IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372.Volume 4, Issue 3 (Jul. - Aug. 2013), PP 42-47
www.iosrjournals.org

Screening of indigenous potential antagonistic *Trichoderma* species from tomato rhizospheric soil against *Fusarium oxysporum* f. sp. *lycoperscisi*

Anil Kumar R[1], and RajKumar H. Garampalli[2]
[1,2](Department of Studies in Botany, Manasagangotri / University of Mysore, MYSORE- 570006, Karnataka, India)

Abstract: *The present investigation was undertaken to evaluate potential inhibitory effect of indigenous bio-control agents against Fusarium oxysporum f. sp. Lycopersici, (FOL), a causal organism of wilt disease in tomato crops. Twenty indigenous Trichoderma species were isolated from tomato rhizosphere soil collected from tomato growing fields in and around Mysore district, which is geographically located in Southern part of Karnataka, India. Amongst twenty Trichoderma isolates, eight isolates displayed significant activity against the test pathogen. Among the eight isolates two isolates, T. harzianum and T. viride, exhibited excellent inhibitory effects on the test pathogen in dual culture technique. The obtained preliminary results are valuable and promising enough for further studies towards isolation and characterization of antifungal agent responsible for activity.*

Key words: *Tomato wilt, Fusarium oxysporum, Trichoderma, Rhizospheric soil, Bio control agents.*

I. Introduction

Tomato is one of the most widely grown vegetable crops across the globe. It is an important source of vitamins and an important cash crop for small holders and medium-scale commercial farmers. Tomatoes contribute to a healthy, well-balanced diet. They are rich in minerals, vitamins, essential amino acids, sugars and dietary fibres. Hence, tomato forms one of the widely used vegetable but during the cultivation, tomato crop is susceptible to various kinds of disease and disorders, among which *Fusarium* wilt is reported to cause one of the most severe disease on tomato resulting in complete damage of crop resulting in yield loss. *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Snyder and H.N. Hans (FOL) is the causative organisms of *Fusarium* wilt [4; 12]. The yield loss due to this disease may be about 30 to 40% [6]. Use of pesticides and other commercially available fungicides have shown a ray of hope on improving the crop yield but at the same time, the large use of these pesticides and fungicides are bound with various limitations such as loss of soil fertility, contamination of both ground and surface water, biomagnifications, health hazards, etc. which are reported to have deleterious effects on health of all living organisms of the biosphere. Therefore, alternative strategies are being widely employed. One such practice is use of bio-control agents. Research on bio-control agents have expanded in recent past as ecofriendly management of targeted crops. One such area is bio-control activity against tomato. As mentioned earlier tomato is one of the widely used vegetable and its safe management is one of the essential aspects, as it is consumed by humans.

Hence, the present study was designed and executed towards isolation of indigenous bio-control isolates of *Trichoderma* species from tomato rhizosphere soil as the performance of the introduced bio-control agent may not be always favourable because of competition for space and resources with the already established microorganism in the microcosm. Literature pursued by far have demonstrated positive ray of bio-control agents against the tomato pathogens but there is a lack of research dealing with the species used in the present study and its commercial application. Consequently, large number of studies must be demonstrated to obtain a potent isolates bearing significant activity even at harsh conditions [10].

II. Materials And Methods

1.1 Seed material

Tomato seeds of PKM variety were obtained from local seed agencies, Mysore district, Karnataka state, India. The seeds were surface- sterilized with 0.5% sodium hypochlorite for 3 min. followed by repeated washing in distilled water.

2.2 Collection of Soil and sampling

Rhizospheric soil samples were collected from twenty one different fields of tomato which are located in different geographical regions of Mysore district, Karnataka state, India. Mysore district is located between

latitude 11°45' to 12°40' N and longitude 75°57' to 77°15' E. Four composite soil samples were collected from each site and were marked accordingly and carried to laboratory within twenty four hours.

