In vitro antagonism of *Trichoderma spp.* against *F. oxysporum f. sp. Ciceris*

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Abstract: Trichoderma spp. have been developed into several commercial biological control products used in field crop and greenhouse systems and are known to control numerous soil-borne diseases, such as those caused by *Fusarium oxysporum f. sp. Ciceris* . The present study was carried out to assess the efficacy of microbial antagonist for Fusarium wilt of chickpea . Biological control agents for plant diseases are currently being examined as alternatives to synthetic pesticides due to their perceived increased level of safety and minimal environmental impacts. Fungal biological control agents have several mechanisms of action that allow them to control pathogens, *Trichoderma* spp. has been widely used as antagonistic fungal agents against several pests. Among the three *Trichoderma* spp. tested in vitro (*T. harzianum*, *T. viride* and *T. koningii*), for antagonistic potentials against the fungus T. harzianum showed the best performance (89.8% inhibition) followed by T. viride (85.7% inhibition), and T. koningii (53 % inhibition).

Keywords: *Trichoderma* spp., Antagonism,Fusarium, *oxysporum. sp. Ciceris*, Chickpea

1 INTRODUCTION

Chickpea (Cicer arietinum L.) is a vital source of plant- derived edible protein in many countries. Chickpea also has advantages in the management of soil fertility, particularly in dry lands and the semiarid tropics (Singh and Saxena, 1996). Indian subcontinent accounts for 90% of the total world chickpea production (Juan et al., 2000). Yet chickpea yields (0.88 tons/ha) in Bangladesh (BBS, 2011) have fallen below expectation. Low yield of chickpea attributed to its susceptibility to several fungal, bacterial, and viral diseases. Among the diseases affecting chickpea, vascular wilt caused by an important obligate biotroph *Fusarium oxysporum f. sp*. Ciceris. The disease is wide spread in the chickpea growing areas of the world, it is most prevalent in the Mediterranean Basin and the Indian subcontinent (Jalali and Chand, 1992). Fusarium wilt epidemics cause significant annual losses of chickpea yields which, account for 10 to 15% of the total yield and sometimes escalate to 100% under conditions favorable for disease (Navas-Cortés et al., 2000). F. oxysporum f. sp. ciceris infects chickpea at seedling as well as at flowering and pod forming stage (Grewal, 1969), with more incidence at flowering and podding stage if the crop is subjected to sudden tem

perature rise and water stress (Chaudhry et al., 2007). Following infection of host roots, the fungus enters the xylem tissues and spreads rapidly up through the vas cular system, becoming systemic in the host tissues, and may directly infect the seed. Translocation of water and nutrients is severely prevented by blockage of vessels, resulting in stomatal closure, wilting and death of leaves, often followed by death of the whole plant (Cho and Muehlbauer, 2004). Early wilting causes more loss than late wilting, but seeds from late-wilted plants are lighter, rough and dull than those from healthy plants (Haware and Nene, 1980). F. oxysporum f. sp. ciceris can survive as mycelium and chlamydospores in seed and soil, and also on infected crop residues, roots and stem tissue buried in the soil for up to 6 years (Singh et al., 2007). The disease is primarily managed by resistance bree ding programs. But high pathogenic variability and muta bility limit the sustainability and effectiveness of any naturally selected resistance against the pathogen (Nimalkar et al., 2006). Management of Fusarium wilt with fungicides is uneconomical and difficult to achieve because of the soil and seed-borne nature of the pathogen (Ahmad et al., 2010). Moreover, the application of fungicides causes groundwater pollution, loss of non- target beneficial flora and evolving fungicidal resistance variants of the pathogen. The recontamination of the pathogen in the fungicide-treated soil often flourishes faster due to the absence of competitive microflora leading to higher incidence of disease in susceptible host (Jamil et al., 2010). As such in the present context, biological management of wilt with bioagents offers a great promise. Trichoderma harzianum is one efficient biocontrol agent that is successfully used to suppress Fusarium wilt (Khan et al., 2004; Dubey et al., 2007). The *Trichoderma* species (*T. viride*, *T. harzianum*, *T. longibrachiatum*, *T. hamatum*, *T. koningii* and *T. longibrachiatum*) are very promising against phtyopathogenic fungi such as *F.oxysporum*, *Pythium ultimum* and *Sclerotinia sclerotium* (Manczinger *et al*., 2002). For isolation of antagonist soil should be collected whenever possible from the rhisosphere of the host to be protected rather than from the soil mass (Baker *et al* 1979). Wang *et al*. (1999) described the fast growth rate of *Trichoderma* species.

