Int.J.Curr.Microbiol.App.Sci (2017) *6*(2): 258-265

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 2 (2017) pp. 258-265**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas. 2017.602.031

Antagonistic Action of *Trichoderma* Isolates against *Fusarium oxysporum* f. sp. *lycopersci*

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A B S T R A C T

Keywords

Trichoderma, Biocontrol, *Fusarium*, Tomato

Article Info

Accepted:
12 January 2017
Available Online:
10 February 2017

In the present study, 25 soil samples were collected from the different location of U.P and M.H. and 17 *Trichoderma* isolates were obtained belonging to three different species *T. harzianum*, *T. viride* and *T. koningi*. All the obtained isolates were studied microscopically for their species level identification. Morphological characterization of all the isolates was done. All the isolates were screened against *Fusarium oxysporum* f. sp. *lycopersci* for their efficacy through dual culture technique. Highest mycelium inhibition was recorded with SVPU-Thar7, (81.40 %) and minimum with SVPU-Thar4 (57.05%).

Introduction

Modern agricultural practices are getting affected by various problems such as disease, pest, drought, decreased soil fertility etc. due to the increasing use of chemical pesticides. The fungal disease are one of the major cause of crop productivity lose in India. This damage is estimated to be Rs.50,000 crores annually. Tomato (*Lycopersicon esculentum* Mill.) is the second most important vegetable crop next to potato grown in almost all parts of India. It is a rich source of vitamins A and C. The present world production is about 100 million tons of fresh fruit produced on 3.7 million hectare. It is affected by several diseases, wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) is the most important disease (Agrios, 2006; Rich, 1976; Srinon *et al*., 2006). It is a devastating disease causing considerable economic losses ranging from 10-80% yield loss in tomato production (Keshwan and Chaudhary, 1977). *Fusarium* wilt is soil-borne in nature application of fungicides to control this disease is not very effective. However, the regular use of chemical fungicides is harmful for the environment (Lewis *et al*., 1996). Hence, there is a need to develop eco-friendly practices for the control of soil borne phytopathogens. Recent trends favor the use of natural plant extracts, biological control agents and several others components. The various types of biological control agents such as bacteria and fungi are involved in biocontrol activity. Biological control agents (BCAs) offer an alternative to the chemical based control of fungal phytopathogens as they can kill or limit the growth of pathogens without posing threat to the environment. Various bacteria, fungi and protists are known to have such features. Various mechanisms involved in biocontrol process are: competition for nutrients, secretion of lytic enzymes, secretion of toxic metabolites and direct parasitism on host (Agrios, *2005*). Members of the genus *Trichoderma* are well known BCAs (Elad *et al*., 1983; Chet, 1987). It has been very effectively used for the control of large number of soil borne plant pathogen like *Phytophthora, Rhizoctonia, Sclerotium, Phythium, Fusarium, Sclerotinia, and Galumannomyces*. Presently, for commercialization purpose there are mainly three species used viz, *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma koningii.*

In recent years biological control of soil borne plant pathogens is very popular (Hanafi, 2003; Giotis *et al*., 2009). Successful reductions of *Fusarium* wilt in many crops with application of different species of *Trichoderma* have been found. However, it is also reported that all the isolates of *Trichoderma* spp. are not equally effective in control of pathogen *in vitro* and *in vivo* conditions to control diseases. Therefore, specific isolates are needed for successful control of a particular pathogen.

The main objective of the present investigation is to check the antagonistic potential of *Trichoderma* isolates against *F.o.l* under *in vitro* conditions.

Materials and Methods

Collection, isolation identification and purification of fungi isolated from rhizospheric and non rhizospheric soil

Soil samples were collected from the rhizosphere soil of different crop niches in Uttar Pradesh (India). Five- fold serial dilutions as described by Singh and Singh, (1970) for each soil sample was prepared in sterilized distilled water and 0.5 ml diluted sample was poured on the surface of *Trichoderma* Specific Medium (TSM) (Elad *et al*., 1981). Plates were incubated at 28 ± 2°C for 96 h, morphologically different colonies appearing on the plates were purified on Potato Dextrose Agar Medium (PDA) (HiMedia, India)

Test pathogen i. e. *Fusarium oxysporum* f. sp. *lycopersici* (ITCC no. 1322) was procured from division of Plant Pathology, IARI, New Delhi.