2.3 Isolation and identification of *Fusarium oxysporum f. sp. lycoperscisi* and their pathogenicity testing

Different parts of infected tomato plants were collected and plated onto wet blotter disc via standard blotter protocol (ISTA, 2003). The plates were incubated for 7 days at 22° C and under 12h / light: 12h dark. After incubation, fungi developed on each root and stem samples, were isolated as pure cultures and maintained on Potato Dextrose Agar (PDA) slants at 4° C. After the incubation period the pathogen was identified by consulting suitable keys and was evaluated for pathogenicity test by inoculating the pathogen to tomato test variety. The spore suspension of actively growing fungal pathogen was prepared in sterile distilled water. The concentration of spore was standardized and optimized (spores/ mL) using haemocytometer. Under green house conditions the young seedlings were inoculated by root dip method and plants were grown under transparent polythene covers for a period of 48 hrs. Periodic observations were monitored for one week for symptom and development of the disease [7; 15].

2.4 Isolation and identification of native bioactive isolates

One gram of rhizosphere soil sample collected from diseased and healthy tomato plants was taken and added to 10 ml of sterilized distilled water forming a stock solution. Later the sample was serially diluted to obtained dilution factor from 10^{-1} to 10^{-8}. From the dilutions viz., 10^{-3} to 10^{-6} was plated onto PDA. The Petri plates were then incubated at 24 ± 2 °C for 7 days. Bioactive isolated obtained were maintained with accession number, sealed and preserved [14].

2.5 *In vitro* screening of antagonist native bio-agents against *F. oxysporum* f. sp. *Lycopersici*

The initial screening for antagonistic activity of native bioactive isolates was evaluated against *F. oxysporum* f. sp. *lycopersici* (FLO) by dual culture technique. 5 mm mycelia disc of actively growing *F. oxysporum* and antagonist native bio-agents were tested for activity. Totally eight *Trichoderma* isolates were used in the present study viz. *T. harzianum* (2 isolates), *T. Viride* (2 isolates), *T. hamatum* (2 isolates) and *T. virens* (2 isolates). Seven days old mycelia disc (5 mm diameter) of test pathogen (*F. oxysporum* f. sp. *lycopersici)* was placed alone on plate serve as control and FOL with bio-agent placed juxtapose in petriplates containing PDA medium served as treated. Plates were incubated at 28 ± 1^0C and the radial growth of test pathogens in treated and control plates were recorded after 7 days of incubation and the per cent inhibition of mycelial growth of the pathogens was calculated by using following formula;

$$L = [(C - T)/C] \times 100$$

Where, L is the percent inhibition; C is the colony radius in control plate and T is radial growth of the pathogen in the presence of *Trichoderma* isolates [11]. Three replicates of each treatment were maintained.

2.6 Degree of antagonisms

The degree of antagonisms between each bio-agent and test pathogen in dual culture was scored on scale of 1-5 as proposed by [2].

1. Antagonist completely overgrew the pathogen and covered the entire medium surface.
2. Antagonist overgrew at least two third of the medium surface.
3. Antagonist and the pathogen each colonized one half of the medium surface (more than one third and less than two third) and neither organism appeared to dominate each other.
4. The pathogen colonized at least two third of the medium surface and appeared to withstand encroachment.
5. The pathogen completely overgrew the antagonist and occupied the entire medium surface.

2.7 Statistical analysis

All the data were statistically analyzed using one-way ANOVA (Analysis of Variance) by SPSS. Differences among treatments were determined using Tukey's multiple range tests (TMRT) at a significant level of P=0.05. Data are presented as means ± standard deviation (SD).

III. Results

The results obtained in the present investigation were promising enough towards isolation of twenty bioactive isolates of *Trichoderma* sp. Upon evaluation, among twenty isolates, eight isolates displayed activity.

3.1 Isolation and Identification of *F. oxysporum* f. sp. *lycopersici*

Evaluation of infected part of tomato plants resulted in isolation of fungal pathogen which was identified and confirmed based on the examination under different magnifications of a stereomicroscope. The characteristic growth of the fungus on root and stem samples and the morphological characters of micro conidia and macro conidia and with chlamydospores, observed under a compound microscope, further confirmed the pathogen [3; 12] (Fig. 1).Further confirmation was carried out via pathogenicity test which displayed the infection upon treating the healthy plant with spore suspension by root dip method.