2MATERIALS&METHODS

2-1 Isolation of *Fusarium oxysporum*

2-1-1 Isolation from Plant materials

Infected chickpea roots showing symptoms of the disease were obtained from sick blots from Shambat Research Station in September, 2009. The roots were cut into small sections (0.5-1.0 cm), washed thoroughly with tap water, surface sterilized with Clorox (NaOCL) for 5 minutes, rinsed three time in changes of sterilized distilled water and dried on sterilized filter papers. The sterilized roots sections were plated at the rate of five sections/ plate onto potato dextrose agar (PDA) medium supplemented with chloramphenicol (0.05 g/L) in 9-cm Petri dishes. The Petri dishes were incubated at 25^0C. After incubation for 7 days, isolated fungi were subculture on PDA. When free from contamination; Isolates were maintained on PDA slants and examined visually for their growth patterns and pigmentation on the adverse side of the agar. Further microscopic examinations were carried out for mycelia and conidia structure using pure culture of *F. oxysporum* f.sp.*ciceri* was obtained by using Hyphal Tip Technique. Pure culture of the isolated fungi was transferred to PDA slants and kept in refrigerator at 40°C for further use. Sample of the obtained colonies were sub cultured by transferring small mycelia from the colony margins. Pure cultures were obtained by sub-culturing three times and slides were prepared and examined microscopically to confirm identity (x: 40).

2-1-2 Isolation from Soil Sample

Soil samples, 1gram each, were collected from the vicinity of the roots of infected plants. The samples, bulked, were thoroughly mixed and 1g sample was randomly taken. The soil suspensions of different dilutions were prepared. One milliliter of each dilution was uniformly spread over PDA. The obtained colonies were sub cultured on PDA plates by transferring small mycelial from the colony margins. Pure culture was obtained by sub-culturing three times. The fungus was identified based on morphology and colony characteristics using the method of Watanabe (2000).

2-2 Identification of the pathogens

The identification of the fungus was based on visual culture characteristics, mainly the growth patterns and pigmentation. Furthermore, microscopic examinations were carried out for mycelial and conidia structure based on the methods of Booth's key (1977).

2-3 Effects of Trichoderma spp. On the growth of F. oxysporum in vitro

The experiment was laid out in Petri dishes containing sterilized PDA. Half of the solidified medium was inoculated with the fungus from 7 days old culture as in 5 mm discs. The second half was inoculated with a *Trichoderma* spp (*T harzianum, T.viride and T. koningii*). Thus, both organisms would get equal opportunities for growth. One plate containing the test fungus only was included as control. The individual treatments were replicated three times. The petridishes were incubated at 25^{o}C and fungal growth was estimated daily and percent growth inhibition was calculated .

2-4 Statistical Analysis Procedure

Data generated was subjected to Gen stat Two-way ANOVA .Design analysis

3 RESULTS

3-1 Effects of *Trichoderma spp* on radial growth of *F. oxysporum*

The results indicated that all Trichoderma species suppressed *F. oxysporum* growth rate (Fig.1). Growth of *F. oxysporum* was inhibited by encroachment of Trichoderma from all sides of the pathogenic fungus (Fusarium).At 3days after incubation Fusarium growth was similar in all treatments. However, at 5and 7days the fungus growth was significantly inhibited by all *Trichoderma spp* (Plates 1 to 3). The fungus on the control medium was 8.2cm in Petri dishes at 7 days after commencement of the experiment. All the *Trichoderma spp* resulted in significant inhibition of the fungus *T.harzianum ,T. viride*, and *T. koningii* inhibited the fungus mycelial growth by 1, 1.6 and 2.1%, respectively.

