Evaluation of antagonistic of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *lycopersici*and *Fusarium oxysporum* f. sp. *ciceri in in vitro*

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ABSTRACT *The objective of this paper was evaluatedto antagonistic of 14 different strain of Trichoderma species against Fusarium oxysporum f. sp. lycopersiciand Fusarium oxysporum f. sp. ciceri in in vitro.However, Fusarium wilt is a disease and one of the major yield limited factorswhich are harmful to economic important crop (Tomato and Chickpea).The antagonist of Trichoderma had been determined base on dualand its interaction. Here, II-method of dual culture was measured by Percent Growth Inhibition (Korsten L.) and MIC (Sangoyomi T.). Consituently,Invitro result of all Trichoderma strain was excellent as biological control agents due to PGI value more than 80%. However,Trichoderma virens(96.27%) was reduced to mycelium of Fusarium oxysporum f. sp. lycopersici as compared to other strain. In another hand,Trichoderma harzianumand Trichoderma viride(93.75%) was reduced to Fusariumoxysporum f. sp. ciceri.Therefore, Scale of MIC values were effective for all strain of Trichoderma according to Sangoyomi T.Conclusively, finding of the study indicates thatdifferent strain of Trichoderma has varied degree of antagonistic for both pathogens.*

Keywords: *Trichoderma strain, Fusarium wilts,PGI, MIC, antagonist etc.*

1. Introduction

This crop, (Chickpea and Tomato) is also very important in diet against common disease and economic value for farmers, Soil-borne pathogens inflict a lot of disease and economic yield loss (Babalola and Glick,2012). Out of phytopathogens, specially, wilt disease caused by species of *Fusarium*remain to be a challenging task in terms of management (Barari,2016). Disease of wilt is a destructive mechanism in regard economically important crops caused by the soil borne fungus *Fusariumoxysporum*(Singha *et al.* 2016). *Fusarium oxysporum* Schlechtend Fr. f. sp. *ciceri* (Padwick) Matuo & K. Sato and *Fusarium oxysporum f. sp. lycopersici* (Sacc.) W.C. Snyder and H.N. Hans (FOL), is the most important soil-borne disease throughout the world and particularly in the Indian Subcontinent.It is a cosmopolitan, soil pathogen and saprophyte that feed on dead and decaying organic matter. Therefore, *Fusarium oxysporum* f. sp. *ciceri* and *Fusarium oxysporum f. sp. lycopersici*, is not easily to control and used of chemicals causes threat to environment and crop quality. However, biocontrol is the effective (Sonawane, Mahajan, and Renake 2015; Brandler *et al.* 2017) with best substitution at the place chemical. Recently, interest in biological control of soil borne fungus *Fusariumoxysporum* has increased and effort to find alternatives to use of chemicals (Akila *et al.* 2011;Vinale *et al.* 2014).

Recently,Soil-dwelling filamentous fungy, *Trichoderma* spp. are used in agriculture as biofungicides and induction of plant defense. Morever, Mycoparasitism of *Trichoderma* are considered to be the most important mechanisms, known as *Trichoderma*-mediated biological control (Mukherjee *et al*. 2012, Cucinotta 2014). Therefore, *Trichoderma* spp. is chosen to be the most promising biocontrol agents of plant diseases might be exploited for suitable disease management programs to save environmental risk.

The control of *Fusarium oxysporum* can be done by most promising *Trichoderma* spp., widely used as putative BCAs. In the present study, the biological potential of *Trichoderma* strain was evaluated with in *in vitro* experiments against two different *Fusarium* wilt pathogen (*Fusarium oxysporum* f. sp. *lycopersici*and *Fusarium oxysporum* f. sp. *ciceri*) (Altinok and Erdogan, 2015). Understanding the interaction of *Trichoderma-Fusarium oxysporum* in *invitro*, would be help in developing strains with superior biocontrol properties.

2.Materials and methods

2.1. Source of *Trichoderma* isolates

Trichoderma spp. were collected from rhizosphere zone of healthy plant tomato and chickpea plant according to Akhtar (1996) than

Purified on PDA plate according to watts *et. al*. and Rifai. After 5 days incubate, growing Microflora was observe under compound microscope, identified with available literature Rifai and Webster (1969), Barnett (1998) and identified *Trichoderma* spp. were purified by hypal tip culture technique and preserved in refrigerator at 5^0C for further use (Mustafa*et.al*.,2009; Khang*et.al*.2013).

2.2. Isolation and identification of *Fusarium oxysporum*

F. oxysporum was isolated from both infected tomato and chickpea plants according to Rahman *et.al.*2009; Ignjatov *et al.* 2012.and growing Microflora was observe under compound microscope,identified with available literature. All culture was preserved at 4 ^{0} C until further study.

