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RESEARCH PAPER

In vitro efficacy of fungal and bacterial antagonists against *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt

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Abstract : *Fusarium oxysporum* f. sp. *ciceri* is one of the most destructive pathogen, causing wilt disease in chickpea and there by inflicting accountable quantitative (48.29%) as well as qualitative losses. All the six fungal and two bacterial bioagents tested *in vitro*, exhibited significant mycelial growth inhibition of *Fusarium oxysporum* f. sp. *ciceri*. However, *Trichoderma viride* recorded significantly highest mycelial growth inhibition (75.55%), followed by *Trichoderma harzianum* (73.77%) *Trichoderma koningii* (71.88%) and *Pseudomonas fluorescens* (43.77%). Rest of the bioagents tested also caused significant mycelial inhibition of the test pathogen.

Key Words : Fusarium wilt, Fungal bioagents, Bacterial bioagents, Chickpea

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important pulse crop, which belongs to Leguminoceae family, ranking third after dry beans (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum* L.). The centre of origin of chickpea is in Eastern Mediterranean (Aykoid and Doughty, 1964). The kabuli and desi chickpea is grown throughout the world with different names *i.e.*, Chickpea (UK), Garbanzo (Latin America), Bengal gram (India), Hommes Hamaz (Arab world), Shimbra (Ethiopia) and Nohud and Loblebi (Turkey). India is largest producer of chickpea in world sharing 65.25 per cent in area and 65.49 per cent in production. In India, chickpea is grown on 10.23 million ha area with production 9.88 million tonnes and productivity 967 kg/ha. The production of chickpea in Maharashtra is 1.62 million tonnes with productivity 891 kg/ha which covered nearly 1.82 million ha of area. Maharashtra contributes about 16.42 per cent share in total production of country (Anonymous, 2014).

Chickpea grows best as a post-monsoon cool season crop in semi-arid regions of the sub-continent. It takes 80 to 170 days to mature. Optimum conditions for growth include 21 to 29°C nights and 18 to 26°C day's temperature with 600-1000 mm annual rainfall (Muehlbauer *et al.*, 1988 and Duke, 1981). In the dry land areas it fixes atmospheric nitrogen in the soil and helps in the management of soil fertility (Sharma and Jodha, 1984). In addition to source of proteins it has carbohydrate 38-59 per cent, fibre 3 per cent, oil 4.8-5.5 per cent, ash 3 per cent, calcium 0.2 per cent, and

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phosphorus 0.3 per cent. Its protein and carbohydrate digestibility varies from 76 to 78 per cent and from 57 to 60 per cent, respectively (Hulse, 1991; Huisman and Vanderpoel, 1994).

The major limiting factor in chickpea production is Fusarium wilt which is caused by *F. oxysporum* Schlechtend. Fr. f. sp. *ciceris* (Padwick) Matuo and K. Sato (Jalali and Chand, 1992; Haware, 1990 and Nene and Reddy, 1987). It was first reported in Indo-Pak sub-continent (Butler, 1918). McRae (1932) as well as Prasad and Padwick (1939) reported *F. oxysporum* f. sp. *ciceris* pathogenic to chickpea crop which is now accepted worldwide as the causal agent of *ciceri* spp. In general, the disease causes substantial yield losses which may reach even 100 per cent under favourable weather conditions (Jalali and Chand, 1992). The chickpea is cultivated as a rain fed crop in Maharashtra state and yield losses amounted to 10 to 15 per cent (Khilare *et al.*, 2009).

MATERIAL AND METHODS

Dual culture technique :

Six fungal antagonists *viz., Trichoderma viride, T. harzianum, T. hamatum, T. virens, T. koningii, Aspergillus nigar* and two bacterial antagonists *viz., Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated *in vitro* against *Fusarium oxysporum* f. sp. *ciceri*, applying dual culture technique (Dennis and Webster, 1971). Seven days old culture of the test bioagents and the test pathogen (*Fusarium oxysporum* f. sp. *ciceri*) were used for the study. Culture discs (7 mm dia.) of the test pathogen and bioagents (7 mm diameter) were cut out with sterilized cork borer. Then two culture discs, one each of the test fungus and bioagent were placed aseptically at equidistance and exactly opposite with each other on solidified PDA medium in Petri plates and plates were incubated at 28 ± 2^{0}C. Three plates / treatment / replication were maintained. PDA plates inoculated only with culture disc of the test pathogen were maintained as untreated control.

Observations on linear mycelial growth of the test pathogen and bioagent were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth of the test pathogen. Per cent inhibition of the test pathogen by the bioagent over untreated control was calculated by applying formula (Arora and Upaddhyay, 1978).

RESULTS AND DISCUSSION

The results obtained on mycelial growth and inhibition of *Fusarium oxysporum* f. sp. *ciceri* with six fungal and two bacterial antagonists are presented in Table 1. Results revealed that all the bioagents evaluated, exhibited fungistatic/antifungal activity against *Fusarium oxysporum* f. sp. *ciceri* and significantly inhibited its growth over untreated control.

