Int.J.Curr.Microbiol.App.Sci (2018) Special Issue-7: 595-599

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-7 pp. 595-599**
Journal homepage: http://www.ijcmas.com

Original Research Article

Evaluation of *Trichoderma* sp. against Fusarium Wilt of Chickpea Caused by *Fusarium oxysporum* f. sp. *ciceris* under in vitro Condition

Kunal Mahajan[1*], Jitendar Kumar Sharma[1] and Ankita Dhage[2]

[1]Department of Plant Pathology, School of Agriculture, Rai University, Ahmedabad, India
[2]Department of Microbiology and Biotechnology, Nabira Mahavidhyalaya, Katol, Dist. Nagpur, India
**Corresponding author*

Keywords

Chickpea, Fusarium oxysporum f. sp., ciceri, Trichoderma and wilt

A B S T R A C T

Fusarium wilt (*Fusarium oxysporum* f. sp., *ciceri*) is one of the major yield limiting factors of chickpea (*Cicer arietinum*). For eco-friendly and sustainable management of the disease, Five species of different antagonists (*Trichoderma fasciculatum*, *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma Koningi* and *Trichoderma atroviride*) were evaluated against the pathogen. The study was carried out under *Invitro* conditions. Results showed that TVS-1 alone significantly inhibited the mycelial growth of the pathogen. Results of the study show that bio-agents significantly reduced the wilt incidence of chickpea.

Introduction

Pulses are important sources of protein for vegetarian population. Chickpea (*Cicer arietinum* L.) commonly known as gram is an important pulse crop. It is the world's fourth most important pulse crop after soybeans (*Glycine max* L.), beans (*Phaseolus vulgaris* L.) and peas (*Psium sativum* L.)(FAO 2012). In India, chickpea is ranked first in terms of production and consumption in the world. About 65% of global area with 68% of global production of chickpea is contributed by India (Amarender, *et al.*, 2010). Low yield of chickpea is attributed to its susceptibility to several fungal, bacterial and viral diseases. *Fusarium* wilt caused by *Fusarium oxysporum* Schlechtend Fr. f. sp. *ciceri* (Padwick) Matuo & K. Sato, is the most important soil borne disease of chickpea throughout the world and particularly in the Indian Subcontinent, the Mediterranean Basin and California (Nene, *et al.*, 1987). At the national level, chickpea yield losses encounter due to wilt may vary between five to ten percent (Dubey, *et al.*, 2007). Since the pathogen is both seed and soil borne, drenching with fungicides is very expensive and impractical.

Fusarium oxysporum f. sp. *Ciceri* is a facultative saprophytic and it can survive as mycelium and chlamydospores in seed, soil and also on infected crops residues, buried in the soil for up to five to six years

(Haware, *et al.*, 1982) Therefore, integrated disease management strategies are the only solution to maintain plant health. These strategies should include minimum use of chemicals for checking the pathogen pollution, encouragement of beneficial biological agents to reduce pathogen inoculum, modification of cultural practices and use of resistant varieties (Bendre *et al.*, 1998). In beneficial biological agent, *Trichoderma*, is a filamentous fungi which have attracted the attention because of their multi prong action against various plant pathogens (Harman, *et al.*, 2004). Several modes of action have been proposed to explain the biocontrol of plant pathogens by *Trichoderma*, these include production of antibiotic and cell wall degrading enzymes, competition for key nutrients, parasitism, stimulation of plant defense mechanisms and combination of those possibilities (cook, *et al.*, 1985) Trichoderma spp. generally grows in its natural habit on plant root surface and therefore it controls root diseases in particular (Faruk, *et al.*, 2002, Kamlesh and Gujar 2002 and Monte, 2001). The species of *Trichoderma* have been evaluated against the wilt pathogen and have exhibited greater potential in managing chickpea wilt under field condition (Podder *et al.*, 2004) Considering these points, the present study was conducted to find out the most effective species of *Trichoderma* and fungicide against chickpea *Fusarium wilt.*

Materials and Methods

Isolation and purification of pathogens

Infected vascular tissues from stem and root regions of chick pea (*Cicer arietinum*). Showing wilt symptoms were collected separately from farmer's field. Tissue bits were surface sterilized with 10 per cent sodium hypochlorite for 5- 10 min. and subsequently three washings with sterile distilled water. Then, they were placed on potato dextrose agar (PDA) medium separately and incubated at the laboratory conditions at 25 ± 3^0 C for five day.

