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Management of chickpea wilt pathogen *Fusarium oxysporum* f. sp. *ciceris* by *Trichoderma*, *Pseudomonas* and *Bacillus* under *in vitro*

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ABSTRACT: Chick pea (*Cicer arietinum* L.) is one of the leading legume crops, which contributes 18% of the worlds grain legume production, it serves as an important dietary protein source. Since last two decades, due to several biotic and abiotic constraints reduction in the cropping area and production was observed. Among the different biotic constraints crop production was majorly affected with vascular wilt disease caused by fungus *Fusarium oxysporum* f. sp. *ciceris*. The potential use as biocontrol agents against economically important soil borne plant pathogens with protective activity has been highlighted in recent decades as effective alternative to chemical fungicides. In the present investigation, the inhibition action of four antagonists *Trichoderma* spp. (Th-1 and Tv-1), *P. fluorescens* (Pf-1) and *B. subtilis* (Bs-1) was evaluated against the radial growth of *F. oxysporum* f. sp. *ciceris* was studied under *in vitro* conditions. The culture filtrates of all the four tested bio control agents were proved to be inhibitory to the pathogen. Among the two different *Trichoderma* spp. antagonists the culture filtrate of Th-8 was found effective with 23.70% of growth inhibition, followed by Tv-1 with 19.26% at 5 per cent concentration in comparison with antagonists Pf-1 and Bs-1.

Keywords: Chick pea, pathogen, *Fusarium oxysporum* f. sp. *ciceris*, vascular wilt disease.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most economically important legume food crops grown in about 14.96 Mha in the tropical and sub-tropical regions of the world. India, in accounts for approximately 70 per cent of world's chickpea production (FAOSTAT, 2019). Last year, chickpea was grown in about 98.86 Lha with a production of 107 Lt at a highest productivity level of 1086 kg/ha.

Chickpea is an essential source of an inexpensive protein in many parts of the world specifically for the vegetarians, as it has the highest nutritional composition of among dry edible legumes. In addition to it's role in improving the fertility of soil by increasing the nitrogen concentration when practiced under crop rotation, it also assists in breaking the disease cycle of several important soil borne plant pathogens.

Despite of it's high total production, yields of chickpea are reducing due to many biotic and abiotic constraints. Among the different biotic constraints about 172 pathogens have been reported from 55 chickpea growing countries of world, out of which 89 pathogens have been reported from India alone (Andrabi *et al*., 2008). Among these, serious diseases caused by pathogens especially soil borne are become major constraints in agricultural production. The diseases caused by major soil borne plant pathogens, like *Fusarium oxysporum* f. sp. *ciceris* (FOC), *F. solani, Macrophomina phaseolina, Sclerotium rolfsii, Rhizoctonia solani* and *Pythium ultimum* is are the most important, devastating and challenging the crop growth at any stage (Parmer and Gohel 2019). This problem is widely spread in several chickpea growing countries of the world. Among the different soil borne diseases Fusarium vascular wilt caused by *Fusarium oxysporum* f. sp. *ciceris* (Foc) is affecting the chickpea production globally. Fusarium vascular wilt disease was first reported in India by Butler in 1918, and its etiology was explained by Padwick in 1940 (Cunnington *et al.,* 2007). Fusarium wilt can be epidemic disease and may causes up to 100% yield loss if infested under suitable conditions. Symptoms of the disease can visible from 25 days after sowing to podding stage and affected plants may be grouped in patches or appear spread across a field.

