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In vitro efficacy of bioagents against *Fusarium oxysporum* f. sp. *ciceri*, causing chickpea wilt

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Abstract
Chickpea (*Cicer arientum* L.) is the most important pulse crop cultivated in India and affected by many fungal and viral diseases and among all the diseases, wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is one of the most common fungal pathogen which causes ultimate yield loss in chickpea crop. Results revealed that all the bioagents evaluated, exhibited fungistatic/antifungal activity against test pathogen and significantly inhibited its growth over untreated control. *Trichoderma harzianum*, most effective with highest mycelial growth inhibition (90.56%) of the test pathogen. The second and third best inhibitor antagonists found were *T. hamatum* and *T. virens* with mycelial growth inhibition (14.97 mm and 83.37%), respectively. These were followed by *Aspergillus niger* (83.29%), *T. asperellum* (81.70%), *T. koningii* (70.67%), and *Metarrhizium anisopliae* (60.90%), respectively. However, *Pseudomonas fluorescens* and *Bacillus subtilis* were found least effective with highest mycelial growth and minimum mycelial growth inhibition of (29.03%) and (28.37%), respectively.

Keywords: Chickpea, wilt, *F. oxysporum* f. sp. *ciceri*, bioagents

1. Introduction
Chickpea (*Cicer arietinum* L.) is one of the most important *Rabi* pulse crop in India sharing hectarage next to pigeonpea and makes up for 20% of the world pulse production. It belongs to family *Leguminoceae*. Chickpea is commonly known as bengal gram, garbanzo bean, gram, chana, chole and harbhara. It plays a vital role in the diet of poor people which serves as a major source of vegetable protein (21.1%), carbohydrates (61.5%) and fat (4.5%). It does not contain any anti-nutritional factor. It is mainly used for human consumption as well as for animal feeds. It is consumed as whole seed, dal, boiled, fried, salted or more generally which is cooked.

In India, chickpea is an important pulse crop, occupies 9.85 million hectares area, production 11.99 million tonnes and yield 1217 kg/ha. In Maharashtra, area under chickpea cultivation is 2.15 million hectares with production 2.37 million tonnes and yield 1105 Kg/ha.
(Agril. Statistics At a Glance, 2021) [1]. In India chickpea accounts for about 45% of total pulses produced in the country. India is the largest producer, with about 10 million tonnes accounting of about 70% of total world production.

Various diseases affect the chickpea *viz*. *Ascochyta* blight, damping off, *Botrytis* grey mold, *Phytophthora* root rot, seed rot / *Pythium* rot and rust but wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is the most serious disease. The chickpea wilt fungus, *Fusarium oxysporum* f. sp. *ciceri* is a vascular pathogen. This pathogen is soil and seed borne (Haware *et al*., 1978) [4]. Under severe conditions, the wilt disease can damage the crop completely and causes losses upto 100% (Navas-cortes *et al.*, 2000) [7]. Considering, the importance of disease in the state, the losses incurred in the farmer's field and the problem has increased in past 6-7 years with heavy economic losses. Therefore, it was felt necessary to investigate on *in vitro* efficacy of bioagents against *Fusarium oxysporum* f. sp. *ciceri*, causing chickpea wilt.

2. Materials and Methods
Seven fungal and two bacterial bioagents were evaluated in *in vitro* against *Fusarium oxysporum* f. sp. *ciceri* by applying dual culture technique (Dennis and Webster, 1971) [3]. Seven days old culture of bioagents and pathogen (*Fusarium oxysporum* f. sp. *ciceri*) were used for the further study.

The pathogen and bioagents were placed aseptically at equidistance exactly opposite with each other on solidified PDA medium in petri plates. For bacterial biocontrol agents, a culture disc (5 mm) for the test fungus was placed along periphery of the PDA plate and exactly opposite

to it pure culture suspension of the test bacterial biocontrol agent was streaked with wire inoculation needle loop. The PDA plates inoculated (in the centre) alone with pure culture disc of the test fungus were maintained as untreated control.

2.1 Experimental details

Design: Completely Randomized Design (CRD)
Replications: Three
Treatments: Ten

Treatments details

Tr. No.	Treatments	Tr. No.	Treatments
T1	*Trichoderma koningii*	T6	*Trichoderma virens*
T2	*Trichoderma hamatum*	T7	*Aspergillus niger*
T3	*Trichoderma harzianum*	T8	*Pseudomonas fluorescens*
T4	*Trichoderma asperellum*	T9	*Bacillus subtilis*
T5	*Metarrhizium anisopliae*	T10	Control (untreated)

2.2 Observations

Observations on radial mycelial growth / colony diameter of the *Fusarium oxysporum* f. sp. *ciceri* were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth. Per cent mycelial growth inhibition of the pathogen with the bioagents over the untreated control was calculated by using the following formula (Arora and Updhyay, 1978) [2].

$$\text{Per cent growth inhibition} = \frac{\text{Colony growth in Control plate} - \text{Colony growth in Intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

3. Results and Discussion

The results obtained on mycelial growth and inhibition of *Fusarium oxysporum* f. sp. *ciceri* with seven fungal antagonists *viz*., *Trichoderma koningii*, *T. hamatum*, *T. harzianum*, *T. asperellum*, *Metarrhizium anisopliae*, *T. virens*, *Aspergillus niger* and two bacterial antagonists *viz*., *Pseudomonas fluorescens* and *Bacillus subtilis* are presented (Plate 1, Table 1 and Fig. 1).

