated)

In vitro efficacy of fungicides

Efficacy of seven combi fungicides (each @ 1500, 1750 and 2000 ppm) was evaluated *in vitro* against *Fusarium oxysporum* f. sp. *ciceri* by applying poisoned food technique (Nene and Thapliyal, 1993) [9]. Three Petri plates / treatment / replication were maintained and also untreated control plates were maintained.

Observations on radial mycelial growth / colony diameter of the *Fusarium oxysporum* f. sp. *ciceri* was recorded when untreated control plates were fully covered with mycelial growth. Per cent mycelial growth inhibition of the pathogen with the test fungicides over the untreated control were calculated by using the formula given by (Vincent, 1927).

$$\text{Percent growth inhibition (\%)} = \frac{C - T}{C} X\ 100$$

Where,
C = Growth of test pathogen in control
T = Growth of test pathogen in treated plates.

Details of the experiment
Design: CRD
Treatments: Eight
Replication: Three

Treatment details

Tr. No.	Chemicals
T_1	Penflufen 13.28% + Trifloxystrobin 13.28% w/w
T_2	Procloraz 5.7% + Tebuconazol 1.4% w/w
T_3	Tebuconazole 10% WP + Sulphur 65% WG
T_4	Mancozeb 50% + Carbendazim 25% WP
T_5	Carboxin 37.5% + Thiram 37.5% DS
T_6	Thiophanate Methyl 45% + Pyraclostrobin 5%
T_7	Carbendazim 12% + Mancozeb 63% WP
T_8	Control (Untreated)

Results and Discussion

Efficacy of bioagents against *Fusarium oxysporum* f. sp. *ciceri*

The results reveled that, the colony diameter of mycelial growth and per cent inhibition of the *F. oxysporum* f. sp. *ciceri* with fungal antagonist ranged from 11.18 mm and 87.57% with *T. harzianum* to 48.12 mm and 46.53% with *Bacillus subtilis.*

Radial mycelial growth

Results (Table 1) revealed that among the seven bioagents tested, (T_2) *Trichoderma harzianum* with 87.57 per cent mycelial growth was found most effective in controlling the mycelial growth of *F. oxysporum* f. sp. *ciceri* (11.18 mm) which was found significant over all of the treatments and (90.00 mm) untreated control (T_8), (T_6) *Aspergillus niger* was next best bioagent restricting mycelial growth of the *F. oxysporum* f. sp. *ciceri* (14.36 mm), followed by (T_7) *Pseudomonas fluorescens* (22.27 mm), (T_1) *T. asperellum*(28.44 mm), (T_3) *Verticillium lecanii* (34.63 mm), (T_5) Pink Pigmented Facultative Methylotrophs (PPFMs) (34.67 mm) and (T_4) *Bacillus subtilis* (48.12 mm).

All the bioagents (Table 1) evaluated, exhibited fungistatic /antifungal activity against test pathogen and significantly inhibited its growth, over untreated control.

Table 1: Efficacy of different bioagents against *Fusarium oxysporum* f. sp. *ciceri*

Tr. No.	Treatments	Pathogen Colony Dia. (mm)	Per cent Growth Inhibition
T_1	*Trichoderma asperellum*	28.44	68.40 (55.80)*
T_2	*T. harzianum*	11.18	87.57 (69.36)
T_3	*Verticillium lecanii*	34.63	61.52 (51.66)
T_4	*Bacillus subtilis*	48.12	46.53 (43.01)
T_5	Pink Pigmented Facultative Methylotrophs (PPFMs)	34.67	61.47 (51.63)
T_6	*Aspergillus niger*	14.36	84.04 (66.45)
T_7	*Pseudomonas fluorescens*	22.27	75.25 (60.16)
T_8	Control (Untreated)	90.00	00.00 (00.00)
SE(m) ±		00.82	01.85
C.D (P=0.01)		02.38	03.83

* Figures in parenthesis are angular transformed value.

Inhibition of mycelial growth

Results (Table 1) revealed that out of the seven bioagents tested, (T_2) *Trichoderma harzianum* was found most effective in inhibiting the mycelial growth of *F. oxysporum* f. sp. *ciceri* (87.57%) which was significant over rest of the treatments and untreated control (00.00%), The next best bioagent found in inhibiting the mycelial growth of the *F. oxysporum* f. sp. *ciceri* was (T_6) *Aspergillus niger* (84.04%), which was followed by (T_7) *Pseudomonas fluorescens* (75.25%), (T_1) *T. asperellum* (68.40%), (T_3) *Verticillium lecanii* (61.52%), (T_5) Pink Pigmented Facultative Methylotrophs (PPFMs) (61.47%) and (T_4) *Bacillus subtilis* (46.53%).

The results are in agreement with the finding of several workers who reported that bioagents *viz.*, *Trichoderma harzianum*, *Aspergillus niger*, *Pseudomonas fluorescens*, and *T. asperellum* had significantly inhibited mycelial growth of F. *oxysporum* f. sp. *ciceri* by Singh *et al*. (1977 and Sonawane and Pawar (2001) [16, 17].

