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In vitro Evaluation of *Trichoderma* spp. against Chickpea Wilt

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ABSTRACT

Management of chickpea wilt by chemical fungicides can be spectacular but this is relatively short term measure. So, in the present study, the mycoparasitism inhibitory effects of three Trichoderma species (T. harzianum, T. virideand T. koningii) on the growth of the causal agent of Fusarium wilt of chickpea (Fusarium oxysporum f.sp. ciceri) were investigated by dual culture under in vitro conditions. Results showed that bio-agents significantly reduced the wilt incidence of chickpea.

Key words: *Chickpea, antagonism, Fusariumoxysporum, Trichoderma, wi*lt.

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INTRODUCTION

Pulses are important sources of protein for vegetarian population [9]. Out of all pulses, chickpea (*Cicer arietinum* L.) is an annual legume and the only cultivated species within genus *Cicer* [1, 12]. In India, chickpea accounts for about 45% of total pulses production [10]. Chickpea is valued for its nutritive seed composition which is high in protein content and provides nutritious food for an expanding world population and will become increasingly important with climate change [11]. More than fifty soil and seed borne pathogens have been reported on chickpea which act as a constraint in their production [13].

Chickpea wilt is caused by *Fusarium oxysporum* Schlechtend Fr. f. sp. *ciceri* (Padwick) Matuo& K. Sato, is the most important soil-borne disease [16]. Efficacy of wilt management was improved when bio-control agents were combined with cultural practices such as sowing date [8]. Biological control provides an alternative to the use of synthetic pesticides with the advantages of greater public acceptance and reduced environmental impact [17]. *Trichoderma* spp. have gained wide acceptance as effective bio-control agents against several phytopathogens [23]. The species of *Trichoderma* have been evaluated against the wilt pathogen and have exhibited greater potential in managing chickpea wilt under field condition [15]. Considering these points in mind, the present study was conducted to find out the most effective species of *Trichoderma* and fungicides against *Fusarium* wilt of chickpea.

MATERIAL AND METHODS

The experiment was conducted in the Laboratory of School of Agriculture, Lovely Professional Univesity, Punjab, India. Isolation and purification of the test pathogen was done from infected vascular tissues of stem and root regions of chickpea (*Cicer arietinum*).
Soil from the rhizospheric zone of healthy chickpea plants were collected from different locations. The identified Trichoderma antagonists *viz.*, *Trichoderma harzianum*, *Trichoderma viride, Trichoderma koningii* and *Trichoderma longibrachiatum* were isolated by serial dilution technique using *Trichoderma* selective medium (TSM) and compared with the isolate maintained in the laboratory [3].

Dual Culture Assay of *Trichoderma* against the test pathogen:

Species of *Trichoderma viz.,Trichoderma harzianum*, *Trichoderma viride, Trichoderma koningii* and *Trichoderma longibrachiatum* were evaluated for relative viability against *Fusarium oxysporum* f. sp. *ciceri*by dual culture techniques [1].About 9 mm breadth circle of test growth and the hostile organisms, cut as bit from the edge of five days old culture were put inverse to each other at a separation of 5 mm from the fringe of Petriplate. Same circle of test organism was set another Petri plate one side on PDA plate, which filled in as control. Every treatment was recreated 3 times and hatched at 25±2°C. The plates were analyzed following 7 days for the development of restraint zones amongst Trichoderma and pathogen. The experiment was replicated thrice and percent growth inhibition was calculated by using the formula [22]:

$$L = \frac{C - T}{C_{-\times}} \times 100$$

Where:
L = Inhibition of radial mycelial growth
C = Radial growth measurement of the pathogen in the control
T = Radial growth measurement of the pathogen in the presence of antagonists

RESULTS

In vitro evaluation of different biocontrol agents against *Fusarium oxysporum* f. sp. *ciceri*: Biological management of plant pathogens by employing potential bioagents has been an important component of non-chemical plant disease management. Extensive study was under taken through in vitro screening of four bioagents to as certain their potential as suitable bio-pesticides against *F. oxysporum* f. sp. *ciceri*. Analysis of data presented in Table 1 indicated that *Trichoderma koningii* was very effective after 48 hours in controlling *F. oxysporum* f. sp. *ciceri* where inhibition zone formation was highest (79.28%) followed by *Trichoderma viride* and *Trichoderma harzianum* with inhibition zone of 77.14% and 74.28 %, respectively.