Plate 1: *In vitro* efficacy of bioagents against *Fusarium oxysporum* f. sp. *cicero* causing chickpea wilt

Table 1: *In vitro* efficacy of bioagents against *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt

Tr. No.	Treatments	Colony Diameter* of Pathogen (mm)	% Inhibition*
T1	*Trichoderma koningii*	26.40	70.67 (57.20)**
T2	*Trichoderma hamatum*	13.46	85.04 (67.24)
T3	*Trichoderma harzianum*	8.50	90.56 (72.10)
T4	*Trichoderma asperellum*	16.46	81.70 (64.67)
T5	*Metarhizium anisopliae*	35.20	60.90 (51.29)
T6	*Trichoderma virens*	14.97	83.37 (65.93)
T7	*Aspergillus niger*	15.03	83.29 (65.87)
T8	*Pseudomonas fluorescens*	63.87	29.03 (32.60)
T9	*Bacillus subtilis*	64.47	28.37 (32.18)
T10	Control (untreated)	90.00	00.00 (00.00)
	S.E. ±	0.51	0.53
	C.D. (P=0.01)	1.53	1.59

*Mean of three replications **Figure in parenthesis are arcsine transformed values

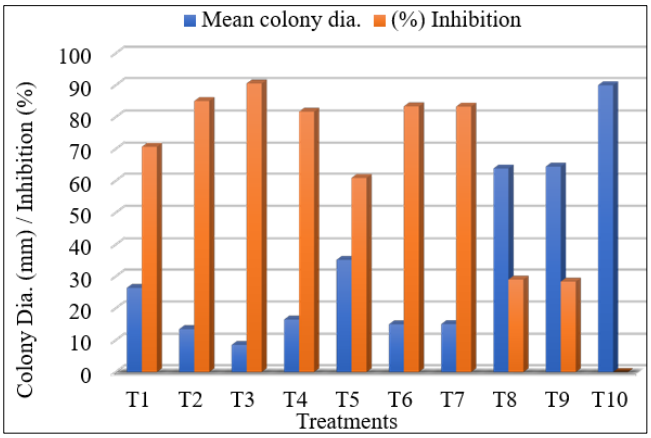

Fig 1: *In vitro* efficacy of bioagents against *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt

Results (Plate 1, Table 1 and Fig. 1) revealed that, all the bioagents evaluated, exhibited fungistatic/antifungal activity against test pathogen and significantly inhibited its growth over untreated control. *Trichoderma harzianum* shown significantly least linear mycelial growth (8.50 mm) and found most effective with highest mycelial growth inhibition (90.56%) of the test pathogen. The second and third best inhibitor antagonists found were *T. hamatum* and *T. virens* with mycelial growth and inhibition (13.46 mm and 85.04%) and (14.97 mm and 83.37%), respectively. These were followed by *Aspergillus niger* (15.03 mm and 83.29%), *T. asperellum* (16.46 mm and 81.70%), *T. koningii* (26.40 mm and 70.67%), and *Metarrhizium anisopliae* (35.20 mm and 60.90%), respectively. However, *Pseudomonas fluorescens* and *Bacillus subtilis* were found least effective with highest mycelial growth and minimum mycelial growth inhibition of (63.87 mm and 29.03%) and (64.47 mm and 28.37%), respectively.

Thus, the bioagents *viz.*, *Trichoderma harzianum, T. hamatum* and *T. virens* were found as most potent antagonists against *Fusarium oxysporum* f. sp. *ciceri.*

These results are in conformity with the earlier finding of those workers who reported bioagents *Trichoderma harzianum, T. hamatum* and *T. virens* were effective against *Fusarium oxysporum* f. sp. *ciceri*. Kumar *et al.* (2017) [6] reported that *T. harzianum* resulted with highest mycelial growth inhibition, followed by *T. asperellum* against *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt. Thaware *et al.* (2017) [8] reported that *T. asperellum* resulted with highest mycelial growth inhibition, followed by *T. harzianum* against *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt. Khillare *et al*. (2020) [5] reported that *T. harzianum* resulted with highest mycelial growth inhibition, followed by *Trichoderma hamatum* and *T. asperellum* against *Fusarium oxysporum* f. sp. *ciceri* causing pigeonpea wilt.

4. Conclusion

Results indicated that, *Trichoderma hamatum*, *T. harzianum* and *T. virens* were proved to be strong antagonists against *F. oxysporum* f. sp. *ciceri*, causing chickpea wilt.

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