2.3. Dual test

Trichoderma strains were tested against to both isolate *Fusarium oxysporum* from tomato and chickpea, known as *Fusarium oxysporum* f. sp. *lycopersici*(FOL) and *Fusarium oxysporum* f. sp. *ciceri*(FOC) based on host. The screening of antagonist was based on dual according to method of Dickinson and Skidmore (1976) and Rahman *et.al.*2009. Each antagonist and pathogen was set up in II-method according to Rahman *et.al.*2009 with triplicated. The inoculated plates were incubated at 24±2^{0}C with a photoperiod.

Percent Growth Inhibition (PGI) of pathogen was also calculated as describe by korsten L.

$$PGI = \frac{R_1 - R_2}{R_1} \times 100$$

R_1 = Mycelium growth of both pathogen (*Fusarium oxysporum* f. sp. *lycopersici*(FOL) and *Fusarium oxysporum* f. sp. *ciceri*(FOC) without *Trichoderma* sp.

R_2 = Mycelium growth of both pathogen (*Fusarium oxysporum* f. sp. *lycopersici*(FOL) and *Fusarium oxysporum* f. sp. *ciceri*(FOC) with *Trichoderma* sp.

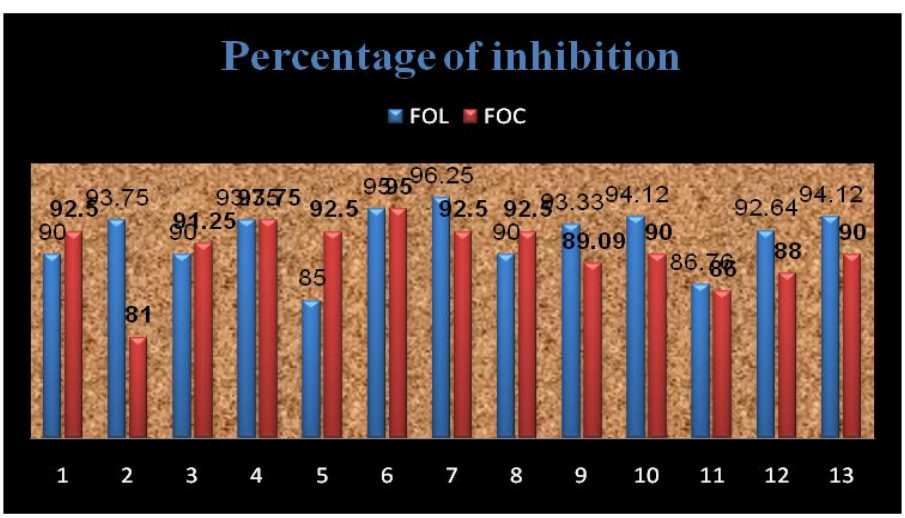
After PGI was calculated, used to value in MIC (Minimum Inhibition Concentration) as measure effective in inhibition of Mycelium growth of both pathogen (*Fusarium oxysporum* f. sp. *lycopersici*(FOL) and *Fusarium oxysporum* f. sp. *ciceri*(FOC). Scale of measure was given by Sangoyomi T. as below (Nwankiti and Gwa; 2018)

≤ 0% Inhibition (Not effective)
> 0-20% Inhibition (Slightly effective)
>20-50% Inhibition (Moderately effective)
>50-<100% Inhibition (effective)
100% Inhibition (Highly effective)

Statistical Analysis

All the data were statistically analyzed using by SPSS. Differences among treatments were determined by XLSTAT. Data are presented as mean values.

3. Result and discussion



Pic.1. Percentage of inhibition represented between two pathogen Fol and Foc.

Here, we had been used to two type pathogen and monitored of percentage of inhibition based on dual technique. All Result of percentage inhibition was more than 80%. Thus all Trichoderma strain was excellent effective by measuring scale of Sangoyomi T. However,*T. virens*(2) was maximum (96.25) percentage of inhibition in case Fol, in compared to other species while *T. harzianum* (3) and *T. piluliferum* (3) was second highest (94.12) in % of inhibition. In case Foc, *T. harzianum* (2) was maximum 95% percentage of inhibition where *T. harzianum* (1) and *T. viride* (2) was

93.75. *T. harzianum* (1) and (2) was same for both pathogen Fol and Foc.

Data are availabled according to list of Table.1.with Pic.1.