Efficacy of fungicides against *Fusarium oxysporum* f. sp. *Ciceri*

Radial mycelial growth

The results (Table 2) revealed that at 1500 ppm concentration of the combi-product fungicides the radial mycelial growth of *F. oxysporum* f. sp. *ciceri*, ranged from 00.00 mm (T_7) to 62.33 mm (T_3). Minimum mycelial growth of *F. oxysporum* f. sp. *ciceri* was recorded in (T_7) Carbendazim + Mancozeb (00.00 mm) which was followed by (T_5) Carboxin + Thiram (12.28 mm). Whereas at 1750 ppm concentration of the combi-product fungicides the radial mycelial growth of *F. oxysporum* f. sp. *ciceri*, ranged from 00.00 mm (T_7) to 43.79 mm (T_3). Minimum mycelial growth of *F. oxysporum* f. sp. *ciceri* was recorded in (T_7) Carbendazim + Mancozeb (00.00 mm) which was followed by (T_5) Carboxin + Thiram (06.67 mm). Also, at 2000 ppm concentration of the combi-product fungicides the radial mycelial growth of *F. oxysporum* f. sp. *ciceri*, ranged from 00.00 mm (T_7) to 31.22 mm (T_3). Minimum mycelial growth of *F. oxysporum* f. sp. *ciceri* was recorded in (T_7) Carbendazim + Mancozeb (00.00 mm) which was followed by (T_5) Carboxin + Thiram (01.72 mm).

Table 2: Effect of different fungicides against *Fusarium oxysporum* f. sp. *ciceri*

Sr. No.	Chemicals	Different concentrations (ppm)					
		1500		1750		2000	
		Colony Dia. (mm)	Per cent Growth Inhibition	Colony Dia. (mm)	Per cent Growth Inhibition	Colony Dia. (mm)	Per cent Growth Inhibition
T_1	Penflufen 13.28% + Trifloxystrobin 13.28% w/w	39.17	56.48 (48.72) *	28.34	68.51 (54.04) *	13.03	85.52 (65.33) *
T_2	Procloraz 5.7% + Tebuconazol 1.4% w/w	44.36	50.71 (45.71)	33.41	62.87 (52.46)	18.69	79.23 (62.88)
T_3	Tebuconazole 10% WP + Sulphur 65% WG	62.33	30.74 (33.67)	43.79	51.34 (45.77)	31.22	65.31 (53.92)
T_4	Mancozeb 50% + Carbendazim 25% WP	40.34	55.18 (47.97)	29.77	66.92 (54.89)	18.34	79.62 (63.16)
T_5	Carboxin 37.5% + Thiram 37.5% DS	12.28	86.36 (68.33)	06.67	92.58 (74.19)	01.72	98.01 (81.89)
T_6	Thiophanate Methyl 45% + Pyraclostrobin 5%	14.48	83.91 (66.35)	08.92	90.00 (71.57)	03.31	96.03 (78.51)
T_7	Carbendazim 12% + Mancozeb 63% WP	00.00	100.00 (90.00)	00.00	100.00 (90.00)	00.00	100.00 (90.00)
T_8	Control (Untreated)	90.00	00.00 (00.00)	90.00	00.00 (00.00)	90.00	00.00 (00.00)
SE(m) ±		00.93	01.08	01.08	01.87	01.04	01.45
C.D (P=0.01)		02.71	03.42	03.18	04.41	03.30	04.06

* Figures in parenthesis are angular transformed value.

Inhibition of mycelial growth

The results (Table 2) revealed that at 1500 ppm concentration of the combi-product fungicides the per cent inhibition of mycelial growth of *F. oxysporum* f. sp. *ciceri*, ranged from 100.00 per cent (T_7) to 30.74 per cent (T_3). Maximum inhibition of *F. oxysporum* f. sp. *ciceri* was recorded in Treatment (T_7) Carbendazim + Mancozeb with cent per cent (100.00%) inhibition. Whereas at 1750 ppm concentration of the combi-product fungicides the per cent inhibition of mycelial growth of *F. oxysporum* f. sp. *ciceri*, ranged from 100.00 per cent (T_7) to 51.34 per cent (T_3). Maximum inhibition of *F. oxysporum* f. sp. *ciceri* was recorded in Treatment (T_7) Carbendazim + Mancozeb with cent per cent (100.00%) inhibition which was then followed by (T_5) Carboxin + Thiram (92.58%). Also, at 2000 ppm concentration of the combi-product fungicides the per cent inhibition of mycelial growth of *F. oxysporum* f. sp. *ciceri*, ranged from 100.00 per cent (T_7) to 65.31 per cent (T_3). Maximum inhibition of *F. oxysporum* f. sp. *ciceri* was recorded in Treatment (T_7) Carbendazim + Mancozeb with

cent per cent (100.00%) inhibition which was then followed by (T_5) Carboxin + Thiram (98.01%).

The results in the present study were almost identical with previous researchers like Ravichandran and Hegde (2015) [13] who studied *in vitro* efficacy of contact, systemic and combi fungicides against pathogen causing chickpea wilt and observed that among these Carbendazim 12% + Mancozeb 63% (SAAF) was found effective at all concentrations (1000, 2000, 3000 ppm) with 100 per cent inhibition of *Fusarium oxysporum* f. sp. *ciceri* and least inhibition was recorded in Zineb 68% + Hexaconazole 4% WP (Avatar) 21.85% at 0.1% concentration.

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