Of the six fungal antagonists tested, *Trichoderma viride* was found most effective and recorded least linear mycelial growth (22.00 mm) with highest mycelial inhibition (75.55%) of the test pathogen. The second and third best antagonists found were *Trichoderma harzianum* and *Trichoderma koningii*, which recorded mycelial growth of 23.60 mm and 25.30 mm and mycelial inhibition of 73.77 and 71.88 per cent, respectively. This was followed by fungal antagonist *Aspergillus niger, Trichoderma hamatum* and *Trichoderma virens* were found least effective which recorded 26.30, 27.00 and 31.30 mm linear mycelial growth and 70.77, 70.00 and

Table 1 : *In vitro* bio-efficacy of bioagents on mycelial growth and inhibition of *Fusarium oxysporum* f. sp. *ciceri*

Treatments	Treatments	*Growth of the pathogen (mm)	Average inhibition (%)
T_1	*Trichoderma viride*	22.00	75.55 (60.36)
T_2	*Trichoderma harzianum*	23.60	73.77 (59.19)
T_3	*Trichoderma koningii*	25.30	71.88 (57.97)
T_4	*Trichoderma hamatum*	27.00	70.00 (56.79)
T_5	*Trichoderma virens*	31.30	65.22 (53.86)
T_6	*Aspergillus niger*	26.30	70.77 (57.27)
T_7	*Pseudomonas fluorescens*	50.60	43.77 (41.42)
T_8	*Bacillus subtilis*	52.00	42.22 (40.52)
T_9	Control (untreated)	90.00	00.00 (00.00)
	C.D. (P=0.01)	0.36	
	S.E. ±	0.09	

*Mean of three replications Figures in parenthesis are arc sine transformed value

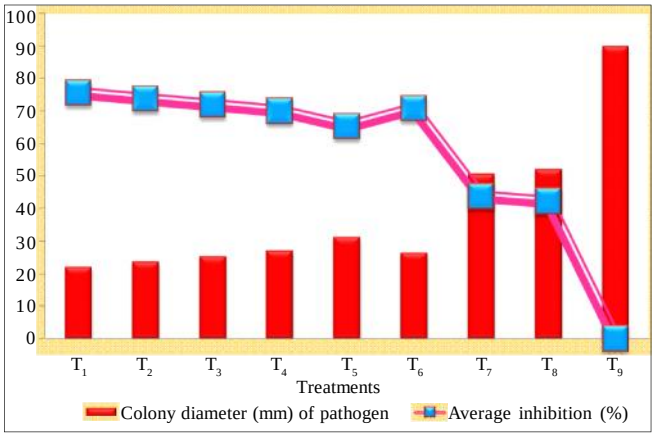

Fig. 1 : Bio-efficacy of bioagents on mycelial growth and inhibition of *Fusarium oxysporum* f. sp. *ciceri*

65.22 per cent mycelial inhibition, respectively. The bacterial antagonists *Pseudomonas fluorescens* and *Bacillus subtilis* were also found fungistatic and recorded 50.60 mm and 52.00 mm linear mycelial growth and 43.77 and 42.22 per cent mycelial inhibition, respectively of the test pathogen.

Thus, all the fungal and bacterial bioagents tested were found fungistatic against *Fusarium oxysporum* f. sp. *ciceri* and significantly inhibited its mycelial growth over untreated control. However, fungal and bacterial bioagents found most effective in the order of merit were *Trichoderma viride*, *T. harzianum*, *T. koningii*, *Aspergillus niger*, *T. hamatum*, *Trichoderma virens*, *Pseudomonas fluorescens* and *Bacillus subtilis*.

The effective *Trichoderma* isolates of present study may be utilized in combination with other management practices or with other bioagents for enhancing their effect. A few workers have also tested *Trichoderma* spp. in dual culture against *Fusarium oxysporum* f. sp. *ciceri*. Chavan (2004) and Korde (2011) reported that maximum zone inhibition of radial growth of fungus was observed with *Trichoderma viride* followed by *T. koningii*, *T. harzianum* and *P. fluorescens*. Kapoor *et al.* (2012) also reported that maximum zone inhibition of radial growth of fungus was observed with *Trichoderma viride* followed by *T. harzianum* and *A. niger*. Least zone inhibition was recorded with *T. virens*. Magar (2012) and Mehta *et al.* (2012) reported that maximum zone inhibition of radial growth of fungus was observed with *Trichoderma viride* followed by *T. harzianum*, *Aspergillus niger*, *T. virens* and *B. subtitis*. Yadav *et al.* (2014) reported that most effective *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *ciceri* which recorded 71.36 per cent growth inhibition.

The similar results on efficacy of *Trichoderma* spp. and *P. fluorescens* were obtained by Sangle and Bambawale (2004); Srivastava and Mall (2008); Mulik (2009); Patil (2010) and Andrabi *et al.* (2011).

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