Management of chickpea Fusarium wilt is difficult to achieve, as the fungus survives in soil through resistant structure i.e. chlamydospores and remains susceptible throughout all the growth stages (Kaiser *et al.,* 1994 and Haware *et al.,* 1996). Due to several adverse effects associated with chemical control of pest and diseases, attention is being given to development of cost effective and environmentally friendly alternative management practices. Biological control based on mycoparasitism or hyper-parasitism between some microbial organisms has drawn the attention of growers and researchers throughout the world, which provides an alternative to chemical management using microbial antagonists such as *Trichoderma* spp. *Pseudomonas fluorescens*,

Bacillus spp. (Mukhopadhyay *et al.,* 1992; Hervas *et al.,* 1997; Khan and Gangopadhyay, 2008 and Jayalakshmi *et al.,* 2009). The potential use of biocontrol agents against economically important plant pathogens with protective activity has been highlighted in recent decades and making them an important alternative to chemical fungicides (Expósito *et al.,* 2017). These biocontrol agents also help in plant system to cope up with abiotic stresses i.e. drought, salinity, heat etc., and improve their growth as well as nutritional status (Keswani *et al.,* 2016; Singh *et al.,* 2016).

MATERIALS AND METHODS

In the present *in vitro* experiment, the antagonistic activity of four bioagents belong to three different genera i.e., *Trichoderma* sp. (*T. harzianum* and *T. viride*), *Pseudomonas* sp. (*P. fluorescens*-1) and *Bacillus* sp. (*B. subtilis*-1) were evaluated against radial growth of *F. oxysporium* f. sp. *ciceris*. The mechanisms of antagonism were studied under *in vitro* conditions.

Inhibition of *F. oxysporium* f. sp. *ciceris* by culture filtrate

The antagonistic potential of the four test microbial bioagents i.e. *T. viride* (Tv-1), *T. harzianum* (Th-1), *P. fluorescens* and *Bacillus subtilis* against *F. oxysporum* f. sp. *ciceris* were determined under *invitro* conditions.

Sample collection, isolation and purification

Chickpea plants showing typical wilt symptoms were collected in paper bags near to Bikaner area of Rajasthan, labelled properly and brought to the laboratory for isolation of the pathogen. The disease infected portions of the plant were washed properly under running tap water to remove excess soil adhered to the root zone and then dried on blotter paper to avoid contamination and then cut the diseased portion along with healthy portion into small pieces of 1-2 mm size with the help of sterilized blade and surface sterilized with 1% sodium hypochlorite solution for one minute and later washed in sterile distilled water for 3-4 times to remove traces of sodium hypochlorite. Then sterile plant pieces were dried on sterile blotter paper and placed in Petri plates containing potato dextrose agar (PDA) medium and incubated at 25±1°C for 3-5 days. Further pure culture of the pathogen was developed through hyphal tip method (Singh, 1988) and maintained on PDA medium for further studies.

In the present experiment was conducted to test the antagonistic activity of culture filtrates of the four test bioagents against *F. oxysporium* f. sp. *ciceris* as described by Upadhyay and Rai (1987). For this purpose, antagonist were grown in different liquid media both *T. harzianum* and *T. viride* were grown on potato dextrose broth, where as *P. fluorescens* and *B. subtilis* were grown on King's B broth and nutrient broth respectively, at 25±1°C temperature in incubator. In case of both *Trichoderma* sp., the seven days old cultures were first filtered through double layered cheese cloth followed by filtering through Whatman No. 1 filter paper. Later the culture filtrate so obtained was centrifuged at 10000 rpm at 4°C for 15 minutes then supernatant was collected through bacteria proof filters and stored in refrigerator. Whereas, to obtain the culture filtrates of two bacterial antagonists i.e. *P. fluorescens* and *B. subtilis* 72 hours old cultures were used. The broth media containing the bacterial growth were centrifuged at 10000 rpm for 15 minutes in 4°C and then supernatant was passed through bacteria proof filters was collected and stored in refrigerator for further studies.

In order to study the inhibition antagonistic activity of the culture filtrates of four bio control agents, the respective supernatant was added to potato dextrose agar medium at three different concentrations *viz.,* 1, 2.5 and 5 per cent at the time of pouring of the media in Petri plates. The mycelial discs (5 mm diameter) of actively growing *F. oxysporum* f. sp. *ciceriss* was collected from periphery of culture plates and placed at the center of Petri plates containing PDA medium amended with the respective supernatants/culture filtrates of bio control agents. Three replications were kept for each type of supernatant/culture filtrate. The control plate was maintained without adding supernatant to PDA in three replications. The inoculated Petri dishes were incubated at 25±1°C. The radial mycelial growth of the *F. oxysporium* f. sp. *ciceris* was recorded seven days after incubation. The inhibition of radial mycelial growth by the respective bacterial antagonists was calculated by using the following formula.