3.2 Pathogenicity test (Pot experiment)

Tomato seedlings were inoculated with FOL using spore suspension with conidial concentration of $1x10^5$ conidia/mL by root dip method, were found to be an effective and high virulent, where, inoculated plants expressed severe infection with the typical sign of symptom like leaf chlorosis. The diseased leaves wilted and dried up. Dropping and wilting of the stem tip is another characteristic symptom observed. The diseased plants wilted down and dry up completely. Their roots were necrotic and rotten, and the necrosis spread to the lower stem. In contrast, control plants were completely free from disease (Fig. 2).

3.3 In-*vitro* screening of antagonist

A total of twenty isolates of Trichoderma were obtained from the rhizospheric soil. Among these twenty isolates only eight were bioactive and have been reported in the present study (Fig. 2).

T. harzianum (Thz-1), T. harzianum (Thz-2), T. viride (Tv-1), T. viride (Tv-1) and *T. hamantum* (Th-1) proved to be best antagonist inhibiting 83.33% and 80.33% per cent over control, respectively, followed by *Trichoderma hamantum* (Th-2), *Trichoderma virens* (Ts-1) and *Trichoderma virens* (Ts-1) which showed 64.48 %, 55.85 % and 60.73 % inhibition respectively (Table 1).

Tukey's multiple range tests (TMRT) was applied at a significance level of P=0.05 which has been represented in the (TABLE 1).

In comparison with control, eight species of *Trichoderma* which were tested for inhibition of FOL, all showed significance growth inhibition. However, *Trichoderma harzianum* isolate -1 exhibited maximum percentage of inhibition and minimum was recorded in *Trichoderma virens* isolate -1. All native bio-agents were found to have the inhibitory activity significantly when compare to control. Whereas, they were not significant among individual species (between eight *Trichoderma* sps.)

3.4 Degree of Antagonism

The degree of antagonism between each bio-agent and test pathogen in dual culture was scored on scale of 1-5 as proposed by [2]. Four antagonists which completely overgrew the pathogen were placed in class-1, whereas, remaining antagonists which parasitized the test pathogen up to two third of the medium surface after seven days were placed in class 2 (TABLE 2).

IV. Discussion

In this preliminary investigation, an attempt was made to screen the antagonistic potential of rhizospheric native bio agents against *Fusarium oxysporum sp. lycopersici* (FOL), the causal organism of wilt disease in tomato. Earlier reports attribute *Trichoderma* sp. as one of the most efficient strains to be evaluated as biocontrol agent. One such study reports that *Trichoderma harzianum* (Th), *Trichoderma viride* (Tv) and *Trichoderma koningii (*Tk*)* upon evaluation via dual culture technique resulted in suppression of soil borne pathogens of different vegetables viz. *Rhizoctonia solani*, *Sclerotium rolfsii* and *Sclerotinia sclerotiarum* under *in vitro* conditions [9]. Similarly, *T. viride* (Tv-1), *T. viride* (Tv-2), *T. harzianum* (Th-1) inhibited the growth of *Rhizoctonia solani* [8]. *Trichoderma virens* T523 and *Trichoderma* sp. were evaluated against five isolates of soil borne phytopathogenic fungi via dual culture techniques resulted in suppression of *Fusarium graminearum, Rhizoctonia solani (AG4 and AG5), Macrophomina phaseoli* and *Phytophtora cacturum* [1]. These studies reported the role of *Trichoderma* sps as potent biocontrol agents, which were also observed in the present investigation that, the tested *Trichoderma* isolates exhibited antagonistic activity against FOL significantly. The degree of antagonism varied between and within species of *Trichoderma* against FOL. *T. harzianum* and *T. viride* reduced the growth of all the FOL soil borne pathogen significantly and, therefore, can be incorporated for integrated disease management of FOL. It is widely known that environmental parameters such as abiotic (soil type, soil temperature, soil pH, water potential and such like) and biotic (plant species and variety, microbial activity of the soil) factors as well as other factors such as method and timing of applications may have influence on the biological control efficacy of *Trichoderma* isolates. Therefore, it is important to isolate the local bio-agents rather than introducing the bio-agents to the entirely new environment, which may not exhibit

its ability to the fullest extent. Further the bio-control efficacy of the *Trichoderma* isolates used in the present study need to be evaluated in the field condition.