Table 1: *In vitro* antagonistic activity of *Trichoderma* spp. against *F. oxysporum* f.sp.*ciceri* at 48 hours.

Treatments		Radial Growth (mm)		Inhibition Growth Percentage
		Antagonists	FOC	
T1	*Trichoderma harzianum*	18.6	3.60	74.28 %
T2	*Trichoderma viride*	22.8	3.20	77.14 %
T3	*Trichoderma koningii*	13.1	2.90	79.28 %
T0	Control	-	14.0	

(FOC = *Fusarium oxysporum*f.sp. *ciceri***)**

Data presented in Table 2 revealed that *Trichoderma harzianum* was very effective after 72 hours in controlling *F. oxysporum* f.sp. *ciceri* where inhibition zone formation was highest (83.87%) followed by *Trichoderma viride* and *Trichoderma koningii* with inhibition zone of 60.00% and 50.32%, respectively.

Table 2: *In vitro* antagonistic activity of *Trichoderma* spp. against *F. oxysporum* f.sp.*ciceri* at 72 hours.

Treatments		Radial Growth (mm)		Inhibition Growth Percentage
		Antagonists	FOC	
T1	*Trichoderma harzianum*	34.1	5.0	83.87 %
T2	*Trichoderma viride*	33.5	12.4	60.00 %
T3	*Trichoderma koningii*	31.9	15.4	50.32 %
T0	Control	-	31.0	

(FOC = *Fusarium oxysporum*f.sp. *ciceri***)**

Data presented in Table 3 revealed that again *Trichoderma harzianum* was very effective after 96 hours in controlling *F. oxysporum* f. sp. *ciceri* where inhibition zone formation was highest (85.55%) followed by *Trichoderma viride* and *Trichoderma koningiii* with inhibition zone of 69.55% and 62.66%, respectively.

Table 3: *In vitro* antagonistic activity of *Trichoderma* spp. against *F. oxysporum* f.sp.*ciceri* at 96 hours.

Treatments		Radial Growth (mm)		Inhibition Growth Percentage
		Antagonists	FOC	
T1	*Trichoderma harzianum*	39.6	6.50	85.55 %
T2	*Trichoderma viride*	39.0	13.7	69.55 %
T3	*Trichoderma koningii*	35.8	16.8	62.66 %
T0	Control		45.0	

(FOC =*Fusarium oxysporum*f.sp. *ciceri***)**

DISCUSSION

From the above findings it was proved that *Trichoderma harzianum* and *Trichoderma koningii* was very effective against wilt pathogen of chickpea. Earlier it was proved by many scientists that *T. harzianum* were effective in control of *F. oxysporum* f.sp. *ciceri* [4], *F. oxysporum* f.sp. *cubense* [21] *Fusarium solani* [2], *F. oxysporum*f.sp. *dianthi* [8], *F. oxysporum* f.sp. *psidii* [20] and *F. oxysporum* f.sp. *lycopersici* [19]. The present study is in agreement with Padwick [14] who reported that a species of *Trichoderma* was highly antagonistic to gram chickpea wilt pathogen under field conditions. Khodzhayan [6] found that *Trichoderma* spp., released antibiotic substance in the nutrient media which killed *F. oxysporum* pathogen. Kirik and Steblyk [7] stated that *Trichoderma koningii* was strong inhibitor to *Fusarium oxysporum* and *Fusarium culmorium*. Kaur and Mukhopadyay [5] reported that chickpea wilt complex disease was effectively controlled by *T. harzianum* alone and in combination with fungicides.

So, it was concluded that eco-friendly management practices, *i.e.*, use of bio-control agents gave better results and these practices can be economical, long lasting and free from residual side effects.

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