Morphological identification

Cultural and morphological observations of colony were based on *Trichoderma* isolates grown on PDA for 7 days in an incubator at 25± 2°C with altering 12h/12h fluorescent light/ darkness. Characters of the conidium-bearing structures and conidia were assessed for each isolate (Table 2).

Antagonistic Activity of *Trichoderma* Isolates

The dual culture technique described by Morton and Stroube was used to test the antagonistic ability of 17 isolates of three different *Trichoderma* spp. viz; *T. harzianum, T. viride, T. koningii*, against *Fusarium oxysporum* f.sp.*lycopercsi*. The pathogen and *Trichoderma* spp. were grown on PDA for a week at 25 ± 2°C. 5mm disc of the target fungi cut from the periphery was transferred to the Petri dish previously poured with PDA. *Trichoderma* spp. was transferred aseptically in the same plate of opposite end and were incubated at room temperature with alternate light and darkness for 7 days and observed periodically. Control plates were maintained without *Trichoderma.* The experiment was replicated thrice and percent growth inhibition was calculated by the formula of I = (C”T)/C × 100, where C is mycelial growth in control plate, T is mycelial growth in test organisms inoculated plate and I is inhibition of mycelial growth. Vincent *et al.*, (1999).

Results and Discussion

A total of 25 soil samples were collected from the different locations of U.P. and M.H. Out the 25 soil samples 17 isolates of *Trichoderma* were obtained belonging to three different species *T.harzianum*, *T.viride* and *T.koningii*. Microscopic studies were done for the species level identification. Cultural and physiological studies of all the isolates were also done. Antagonistic potential of 17 isolates of three *Trichoderma* species was determined through dual culture technique (Table 3).

Table.1 Isolation of different *Trichoderma* isolates

S.N	Location	District/State	Crop/field	Strain name
1.	Vill-Chirori	Meerut (UP)	Paddy	**SVP-Tkoni 1**
2.	P.D.K.V.	Akola (M.H)	Pitunia	**SVP-Tkoni 2**
3.	P.D.K.V.	Akola (M.H)	Chrysenthimum	**SVP-Tkoni 3**
4.	Anand sagar	Akola(M.H)	Wheat	**SVP-Tharz 1**
5.	P.D.K.V.	Akola (M.H)	Cotton	**SVP-Tharz 2**
6.	Ag. Collage PDKV	Akola(M.H)	Marigold	**SVP-Tharz 3**
7.	P.D.K.V.	Akola (M.H)	Marigold	**SVP-Tharz 4**
8.	P.D.K.V.	Akola (M.H)	Marigold	**SVP-Tharz 5**
9.	Anandsagar	Akola(M.H)	Bamboo	**SVP-Tharz 6**
10	Ganganagar	Meerut (UP)	Black Gram	**SVP-Tharz 7**
11.	Ganganagar	Meerut (U.P)	Garlic	**SVP-Tharz 8**
12.	Ganganagar	Meerut (U.P)	Pea	**SVP-Tharz 9**
13.	Ganganagar	Meerut (U.P)	Pea	**SVP-Tharz 10**
14	Village Rajpura	Meerut (U.P)	Wheat	**SVP-Tharz 11**
15.	Village Rajpura	Meerut (U.P)	Garlic	**SVP-Tviri 1**
16.	Village Rajpura	Meerut (U.P)	Sugarcane	**SVP-Tviri 2**
17.	Village Rajpura	Meerut (U.P)	Sugarcane	**SVP-T**

Table.2 Morphological descriptors used for characterization of native isolates of *Trichoderma* spp