$$\text{Percent inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,

C = Mycelial growth of *F. oxysporum* f.sp. *ciceris* in control

T = Mycelial growth of *F. oxysporum* f.sp. *ciceris* in presence of antagonist

RESULTS AND DISCUSSION

The results of the present experiment antagonistic activity of sterile culture filtrate of four antagonistic organisms *viz., T. viride* (Tv-1), *T. harzianum* (Th-1), *P. fluorescens* (Pf-1) and *B. subtilis* (Bs-1) were tested against *F. oxysporum* f. sp. *ciceris* at three different concentrations i.e. 1, 2.5 and 5 per cent in PDA medium revealed that culture filtrate obtained from different antagonistic organisms significantly inhibited the mycelial growth of *F. oxysporum* f. sp. *ciceris* (Table 1). The results shows that with increase in the concentration of cultural filtrates, the radial growth of the test pathogen *F. oxysporum* f. sp. *ciceris* was also inhibited proportionally to the concentration of the culture filtrates/supernatant (Table 1 and 2). Out of three bio control agents *viz., Trichoderma* spp., *P. fluorescens* and *B. subtilis* used in this study *Trichoderma* spp. was found effective in comparison to two other bio control agents.

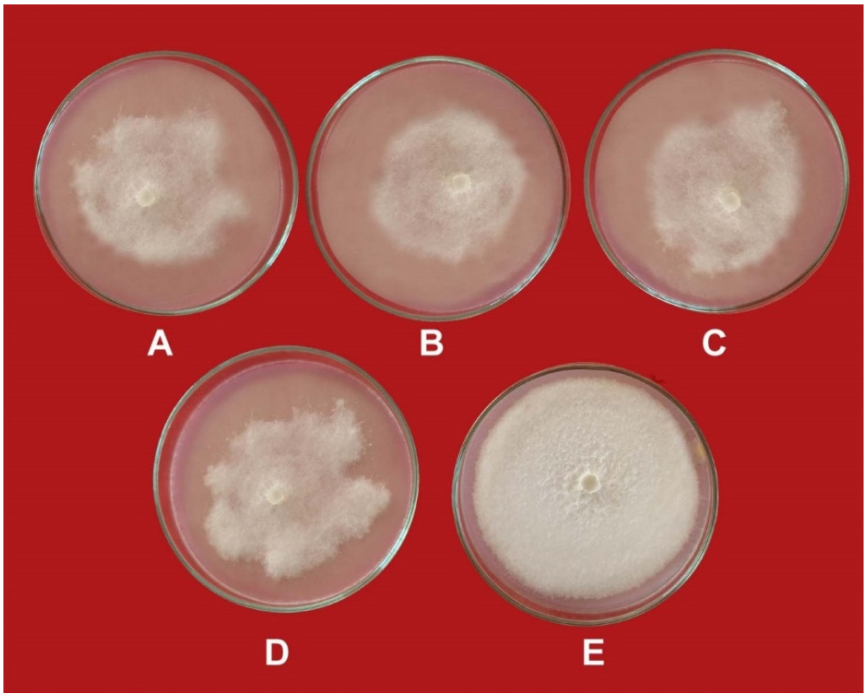
Table 1: Effect of culture filtrate of bioagents on mycelial growth of *F. oxysporum* f. sp. *ciceris* (mm).

Antagonist	Culture filtrate			
	1 per cent	2.5 per cent	5 per cent	Mean
Trichoderma viride (Tv-1)	84.00	78.33	72.67	78.33
T. harzianum (Th-8)	77.67	74.00	68.67	73.44
P. fluorescens (Pf-I)	82.000	80.67	73.33	78.67
B. subtilis (Bs 4-6)	85.00	84.33	84.00	84.44
Control (without culture filtrate)	90.00	90.00	90.00	90.00
Mean	83.73	81.47	77.73	
	S.Em. (+)	CD (P = 0.05)		CV
Antagonist	0.20	0.58		0.75
Culture filtrate concentration	0.16	0.45		
Antagonist X Culture filtrate concentration	0.35	1.01		

Table 2: Inhibition of mycelial growth (%) of *F. oxysporum* f. sp. *ciceris* by culture filtrate of bioagents.