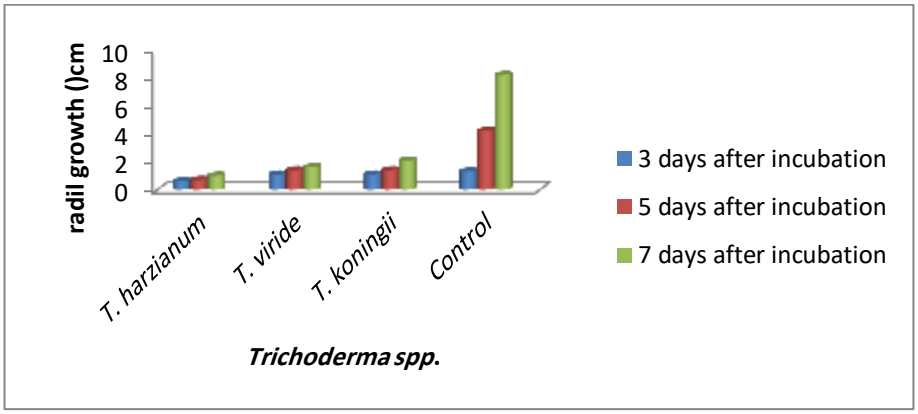

Fig 1 Effects of *Trichoderma spp.* on redial growth of *F. oxysporum*

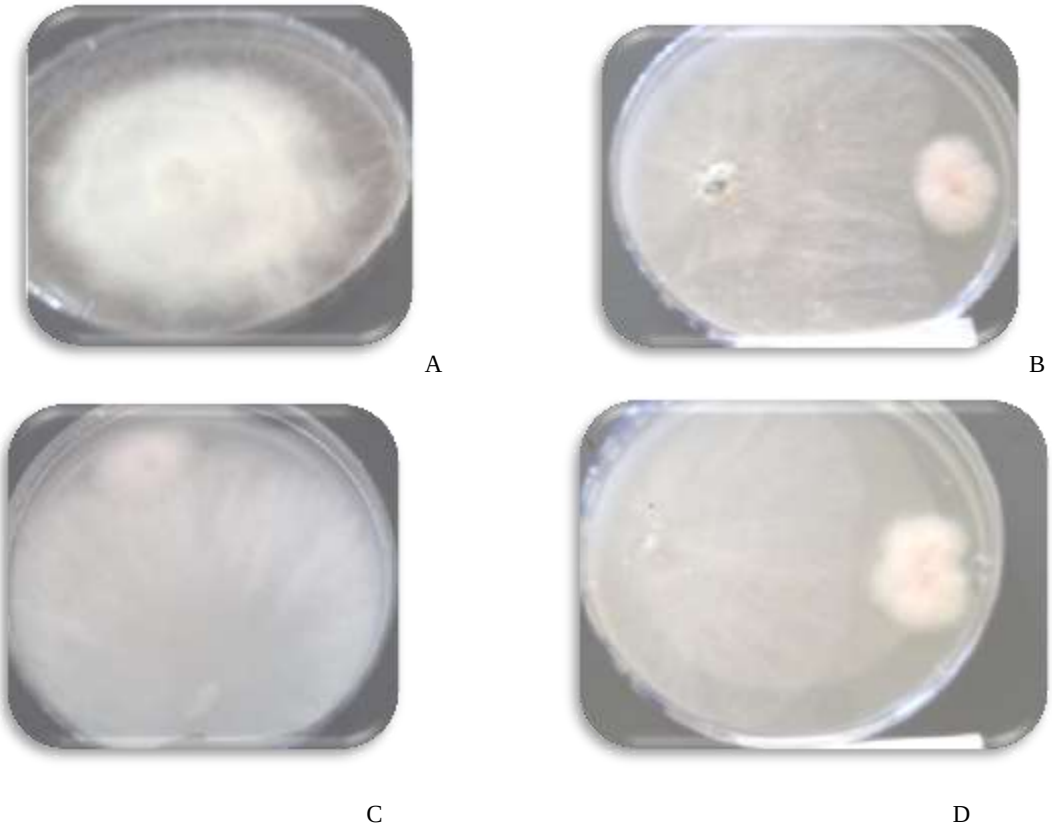

Plate 1 Redial growth of *F. oxysporum* as influence by *Trichoderma spp* (7days-old culture). A- Control B- *T. viride C- T.harzianum D- T. koningii*

