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Research Article

Antagonist's effect evaluation of bio-agents against *Fusarium oxysporum* f.sp. *ciceri* responsible for wilt disease in chickpea pulse crop under *in-vitro* condition

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Abstract Chickpea wilt caused by *F. oxysporum* f. sp. *ciceri*. Antagonist's effect evaluation of four bio-agents were conducted under in- vitro condition and these results were revealed that, the per cent inhibition over control was found maximum (78.02%) into the *Trichoderma viride* followed by *T. harzianum* (72.14%) and it was found minimum into the *T. koningii* (48.61%). *T. hamatum* was found least effective (58.16%) to inhibit the mycelial growth of *Fusarium oxysporum* f. sp. *ciceri.*

Keywords: Antagonist, Bio-agents, Dual culture, Evaluation, *Fusarium* wilt.

Introduction

In India chickpea (*Cicer arietinum* L.) variously known as Gram or Bengal gram. Manly two types ofchickpeas are grown, first one is brown seeded type called as "Desi" and second one is white seeded called as a "Kabuli". According to (Vishwadhar and Gurha, 1998) chickpea is the third most important pulse crop in the worlds after dry beans (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum* L.). Chickpea or chick-pea (*Cicer arietinum* L.) is one of them. Pulse crops play an important role in Indian agriculture systems, besides being a rich source of protein; they sustain the productivity of the crop diversification system. In human it's also consumed as whole seed in the form of fried and boiled for to full fill the protein deficiency. The grains are also used as vegetable (Chhole). This crop (Chickpea) is subjected to attack by several fungal, viral, bacterial, and nematode diseases. The *Fusarium* wilt is one of the most serious one. The disease has been reported from several countries including India, Bangladesh, Burma, Ethiopia, Mexico, Chile, Iran, Nepal, Sudan, Pakistan, Syria, Tunisia, United States, Malawi, Spain, Peru, USSR, Turkey and Italy. In India occurrence of chickpea wilt was first described by Butler (1918**)**.

According to Suman Patra and Mohan Kumar Biswas (2017)they were reported that *F. oxysporum* f. sp. *ciceri* is the most destructive and widespread fungal disease of chickpea and it has drastic effect on yield causing cent per cent loss under favorable conditions.According to Shabir-U-Rahman *et al.* (2013) they were evaluated two bioagents viz; *Trichoderma viride* and *T. harzianum* singly and in the form of combination against *F. oxysporum* f. sp. *ciceri* under *in-vitro* condition. The *in-vitro* results were showed, maximum mycelial inhibition of *F. oxysporum* f. sp. *ciceri* was found with *T. viride* + *T. harzianum* (87.33%) followed by *T. harzianum* (83.3%) and *T. viride* (81.0%) respectively. According to Rani and Mane (2014) both was

evaluated to the efficacy of two fungal bioagents *viz*., *T. viride*, *T. harzianum* and two bacterial bioagents *viz*., *P. flurescens* and *B. subtilis* against *F. oxysporum* f. sp. *ciceri* under *in-vitro* conditions by using dual culture technique. The highest per cent inhibition of growth was recorded by *T. harzianum* (76.66 %) followed by *B. subtilis* (63.14%). The lowest inhibition of redial growth was observed in *P. fluorescens* (53.52%).

According to Thaware *et al.* (2017) they were reported under *in-vitro* condition to check the efficacy of six fungal and two bacterial antagonists against *F. oxysporum* f. sp. *ciceri* and they were reported that the *Trichoderma viride* was recorded significantly highest mycelial growth inhibition (75.55%), followed by *T. harzianum* (73.77%), *T. koningii* (71.88%) and *P. fluorescens* (43.77%) respectively.

Materials and Methods

This experiment was conducted in the laboratory of the Department of Botany, Meerut College, Meerut (U.P.) for two consecutive years of 2019-20 and 2020-21 in completely randomized design with four replications and five treatments included its suitable control (without adding any bio-agents in the petri-plates) under *in-vitro* condition.Total four fungal antagonist bio-agents *viz., Trichoderma viride, T. harzianum, T. hamatum* and *T. koningii* were asses for their efficacy against *F. oxysporum* f. sp. *ciceri* by using dual culture technique (Morton and Strove, 1955). The culture of test fungus and antagonist (bio-agents) was multiplying on potato dextrose agar medium, individually.

By dual culture method (*bio-agents*): Seven days old pure cultured petri-plates of test pathogen were kept separately for this test and all antagonists (bio-agents) cultured petri-palates also were taken, individually during that experiment. Fungal antagonists were evaluated by the placing of 5 mm disc of the test pathogen culture on the one side of pre-poured PDA medium in sterilized autoclavable glass Petri-plates, before the placing of bio-agents disc. After 48 hours, disc of antagonist (bio-agent) with same in diameter (5 mm) was placed, individually at the opposite side of test pathogen disc and it was maintained 3- 4 cm distances between both of the disc. Then all these inoculated plates were incubated at 25 ± 1^0 C in incubation chamber. The plates inoculated only with culture disc of the test pathogen were maintained as untreated control.