Antagonist	Culture filtrate			
	1 per cent	2.5 per cent	5 per cent	Mean
Trichoderma viride (Tv-1)	6.67	12.96	19.26	12.96
	(14.93)*	(21.10)	(26.03)	(20.69)
T. harzianum (Th-8)	13.70	17.78	23.70	18.40
	(21.72)	(24.94)	(29.13)	(25.26)
P. fluorescens (Pf-I)	8.89	10.37	18.52	12.59
	(17.32)	(18.76)	(25.49)	(20.52)
B. subtilis (Bs 4-6)	5.56	6.30	6.67	6.17
	(13.59)	(14.52)	(14.96)	(14.36)
Mean	8.70	11.85	17.04	12.53
	(16.89)	(19.83)	(23.90)	(20.21)
	S.Em. (+)	CD (P = 0.05)	CV	
Antagonist	(0.24)	(0.70)	(3.58)	
Culture filtrate concentration	(0.21)	(0.61)		
Antagonist ×Culture filtrate concentration	(0.42)	(1.21)		

* Figures in parentheses are angular transformed values



A. *T. viride* (TV-1); B. *T. harzianum* (Th-8); C. *P. fluorescens* (Pf-1); D. *B. subtilis* (BS 4-6); E. Control

Fig. 1. *In vitro* evaluation of culture filtrate of antagonists @ 5% against FOC.

Among the two different *Trichoderma* spp. antagonists the culture filtrate of *T. harzianum* (Th-1) was found effective and toxic to the *F. oxysporum* f. sp. *ciceris* by exhibiting the 23.70 per cent of radial mycelial growth inhibition at 5 per cent concentration with minimum radial growth of 68.67 mm over the control (Fig. 1). The radial growth inhibition of *F. oxysporum* f. sp. *ciceris* in *T. viride* (Tv-1) culture filtrate amended medium at 5 per cent concentration was statistically at par with that of *T. harzianum* (Th-1).

Similarly, the culture filtrate of *P. fluorescens* (Pf-1) also showed 20.21% of inhibition the pathogen radial growth However, the magnitude of inhibition was less as compared to *Trichoderma* spp. isolates tested. Whereas, the *B. subtilis* (Bs-1) proved to be least toxic to *F. oxysporum* f. sp. *ciceris,* which showed almost similar per cent of inhibition i.e. 5.56, 6.30 and 6.67 per cent at different tested concentrations 1, 1.5 and 5 per cent respectively. The mycelial growth inhibition of *B. subtilis* (Bs-1) was statistically at par with control at all the three concentration tested. The results of the present investigation shows that among the four different tested antagonistic organisms *Trichoderma* spp. found effective over the *Pseudomonas* sp. and *Bacillus* sp. Our results are in agreement with Animisha *et al.,* (2012) who found that *Trichoderma* was effectively inhibiting the radial growth of *F. oxysporum* f. sp. *ciceriss* under *in vitro* conditions. Previously Somasekhara *et al*. (1996) also reported that bioagents like *T. viride, T.* and *harzianum* were found effective in controlling pigeon pea wilt caused by *F. oxysporum* f. sp. *udum.*

CONCLUSIONS

The use of antagonistic organisms for the management of chickpea Fusarium wilt disease causing pathogen *F. oxysporum* f. sp. *ciceris* is a effective and relatively safer way over the use of chemical fungicides, which affect the human health and also pollute ecosystem. It is observed from our results, that the *T. viride* and *T. harzianum* are found effective strains to promote the chickpea plant growth in *F. oxysporum* f. sp. *ciceris* challenged conditions, these strains may be commercialize as potential biocontrol agent only after a large filed trials.

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