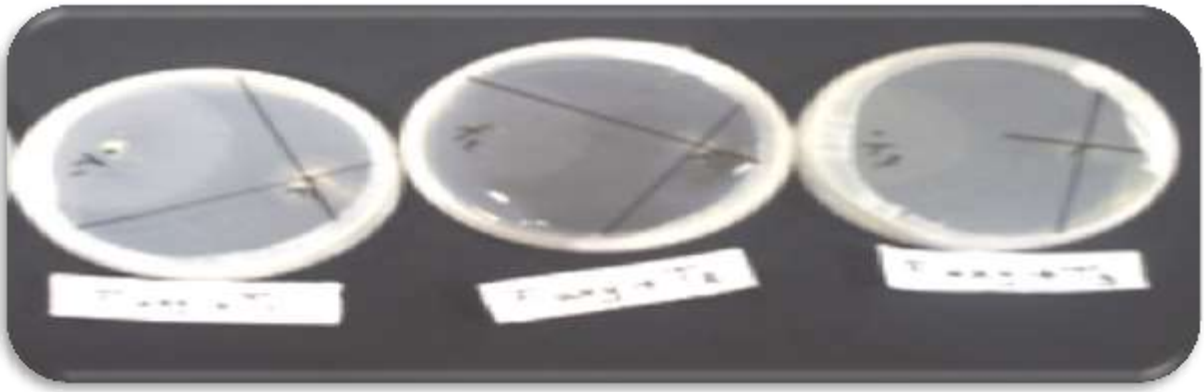

Plate 2 Redial growth of *F. oxysporum* as influence by *Trichoderma spp* (7days-old culture).

Plate 3 Redial growth of *F. oxysporum* as influence by *Trichoderma spp* (7days-old culture).

3-2 Effects of *Trichoderma spp* on chickpea germination and growth

3-2-1Effects on germination:

The results of germination as influenced by *Trichoderma spp.* alone and in presence of the pathogen improved germination considerably (Table 1).Fusarium free Trichoderma treated chickpea seeds displayed 100% germination. *Trichoderma spp* decreased germination of Fusarium treated seeds. Untreated Fusarium free seeds slowed 60 and 73 germination. Fusarium depressed germination to 60 and 53.4% in Shendi and Jebelmara varieties, respectively.

Table 1 Effects of Trichoderma spp. on germination in chickpea

Treatment	Germination (%)	
	chickpea varieties	
	Shendi	Jebelmara
T. Viride	100 %	100 %

T. Harzianum	100 %	100 %
T. Koningii	100 %	100 %
T. viride +F	73.2 %	80.0 %
T. Harzianum +F	80.0 %	80 .0%
T. Koningii +F	80.0 %	80.0 %
Untreated control	60.0 %	73.2%
Fusarium alone	40 .0%	53.4%
SE+	o.1623	
Lsd0.05=	0.4687	
CV%	6.89%	

Effects on plant height:

Fusarium oxysporum reduced chickpea height by 40.00 and 53.40% in Shendi and Jebelmara varieties, respectively. All Trichoderma treatments increased height of chickpea over the untreated controls. The height was highest when the soil treated with *T. harzianum* (30.03 cm). The height was lowest when the soil treated with *T.koningii* +Fusarium (22.55 cm) Table (2). Significant differences were observed between treatments with and without Fusarium and between controls (11.55 cm and 17.92cm) treated and untreated respectively, .The highest height was attained by *Trichoderma spp.* alone .The lowest height was attained by in combination with the pathogen

Effects on dry weight shoots:

The results of the dry weights were recorded in Table (3) ranged between 24.63g to 8.83g in cv. Shendi and 30.67g to 23.43g in cv. Jebelmara. Significant differences were observed between treatments with and without Fusarium, but absent between chickpea. The mean dry weight was highest when chickpea seeds were treated with *T. harzianum*, *T.viride* and *T.koningii* (7.45, 6.78 and 5.97g) respectively, which were all significantly greater than the controls (treated and untreated).

Table 2 Effects of *Trichoderma spp* on chickpea height (cm)

Treatment	Plant height (cm)	
	Time after sowing (WKS)	
	4	8
T. viride	21.20	26.78
T. Harzianum	23.47	30.03
T. Koningii	18.98	25.60
T. viride +F	18.40	24.40

T. Harzianum +F	21.13	26.98
T. Koningii +F	17.03	22.55
Untreated control	17.92	22.30
Fusarium alone	11.55	14.37
SE+	.437	.496
Lsd(.05)=	1.259	1.429
CV%	7.5	4.6

Effects on dry weight roots:

Fusarium reduced dry weight of chickpea by 24.63-8.83g and 30.67-23.43g in Shendi and Jebelmara varieties, respectively (Table 3). Significant differences were observed between treatments with and without fusarium but absent between chickpea. Trichoderma applied to Fusarium free chickpea increased dry weight considerably over the Fusarium free control. All *Trichoderma spp* increased dry weight comparison to the Fusarium infested control. The mean dry weight was highest when chickpea seeds were treated with *T. harzianum*, *T.viride* and *T.koningii* (7.45, 6.78 and 5.97g) respectively, which were all significantly greater than the controls (treated and untreated).