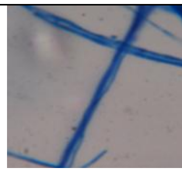
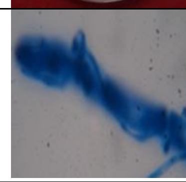
Strain Name	Colony growth rate (cm/day)	Colony colour	Reverse Colour	Colony edge	Mycelial form	Mycelial colour	Conidiation	Conidiophore branching	Conidia wall	Conidial colour	Chlamydos pores
SVP-Tkoni 1	7-8 in 3days	Dirty green	Yellowish	Smooth	Floccose to Arachnoid	Watery white	Ring like zones	Highly branched, regular	Rough	Green	Not observed
SVP-Tkoni 2	8-9 in 3days	Green	Light yellow	Smooth	Floccose to Arachnoid	Watery white	Ring like zones	Branched, regular	Rough	Green	Not observed
SVP-Tkoni 3	6-7 in 3days	Blackish green	Dark brownish	Smooth	Arachnoid	Watery white	Ring like zones	Branched, regular	Rough	Green	Not observed
SVP-Tharz 1	8-9 in 3days	Dark green	Colourless	Wavy	Floccose to Arachnoid	Watery white	Ring like zones	Highly branched, regular	Smooth	Green	Not observed
SVP-Tharz 2	8-9 in 3days	Green to dark green	Yellowish	Smooth	Floccose to Arachnoid	Watery white	Ring like zones	Branched, regular	Smooth	Green	Not observed
SVP-Tharz 3	8-9 in 3days	Light green	Colourless	Smooth	Floccose to Arachnoid	Watery white	Ring like zones	Highly branched, regular	Smooth	Green	Not observed
SVP-Tharz 4	5-6 in 3days	Snow white green	Orange	Smooth	Floccose	Watery white	Ring like zones	Branched, regular	Smooth	Green	Not observed
SVP-Tharz 5	8-9 in 3days	Whitish green	Colourless	Smooth	Floccose to Arachnoid	Watery white	Ring like zones	Branched, regular	Smooth	Green	Not observed
SVP-Tharz 6	8-9 in 3days	Cottony white green	Yellowish	Smooth	Floccose to Arachnoid	Watery white	Ring like zones	Branched, regular	Smooth	Green	Not observed
SVP-Tharz 7	8-9 in 3days	Light green	Light yellow	Smooth	Floccose to Arachnoid	Watery white	Flat	Branched, regular	Smooth	Green	Not observed
SVP-Tharz 8	7-8 in 3days	Watery white	Colourless	Wavy	Arachnoid	Watery white	Ring like zones	Branched, regular	Smooth	Green	Not observed
SVP-Tharz 9	8-9 in 3days	Snow white green	Colourless	Smooth	Floccose to Arachnoid	Watery white	Ring like zones	Branched, regular	Smooth	Green	Not observed
SVP-Tharz 10	8-9 in 3days	Light green	Yellowish	Smooth	Floccose to Arachnoid	Watery white	Ring like zones	Branched, regular	Rough	Green	Not observed
SVP-Tharz 11	8-9 in 3days	Snow white	Colourless	Smooth	Floccose to Arachnoid	Watery white	Flat	Highly branched, regular	Smooth	Green	Not observed

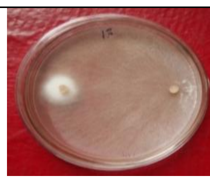
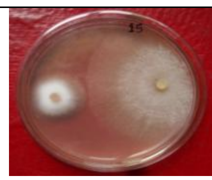
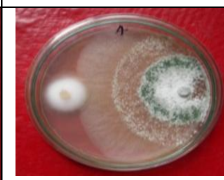
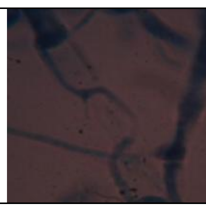
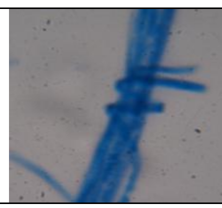
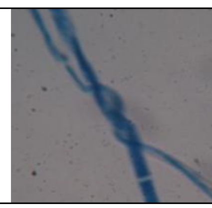
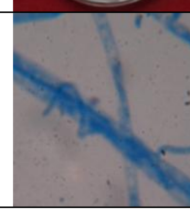
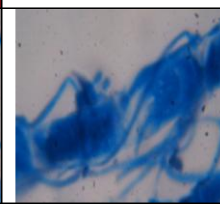
SVP-Tviri 1	8-9 in 3days	Dark green	Colourless	Smooth	Floccose to Arachnoid	Watery white	Ring like zones	Highly branched, regular	Rough	Green	Not observed
SVP-Tviri 2	8-9 in 3days	Greyish green	Colourless	Smooth	Floccose to Arachnoid	Watery white	Ring like zones	Branched, regular	Rough	Green	Not observed
SVP-T	8-9 in 3days	Dirty green	Dark greenish	Smooth	Floccose to Arachnoid	Watery white	Ring like zones	Ball like structure	Rough	Green	Not observed