V. Conclusion

The present study envisions the preliminary results of *in vitro* evaluation of *Trichoderma* sp. as one of the potent bio-control agent against the fungal pathogen *Fusarium oxysporum* f. sp. *lycopersici*. Further studies are promising enough to reveal and characterize the antagonistic agent responsible for bio-control activity. These studies form the base for ecofriendly management of plant diseases which will minimize the usage of pesticides and chemical fungicides.

Acknowledgment

One of the authors is thankful to University of Mysore (Special Cell, SC/ST), for financial support to carry out this work.

References

[1] H. Behzad, T. G. Mousa, R. M. Mohammad, and D. Mahdi, Biological potential of some Iranian *Trichoderma* isolates in the control of soil borne plant pathogenic fungi, African Journal of Biotechnology, 8, 2008, 967-972.
[2] D. K. Bell, H. D. Wells, and C. R. Markham, *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens, Phytopathology, (72), 1982**,** 379-382.
[3] L.W Burgess, B. A. Summerell, S Bullock, K. P. Gott, and D. Backhouse, Laboratory manual for Fusarium research, 3, 1994, 9-11.
[4] J. P. Gilreath, J. P. Jones, A. J. Overman, Soil borne pest control in mulched tomato with alternative to methyl bromide. Proceedings of the Florida State Horticultural Society, 107, 1994, 156-159.
[5] ISTA, International rules for seed testing, Rules Draper (ed) Switzerland, The International Seed Testing Association, 2003, 1- 47.
[6] R. Kumar Kiran, K. S. Jagadeesh, P. U. Krishnaraj, M. S. Patil, Enhanced growth promotion of tomato and nutrient uptake by plant growth promoting rhizobacterial isolates in presence of tobacco mosaic virus pathogen, *Karnataka Journal of Agricultural Sciences*, 2, 2008, 309-311.
[7] M. Marlatt, G. Gilardi, M. L. Gullino, A. Garibaldi, Biological control potential of Achromobacterxylosoxydoms for suppressing Fusarium wilt of tomato, International Journal of Botany, 4, 2008, 369-375.
[8] M. Radwan, Barakat, A. M. Fadel, S. Mohammed, AliShtayeh, I. A. M. Mohammad, Biological Control of *Rhizoctonia solani* by Indigenous *Trichoderma* spp. Isolates from Palestine, *Hebrew University of Jerusalem,* 3, 2007, 1 – 15.
[9] Shafiquzzaman S , Umi K Y , Kausar H, Sarwar J, *In vitro* studies on the potential *Trichoderma harzianum* for antagonistic properties against *Ganoderma boninense, Journal of Food Agriculture and Environment,* 4, 2009, 970 - 976.
[10] V. Shanmugam, N. Kanoujia, Biological management of vascular wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycospersici* by plant growth-promoting rhizo-bacterial mixture, *Biological control*, 57, 2011, 85-93.
[11] H. R. Shivapratap, T. Philip, D. D. Sharma, In-vitro antagonism of *Trichoderma* sps against mulberry leaf spot pathogen *Cercospora moricola*, *Indian Journal of Sericulture*, 2, 1996, 107-110.
[12] Snyder and Hansen, *Fusarium oxysporum* Sch & Sp. *lycopersici* (Sacc), *American Journal of Botany*, 27, 1940, 66.
[13] E. F. Suárez, G. C. Vargas, M. J. Lopez, C. Capel, J. Moreno, Antagonistic activity of bacteria and fungi from horticultural compost against *Fusarium oxysporum* f. sp *melonis*, *Crop Protection*, 26, 2007, 46-53.
[14] F. S. Thatcher, D. S. Ctlar. Microorganism in Foods, Their Significance and Methods of Enumeration*,* 1, 1968, 143.
[15] R. Zaidi Yahiaoui, R. Ladjouzi, S. Benallaoua, Pathogenic variability within biochemical groups of *Pectobacterium carotovorum* isolated in Algeria from seed potato tubers, *International Journal for Biotechnology and Molecular Biology,* 1, 2010, 001-009.

Equations:

(1): $L = [(C – T)/C] \times 100$

Tables

Table 1: Antagonistic inhibition (%) of *Fusarium oxysporum* f. sp. *Lycoperscisi* by different *Trichoderma* sp.