The fungi were purified separately by transferring the tip of the mycelia into PDA slants and maintained as stock cultures for further studies

Isolation and maintenance of fungal native antagonists from tomato rhizosphere soil

Rhizosphere soil from healthy chick pea plants were collected from different locations. The identified *Trichoderma* antagonists *viz., T. Fasciculatum, T. harzianum, T. viride, T. koningi* and *Trichoderma atroviride* were isolated by serial dilution technique using *Trichoderma* selective medium (TSM) and compared with the isolate maintained in the laboratory (Elad and Chet, 1983)

In vitro effect of Trichoderma antagonists against FOL pathogen

Dual culture technique as described earlier was followed. Nine mm disc of fifteen days old fungal cultures were placed on PDA medium one cm away from the edge of the plate, separately. *Trichoderma* spp. (9 mm disc) was placed at opposite side of the Petri plate. Three replicated plates for each treatment was maintained and incubated at 25±3oC. Per cent inhibition over control was calculated (Vincent, J.M. 1927) as per the formulae.

$$PI = \frac{C - T}{C} \times 100$$

Where,

PI = Per cent inhibition over control

C = Growth of test pathogen with absence of antagonist (mm)

T = Growth of test pathogen with antagonist (mm)

Results and Discussion

Effectiveness of native Trichoderma antagonists on wilt incidence under *invitro* conditions. The application of *Trichoderma* native antagonists through dual culture techniques was found effective in suppressing wilt incidence (by 90.67%). Conspicuously, an application of *Trichoderma viride*-1 (Rizospere of Badarkha) antagonistic fungal formulation was recorded least wilt incidence (by 6.26 %) at par THCh-2 (Navsari isolates) by (6.80%), TVS-5 (KVK Amt) by (6.73%), THCh-3(KVK Amt) by (6.83) compared to other isolates (Table 1).

Fungal species belonging to the genus *Trichoderma* are worldwide in occurrence and easily isolated from the soil.

The potential of *Trichoderma* species as bioconrol agents against various plant diseases has been reported by several workers. (Wells, *et al.*, 1972 and Sharon, *et al.*, 2001)

In the present investigation, fungal antagonist TVS-1 isolate caused highly significant reduction in chick pea wilt incidence under in vitro condition. The inhibitory effect of these bioagents against tested pathogen was probably due to competition and/or antibiosis.

Demands for in vitro effectiveness of Trichoderma against species of *Fusarium* have been reported (Padmadaya, *et al.*, 1996).

Table.1 Antagonistic effect of *Trichoderma i*solates against *Fusarium oxysporum* f. sp. *ciceri* (Chickpea wilt)

Sr. No.	Isolates		*Avg. colony dia. of pathogen (mm)	Per cent inhibition of mycelial growth (PIMG)
1	TFC-1	Rizospere of Bareja	20.20	69.90
2	TFC-2	Rizospere of Dholka	11.23	83.27
3	TVS – 1	Rizospere of Badarkha	6.26	90.67
4	TVS – 2	Rizospere of Badarkha	12.56	81.29
5	THCh -1	Rizospere of Bawala	15.30	77.20
6	TACh -1	Rizospere of Paladi	17.83	73.43
7	THCh-2	Navasari Isolates	6.80	89.87
8	TVS-3	Sardar krishi Nagar	9.40	85.99
9	TKNG-1	Sardar krishi Nagar	11.83	82.37
10	TVS-4	Anand Isolates	12.20	81.82
11	TVS-5	KVK Amravati Isolates	6.73	89.97
12	THCh-3	KVK Amravati Isolates	6.83	89.82
13	Control		67.13	-
S.Em.±			0.42	-
C.D.@5%			1.23	-
C.V.%			4.67	-

* Average of three repetitions

TFC-1 & TFC-2: *T. fasciculatum*; TVS: *T. viride*; THCh: *T. harzianum*; TACh-1: *T. atroviride;* TKNG: T. *Koningi*

The antagonist *Trichoderma harzianum*, *T. koningi* and *T. viride* were reported to be equally antagonistic to *F. udum* under in vitro (Bhatnagar, H. 1986 and Sivan and Chet 1987) reported that *Trichoderma* spp. successfully controlled *Fusarium* spp. on cotton, wheat and muskmelon. Sesame seeds treated with three isolates of *T. viride* reduced the pre- and post-emergence damping off caused by *R. solani* and *F. oxysporum* f. sp. sesami under pot culture and field conditions.

Several studies (Jayalakshmi *et al.*, 2009; Muhammad and Amusa, 2003; Bunker and Mathur, 2001; Shabir *et al.*, 2013) reported that inhibition of some soil borne pathogens, including *Fusarium oxysporum* f. sp. ciceri by *Trichoderma* species could probably be due to the secretion of extracellular cell wall degrading enzymes such as chitinase, β-1, 3-glucanase, β-1, 6-glucanase, protease, cellulease and lectin, which help mycoparasites to colonize their host. Also, inhibition of the pathogen may be attributed to the production of secondary metabolites (such as glioviridin, viridin and gliotoxin) the antagonists (Inbar *et al.*, 1994).

In the present investigation, *T. viride* (Badarkh rizospere) is chosen to be the most promising bio-control agent for *F. oxysporum* f. sp. *ciceri*

On the base of present study the bioagents of fungi, might be exploited for sustainable disease management programs to save environmental risk.

The present evaluation thus gave clear indication that the isolates TVS-1(Badarkha isolates) isolated from chickpea rhizosphere are strong and virulent antagonists, which can be effectively used in the management of chick pea wilt caused by *Fusarium oxysporum*.