Experimental Details:

Design	:	CRD
Replication	:	Four
Treatments	:	Five

Treatments Details

Number of treatments	Treatment details
T_1	*Trichoderma viride*
T_2	*T. harzianum*
T_3	*T. hamatum*
T_4	*T. koningii*
T_5	Control (untreated)

The observation on radial mycelial growth/ colony diameter in millimeter (mm) of the test pathogen was assessing at an interval of 24 hours and continued till untreated plates were fully covered with test pathogen mycelial growth, per cent mycelial growth inhibition of the test pathogen with the bio-agents over the untreated control was calculated by using the formula of Vincent (1947).

Percent inhibition

Where, C = Growth of the test fungus in untreated control plates.

T = Growth of the test fungus in treated plates.

* Measuring scale: In millimeter (mm).

Experimental Findings

These results were revealed that, the per cent inhibition over control was found maximum (78.02%) into the Trichoderma viride followed by T. harzianum (72.14%) and it was found minimum into the T. koningii (48.61%). T. hamatum was found least effective (58.16%) to inhibit the mycelial growth of Fusarium oxysporum f. sp. ciceri under In-vitro conditions, **PLATE-1**(Fig. 1A, B & C).

This experiment was conducted during in the years 2019-20 and 2020-21. Total four fungal antagonist bio-agents *viz., Trichoderma viride, T. harzianum, T. hamatum* and *T. koningii* were asses for their efficacy against *F. oxysporum* f. sp. *ciceri* by using dual culture technique (Morton and Strove, 1955) as described under "Materials and Methods". This experiment was conducted in completely randomized block design with four replications and five treatments included suitable control (without adding any botanicals in the petri-plates). These results were presented in table -1.

Table- 1: Antagonist's effect evaluation of bio-agents against *Fusarium oxysporum* f. sp. *ciceri* under *in-vitro* condition.

Treatment No.	Treatments details	% Redial growth (mm)		Mean (%)	Per cent Inhibition over control		Mean (%)
		Year (2019-20)	Year (2020-21)		Year (2019-20)	Year (2020-21)	
T_1	Trichoderma viride	13.47	18.52	15.99	81.44	74.61	78.02
T_2	T. harzianum	17.3	23.25	20.27	76.17	68.12	72.14
T_3	T. hamatum	30.37	30.52	30.44	58.16	58.16	58.16
T_4	T. koningii	37.72	37.07	37.39	48.04	49.18	48.61
T_5	Control (un-treated)	72.60	72.95	72.77	00.00	00.00	0.00
S.E. (m) ±		0.85	0.83	1.75			2.37
CD at 5%		2.61	2.55	6.51			8.82

Results and Discussion

Antagonistic effect of bio-control agents against *Fusarium oxysporum* f. sp. *ciceri* by dual culture method, fourbio-agents *viz., Trichoderma viride, T. harzianum, T. hamatum* and *T. koningii* were used for this experiment. The per cent inhibition over control was found maximum (78.02%) into the *Trichoderma viride* followed by *T. harzianum* (72.14%) and it was found minimum into the *T. koningii* (48.61%). *T. hamatum* was found least effective (58.16) to inhibit the mycelial growth of *Fusarium oxysporum* f. sp. *ciceri under in-vitro* conditions. **The almost similar results were found from earlier reports by** Suman Patra and Mohan Kumar Biswas (2017)**; Rani & Mane, 2014** and **Thaware *et al*. (2017).**

PLATE-1

Antagonist's effect of bio-agents against *Fusarium oxysporum* f. sp. *ciceri*under *in-vitro* condition.

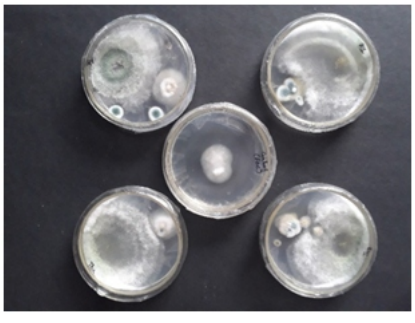

Fig. 1 (A)
Initial stage of Dual culture method (Bio-agents)

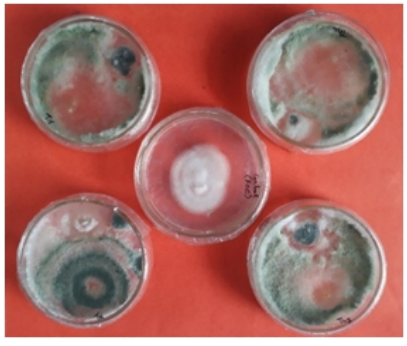

Fig. 1(B)
Mid stage of Dual culture method (Bio-agents)

Fig. 1(C)
Later stage of Dual culture method

Number of treatments	Treatment details
T_1	*Trichoderma viride*
T_2	*T. harzianum*
T_3	*T. hamatum*
T_4	*T. koningii*
T_5	Control (untreated)

Conclusions

Antagonistic effect of the bio-control agents against Fusarium oxysporum f. sp. ciceri by dual culture method under in-vitro conditions clearly indicated that, inhibition per cent over control were found maximum in the *Trichoderma viride* followed by *T. harzianum*.

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