Effects on disease incidence:

In the Fusarium infested controls the disease incidence was 63.33 and 100% 4 and 8 WAS, respectively (Table 4). All *Trichoderma species* reduced disease incidence 10.00 % to 73.33% in cv. Shendi and 6.67% to 53.33% in cv. Jebelmara Table (4). The reductions in disease incidence were 23.33 to 30.00% 4 and WAS, respectively. Significant differences were observed between treatments with and without Fusarium but no significant difference observed between chickpea varieties. The statistical analysis revealed that *T. harzianum* in two varieties significantly reduced the disease incidence compared to the other treatments. The least disease incidence of 46.67% to 70.40% were recorded from chickpea plants at 4 weeks after growing while 66.67% to73.33% was recorded from chickpea plants at the end of 8 weeks after growing of the same season.

Table 3 Effects of *Trichoderma spp* on chickpea dry weight (g) of (Shoots, Roots) plant

Treatment	Dry weight (g)	
	shoots	Roots
T. viride	6.78	2.483
T. Harzianum	7.45	3.050
T. Koningii	5.97	2.233
T.viride +F	5.40	1.883
T. Harzianum +F	6.30	2.150
T. Koningii +F	4.88	2.000
Untreated control	4.92	1.283

Fusarium alone	3.30	.850
SE+	.903	.154
Lsd(.05)=	2.602	.446
CV%	39.3	19.1

Effects on disease severity:

The Fusarium inoculated control displayed a disease severity of 38.3 to 53.33 % 4 and 8 WAS, respectively (Table4). All *Trichoderma species* reduced disease severity significantly (Table4). Significant differences were observed between treatments with and without fusarium but no significant difference observed between chickpea varieties. The least disease severity of (38.37% to 53.33% were recorded from chickpea plants at the first (4 weeks after growing) while 78.33% to 81.69% were recorded from chickpea plants at the end of 8 weeks after growing of the same season. In comparison to the infested control *T. viride* reduced disease severity by 46.70 to 61.20% 4 and 8 WAS, respectively. The corresponding reductions for *T. harzianum* were 50.70 to 73.33%. *T. koningii* reduced disease severity by 46.70 to 60% 4 and 8 WAS, respectively.

Effects on yield

Fusarium wilt reduced grain yield by 73%. All *Trichoderma species* increased yield significantly, *T. viride* increased grain yield by 83.22%. *T. harzianum*, on the other hand, increased yield of chickpea by 92.34%. The corresponding yield increasing affected by *T. koningii* were 80%.

Table 4 Effects of *Trichoderma spp* on chickpea wilt incidence and severity (weeks)

Treatment	Disease incidence (%)		Disease severity (%)	
	Time after sowing (weeks)			
	4	8	4	8
T. viride	0.00	0.00	0.00	0.00
T. Harzianum	0.00	0.00	0.00	0.00
T. Koningii	0.00	0.00	0.00	0.00
T. viride +F	20.00	40.00	25.00	37.50
T. Harzianum +F	13.33	20.00	21.00	25.00
T. Koningii +F	20.00	40.00	25.00	38.33
Control	43.33	70.00	45.80	80.00
Fusarium alone	63.33	100.00	71.70	98.33
SE+	2.357	1.676	3.20	1.250
Lsd(.05)=	6.760	4.801	9.22	3.601
CV%	28.9	21.1	33.2	8.8

Table 5Effects on germination and yield (g)

Treatment	Germination (%)	Yield (g)
T. viride	100	5.07
T. Harzianum	100	5.73
T. Koningii	80	4.33
T. viride +F	76.6	4.40
T. Harzianum +F	83.2	5.22
T. Koningii +F	80	4.21
Untreated control	66.6	2.88
Fusarium	46.6	1.65
SE+	.117	.160
Lsd(.05)=	.339	.462
CV%	7.1	9.4

CONCLUSION:

Trichoderma species play an important role in controlling fungal plant pathogens, especially soil borne fungal pathogens. The use of Trichoderma-based products is not only safe for the farmers and consumers but it is also good for the environment. However, much more work needs to be done to develop stable, cost effective, easy to produce and easy to apply formulations

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