Native Bio Agents	Radial growth of FOL (in mm)	% of inhibition of FOL
Control	4.50±0.00^{a}	--
Trichoderma harzianum (Thz-1)	0.77±0.25^{e}	**83.33±5.51^{d}**
Trichoderma harzianum (Thz-2)	0.90±0.10^{de}	80.33±2.52^{cd}
Trichoderma viride (Tv-1)	1.27±0.25^{cde}	72.33±5.51^{bcd}
Trichoderma viride (Tv-2)	1.03±0.15	77.33±3.06^{cd}
Trichoderma hamantum (Th-1)	1.37±0.15^{cd}	70.00±3.61^{bc}
Trichoderma hamantum (Th-2)	1.73±0.12^{bc}	61.48±2.56^{ab}
Trichoderma virens (Ts-1)	2.00±0.20^{b}	55.85±4.00^{a}
Trichoderma virens (Ts-2)	1.77±0.25^{bc}	60.74±5.59^{ab}
Overall	1.70±1.09	70.17±10.20
Sig@5%	S	S
F-Value	114.636	16.807

Note: Average ± Standard Deviation followed by same super script letter is not significant, according to Tukey's mean range test at 0.05 levels.

Table 2: Degree of antagonism as proposed by Bell *et al*., (1982)

Treatment	FOL
Trichoderma harzianum (Thz-1)	1
Trichoderma harzianum (Thz-2)	1
Trichoderma viride (Tv-1)	1
Trichoderma viride (Tv-2)	2
Trichoderma hamantum (Th-1)	1
Trichoderma hamantum (Th-2)	2
Trichoderma virens (Ts-1)	2
Trichoderma virens (Ts-2)	2

FIGURES

Fig. 1: Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici.*
a. A commercial tomato cultivation plot in Mysore District severely infected with Fusarium wilt, b. Close up view of Fusarium wilt at early plant growth stage, c. Pure culture of *Fusarium oxysporum* f. sp. *lycopersici* on PDA, d. Macro and micro conidia under compound microscope (40x), e. Chlamydospore under compound microscope (40x).

Fig. 2: Fusarium wilt - Pathogenicity test; a. Different degree of infection; b. screening of virulent *Fusarium oxysporum* f. sp. *lycopersici* isolate under greenhouse conditions

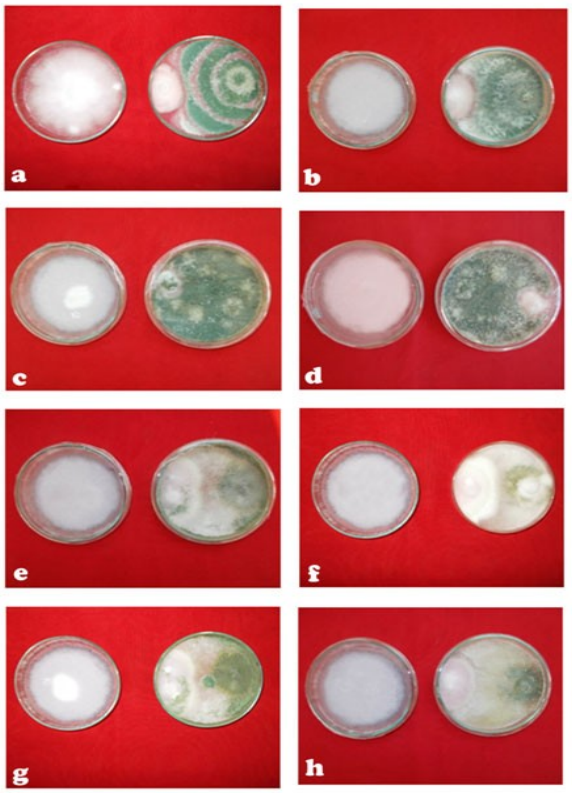

Figure 3: Inhibitory effect of different isolates of *Trichoderma spp* on mycelial growth of *Fusarium oxysporum* f. sp. *Lycopersici* in dual culture technique.
a, b-: *T. harzianum* (isolate 1 and 2), c, d: *T. viride* (isolate 1 and 2), e, f: *T. hamantum* (isolate 1 and 2), g, h : *T. virens*(isolate 1 and 2) with Control.