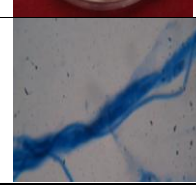
Table.3 In vitro antagonistic activity of *Trichoderma* isolates againsnst Fol

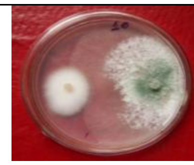
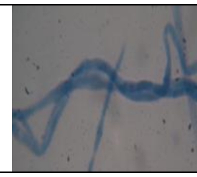
Sl no.	***Trichoderma sp***	**Growth of *Fol* at 72h(cm)**	
		Mycelial growth	**% inhibition in mycelial growth**
1	**SVPT-koni 1**	1.450	72.10
2	**SVPT-koni 2**	1.267	75.60
3	**SVPT-koni 3**	1.467	71.70
4	**SVPT-har 1**	1.233	76.00
5	**SVPT-har 2**	1.300	75.00
6	**SVPT-har 3**	1.300	75.00
7	**SVPT-har 4**	2.233	57.05
8	**SVPT-har 5**	1.433	72.00
9	**SVPT-har 6**	1.433	72.4
10	**SVPT-har 7**	0.967	81.4
11	**SVPT-har 8**	1.367	73.71
12	**SVPT-har 9**	1.367	73.71
13	**SVPT-har 10**	1.333	74.40
14	**SVPT-har 11**	1.467	71.70
15	**SVPT-viri 1**	1.400	73.0
16	**SVPT-viri 2**	1.167	77.5
17	**SVPT-**	1.733	66.60
18	**Control**	4.20	
CD @ 5%		**0.131**	

Fig.1 Antagonistic activity of *Trichoderma* spp. on the *Fusarium oxysporum* f.sp.lycopersici (cm.).

Strain Name	**SVPT-koni 1**	**SVPT-koni 2**	**SVPT-koni 3**	**SVPT-har 1**	**SVPT-har 2**
Mycelium growth inhibition after 72 hours					
Coiling observed under microscopic					NO COILING OBSERVED

Strain Name	**SVPT-har 3**	**SVPT-har 4**	**SVPT-har 5**	**SVPT-har 6**	**SVPT-har 7**
Mycelium growth inhibition after 72 hours					
Coiling observed under microscopic					

Strain Name	**SVPT-har 8**	**SVPT-har 9**	**SVPT-har 10**	**SVPT-har 11**	**SVPT-viri 1**
Mycelium growth inhibition after 72 hours					
Coiling observed under microscopic					

Strain Name	**SVPT-viri 2**	**SVPT-**
Mycelium growth inhibition after 72 hours		
Coiling observed under microscopic		

In the present investigation, screening of the *Trichoderma* isolates was done agains*t* *Fusarium oxysporum* f. sp. *lycopersici*, The potential strains were characterized microscopically. Soil borne fungal plant pathogens are causing economically damage whose pathogenic activities are reducing with the use of fungicide application to minimize losses in plant yield, and quality.

So now the scientists exploit the eco-friendly biological methods of disease control through development of non chemical based biocontrol processes. Among fungal antagonists, *Trichoderma* have been the most commercialized and efficacious inoculants used world over for the control of soil borne fungal plant pathogens (Elad and Kapat, 1999 and Harman, 2010). Biocontrol agents are also known to provide habitat specific suppressive effects.

Several reports indicate that *Trichoderma* species can effectively suppress *Fusarium* wilt pathogens (Vipul *et al.*, 2016). *Trichoderma* species has multiple mechanisms of action, including coparasitism via production of chitinases, ^{2}-1-3 glucanases and ^{2}-1-4 glucanases, antibiotics, competition, solubilisation of inorganic plant nutrients, induced resistance and inactivation of the pathogen's enzymes involved in the infection process (Altomare *et al.,* 1999 and Howel, 2003).

Acknowledgement

The authors are grateful for the laboratory support provided by Sardar VallabhbhaI Patel University of agriculture and technology modipuram Meerut.

References

Agrios, G.N. 2000. *Plant Pathol.,* 5th ed. San Diego, CA, USA. *Academic Press* 2005.