Acknowledgement

The authors express thanks to Dr. Jitendar Kumar Sharma for their keen interest and valuable guidance for initiating the investigation.

References

Amarender, R. and Devraj, M. 2011. Growth and instability in chickpea production in India. www.krisat.org Accessed on 15 February 2011.

Bahatnagar H. 1986. Influence of environmental condition on antagonistic activity of *Trichoderma* spp. against *Fusarium udum*. *Indian Journal of Mycology and Plant Pathology*, 1986; 26: 58-63.

Bendre, N. J. and Barhate, B. G. 1998. A Souvenir on disease management in chickpea. M.P.K.V. Rahuri during 10th Dec. 1998.

Bunker R. N, Mathur K. 2001. Antagonism of local biocontrol agents to *Rhizoctonia solani* inciting dry root-rot of chilli. *J. Mycol Pl. Pathol*. 31 (1):50-53.

Cook, R. J. 1985. Biological control of plant pathogens: *theory to application. Phytopathology*, 12:75- 80.

Dubey, S. S., Suresh, M. and Singh, B. 2007. Evaluation of Trichoderma species against *Fusarium oxysporum* f. sp. *ciceri* for integrated management of chickpea wilt. *Biol. Control*, 40(1):118-127.

Elad Y, Chet I. 1983. Improved selective media for isolation of *Trichoderma* spp. and *Fusarium* spp. *Phytoparasitica*; 11: 55- 58.

FAO, FAOSTAT Database Results (http://apps.fao/ faostat) (2012).

Faruk, M. I., Rahman, M. L. and Bari, M. A., 2002. Management of seedling disease of cabbage through

Trichoderma harzianum amendment in seedbed. Bangl. *J. Plant Pathol*, 18(1-2): 49-53

Harman, G. E., Charles, R. H., Ada, V., Chet, I. and Matteo, L. 2004. *Trichoderma* - opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol*., 2: 43-56.

Haware, M.P. and Nene, Y.L. 1982. Races of *Fusarium oxysporum* f. sp. ciceri. *Plant Dis*. 66: 809-810.

Inbar J, Abramsky M.C.D, Chet I. 1994. Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. *Eur. J. Plant Pathol*. 100:337- 346.

Jayalakshmi S.K, Raju S, Usha R, Benagi VI, Sreeramula K 2009. *Trichoderma harzianum* L1 as a potential source for lytic enzymes and elicitor of defense responses in chickpea (*Cicer arietinum*) against wilt disease caused by *Fusarium oxysporum* f.sp. *ciceri*. *Aust. J. Crop Sci*. 3(1):44-52.

Kamlesh, M. and Gujar, R. S. 2002. Evaluation of different fungal antagonistic, plant extracts and oil cakes against *Rhizoctonia solani* causing stem rot of chilli seedlings. *Ann. Plant Prot. Sci*. 10(2):319-322.

Monte, E., 2001. Understanding Trichoderma: between biotechnology and microbial ecology. *Int. Microb*. 4:1-4.

Muhammad S, Amusa N. A. 2003. In-vitro inhibition of growth of some seedling blight inducing by compost-inhabiting microbes. *Afr. J. Biotechnol*. 2 (6):161-164.

Nene, Y. L. and Reddy, M. V. 1987. Chickpea Diseases and their Control. In: Saxena, M. C. and Singh K. B., the Chickpea. *Oxon, UK: CAB International*. pp. 233- 270.

Padmadaya B, Reddy H.R 1996. Screening of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *lycopersici* causing wilt on tomato. *Indian Journal of Mycology and Plant Pathology*; 26: 288-290.

Podder, R. K., Singh, D. V. and Dubey, S. C. 2004. Integrated application of Trichoderma harzianum mutants and carbendazim to manage chickpea wilt (*Fusarium oxysporum f.sp, ciceri*). *Ind. J. Agric. Sci*., 74: 346-348.

Shabir R, Rubina L, Ebenezer J.K, Talat M.A, Shabir A.G, Waseem A.D, Javid A.B 2013. Eco-friendly management of root-rot of chilli caused by *Rhizoctonia solani* kuehn. *Afr. J. Agric. Res*. 8(21): 2563-2566.

Sharon E, Bar-Eyal M, Chet I, Herra-Estrella A, Kleified O, Spigel Y. 2001. Biological control of the root-knot nematode Meloidogyne javanic by *Trichoderma harzianum*. *Phytopathology*; 91: 687- 693.

Sivan A, Chet I. 1987. Biological control of *Fusarium* crown rot of tomato by *Trichoderma harzianum* under field condition. Plant Disease; 71: 589-592.

Vincent J.M. 1927. Distribution of fungal hyphae in the presence of certain inhibition. Nature, 159: 50.

Wells, H.D., Bell, D.K., Jaworski C.A. 1972. Efficacy of *Trichoderma harzianum* as a biocontrol for *Sclerotium rolfsii*. *Phytopathology*, 62: 442-447.