Table.1. PGI %and MIC of among *Trichoderma* strain

S. No.	Name	PGI % (FOL)	MIC (FOL)	PGI%(FOC)	MIC(FOC)
1.	*Trichoderma virense*(1)	90	>50-<100% Inhibition (effective)	92.5	>50-<100% Inhibition (effective)
2.	*Trichoderma viride*(1)	93.75	>50-<100% Inhibition (effective)	81	>50-<100% Inhibition (effective)
3.	*Trichoderma piluliferum*(1)	90	>50-<100% Inhibition (effective)	91.25	>50-<100% Inhibition (effective)
4.	*Trichoderma harzianum*(1)	93.75	>50-<100% Inhibition (effective)	93.75	>50-<100% Inhibition (effective)
5.	*Trichoderma piluliferum*(2)	85	>50-<100% Inhibition (effective)	92.5	>50-<100% Inhibition (effective)
6.	*Trichoderma harzianum* (2)	95	>50-<100% Inhibition (effective)	95	>50-<100% Inhibition (effective)
7.	*Trichoderma virens*(1)	96.25	>50-<100% Inhibition (effective)	92.5	>50-<100% Inhibition (effective)
8.	*Trichoderma viride*(2)	87.5	>50-<100% Inhibition (effective)	93.75	>50-<100% Inhibition (effective)
9.	*Trichoderma harzianum*(3)	90	>50-<100% Inhibition (effective)	92.5	>50-<100% Inhibition (effective)
10.	*Trichoderma erinaccum*	93.33	>50-<100% Inhibition (effective)	89.09	>50-<100% Inhibition (effective)
11.	*Trichoderma harzianum*(4)	94.12	>50-<100% Inhibition (effective)	90	>50-<100% Inhibition (effective)
12.	*Trichoderma harzianum*(5)	86.76	>50-<100% Inhibition (effective)	86	>50-<100% Inhibition (effective)
13.	*Trichoderma harzianum*(6)	92.64	>50-<100% Inhibition (effective)	88	>50-<100% Inhibition (effective
14.	*Trichoderma piluliferum*(3)	94.12	>50-<100% Inhibition (effective)	90	>50-<100% Inhibition (effective)

A B C

Pic.2. **A and B**: Type of interaction of *TrichodermaIn vitro* (Dual) and
C: Mycelium of pathogen and *Trichoderma* with conidia

3.1. Isolation and Identification both *Trichoderma* and pathogen

Number of isolate *Trichoderma* was 14.Method of Rifai was confirmed Species and also included ITCC, New Delhi. Both pathogens were *Fusarium oxysporum* f. sp. *lycopersici*(FOL) and *Fusarium oxysporum* f. sp. *ciceri*(FOC) which was isolated from infected plant. Identify to Pathogen and *Trichoderma* was based microscopic studies which are presented in Pic.2.C. Mycelium of *Fusarium oxysporum*was thick comparison to mycelium of *Trichoderma* strain, this work is supported by Poornimasharma, 2011, Cordova-Albores *et.al.* 2016.

3.2. Screening of Dual test

Trichoderma species was first screened and evaluated in term for antagonistic ability. AccordingPic.1and Table.1, In this case of

Fusarium oxysporum f. sp. *lycopersici*, highest PGI values were recorded as 96.25% for *T. virens* in comparison other *Trichoderma* strain and lowest PGI value was 85% for *Trichoderma harzianum*. However,*Fusarium oxysporum* f. sp. *ciceri,* highest PGI was 93.75% for *Trichoderma viride*and *Trichoderma piluliferum*and Lowest PGI value was 81% *T. virens*, result of MIC was showed effective for all strain. Degree of antagonist was varied for each strain (Rahman *et.al*., 2009). Each strain of *Trichoderma* was recorded excellent efficacy against *Fusarium oxysporum* f. sp. *lycopersici*(FOL) and *Fusarium oxysporum* f. sp. *ciceri*(FOC) *In-vitro* (Altinok and Erdogan, 2015). These results have implications for use of such *in-vitro* test as a part of general screen for biological efficacy of different biocontrol agents.

Mycelium interaction is a basic method to assess antagonist according Pic.2. During experiment was observed two type interaction of *Trichoderma* strain with FOL and FOC *In vitro.* One was zone of inhibition and second was without zone of inhibition. These results proofed that all strains of *Trichoderma* antagonized FOL and FOC growth to various degrees and that different degrees of inhibition (Jinantara, 1995). This experiment was showed that PGI values varied but the ranking of MIC remained same for all *Trichoderma* strain. This task agreed with the work done by Mokhtar H *et.al*.arereported the inhibition of pathogen. *Trichoderma*, as one of the promising bio-control agent, has been described (Morsy*et.al.,*2009; Sabalpara*et.al*.,2009). Therefore, Many researcher has been proofed that *Trichoderma* are not equally effective in control of the pathogen *in vitro* by Biswas and Das, 1999; Ramezani,2008. The presence Zone of inhibition has been used as evidence of the production of either antibiotics or cell free metabolites by the *Trichoderma* strains (Jackson *et. al*. 1991; Crawford *et.al*., 1993). Barari (2016) has proofed that siderophore and antibiotics was secreted from *Trichoderma* strains which have played a crucial role in plant growth and sustainable disease management programs to save environmental risk.

Conclusion

The study find out the antagonistic potentials of different strain of *Trichoderma* against to*Fusarium oxysporum* f. sp. *lycopersici*and *Fusarium oxysporum* f. sp. *ciceri* is varied and excellent. Morever, degree of PGI value is varied due toavailable different strain of *Trichoderma*antagonist with both pathogens. This investigation is anevidenced that could be easily control to Plantpathogen by *Trichoderma* antagonist behavior, this is proved*in vitro*.

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