Agrios, G.N., Significance of plant disease, pp. 25-37, In: Agrios, G.N. (Ed.), *Plant Pathology*. Academic Press, London.

Altomare, C., Norvell, W.A., Bjorkman, T., Harman, G.E. 1999. Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai. 1292–22. *Appl. Environ. Microbio*., l65: 2926–2933

Chet, I. 1987. *Trichoderma* application, mode of actionand potential as a biocontrol agent of soil borne plantpathogenic fungi. In: (Chet, I. ed.). *Innovative approaches to plant diseases control.* John Wiley and Sons, New York.

Elad, Y. and Kapat, A. 1999. The role of *Trichoderma harzianum* protease in the biocontrol of *Botrytis cinerea*. *Europ. J. Plant Pathol.*, 105: 177- 189.

Elad, Y., I. Chet, Boyle, P., Henis, Y. 1983. Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii* scanning electron microscopy and fluorescence microscopy, *Phytopathol*., 73: 85–88.

Elad, Y., I. Chet, Henis, Y. 1981. A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. *Phytoparas*., 9: 1 59-67.

Giotis, C., Markelou, E., Theodoropoulou, A., Toufexi, E., Hodson, R., Shotton, P., Shiel, R., Cooper, J., Leifert, C. 2009. Effect of soil amendments and biological control agents (BCAs) on soilborne root disease caused by *Pyrenochaetu lycopersici* and *Verticillium alboatrum* in organic greenhouse tomato production systems. *Eur. J. Plant Pathol.*, 123: 387–400.

Hanafi, A. 2003. Integrated Production and Protection in Green-house Tomato in Morocco.p. 192–197. In: “Tomate Sous Abri” Scientific Publication of CTIFL. Editions Centre Technique Interprofessionnel des Fruits et Légumes, 232 pp.

Harman. E.G., Herrera-Estrella, H.A., Horwitz, A.B. and Lorito, M. 2010. *Trichoderma* – from Basic Biology to Biotechnology. *Microbiol.*, 158.

Howell, C.R. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: history and evolution of current concepts. *Plant Dis.*, 89(11): 1195-1200.

Kesavan, V. and B. Chaudhary. Screening for resistance to *Fusarium* wilt of tomato. *SABRO J*., 9: 51-65.

Kumar, V., Kumar, A., Srivastava, M., Pandey, S., Shahid, M., Srivastava, Y.K. and Trivedi, S. 2016. *Trichoderma harzianum* (Th. azad) as a Mycoparasite of *Fusarium* and growth enhancer of Tomato in Glasshouse Conditions. *J. Pure and Appl. Microbiol.*, 10: 1463-1468.

Lewis, J.A., Lumsden, R.D., Locke, J.C. 1996. Biocontrol of damping-offdiseases caused by *Rhizoctonia solani* and *Pythium ultimum* withalginate prills of *Gliocladium virens*, *Trichoderma hamatum* andvarious food bases. *Biocontrol Sci. Technol.*, 6: 163-173.

Rick, C.M., Simmonds, N.W. Evaluation of Tomato Crop Plant,.(Ed.), Longman. *In: New York*, Pp. 263-273,.

Singh, R.S., Singh, N. 1970. Effect of oil cake amendment of soil on population of some wilt causing species of *Fusarium. Phytopath. Zei. Schrift*.,, 26: 160-167.

Srinon, W., Chuncheen, K., Jirattiwarutkul, K., Soytong, K. & Kanokmedhakul, S. 2006. Efficacies of antagonistic fungi against *Fusarium* wilt disease of cucumber and tomatoand the assay of its enzyme activity. *J. Agri. Technol.*, 2(2): 191-201.

Vincent, I., Benhamon, N. and Chet, I. 1999. Induction of defence response in cucumber plants (*cucumis sativas* L.) by the biocontrol agent *Trichoderma harzianum. App. Env. Microbiol*., 55: 1061- 1070.

How to cite this article:

Akash Tomar, Lakshman Prasad, Abhishek Mishra and Sushma Sagar. 2017. Antagonistic Action of *Trichoderma* Isolates against *Fusarium oxysporum lycopersci. Int.J.Curr.Microbiol.App.Sci.* 6(2): 258-265. doi: http://dx.doi.org/10.20546/ijcmas.2017.602.031