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In vitro Antagonism of *Trichoderma viride* against *Fusarium oxysporum* strains

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Abstract
Fusarium oxysporum is a soil borne fungal pathogen that attacks plants through roots at all stages of plant growth, causes major economic losses by inducing necrosis and wilting symptoms. *Trichoderma viride* tested against *Fusarium oxyspoum* strains under *in vitro* conditions. The results revealed that *Trichoderma viride* showed maximum inhibition 71.00% over *Fusarium oxysporum* strain (E) and minimum inhibition 62.50% over Fusarium oxysporum strain (D) in dual culture plate technique. *Trichoderma viride* showed maximum inhibition 45.27% over *Fusarium oxysporum* strain (E) and minimum inhibition 14.72% over *Fusarium oxysporum* strain (C) in sealing agar plate method. It is concluded that the *T. viride* has found to be a potential biocontrol agent against *Fusarium oxysporum* phytopathogenic strains. It may be therefore a promising ecofriendly bio controlling sources and cost effective for the safe agricultural practices as well as to farmers.

Keywords: *Fusarium oxysporum, Trichoderma viride,* inhibition, dual culture plate technique, sealing agar plate method

Introduction
Fusarium oxysporum is a soil borne fungal pathogen that attacks plants through roots at all stages of plant growth, causes major economic losses by inducing necrosis and wilting symptoms in many crop plants with a great overall impact on productivity. The disease caused by this fungus is characterized by wilted plants, yellowed leaves and root rot minimal or absent crop yield. *Fusarium oxysporum* found in its many pathogenic forms, is the most damaging species of the genus where in plants are concerned. Recently a number of new disease reports on fusarium have been submitted to the literature pool on agricultural research (Bokhariand Perveen 2012) [2] *Fusarium oxysporum* is the causal agent of vascular wilt, a disease that affects a large variety of economically important crops worldwide (Ortoneda *et al*., 2004) [10]. Identification of *Fusarium* species by its morphology is notoriously difficult. Especially its conidiogenesis can be easily changed by environment particularly in the composition of the culture medium. Generally the appearance of a fungal culture which results from its metabolism is regulated by pH in association with the nitrogen source in the medium (Kwasna and Bateman 2005) [7]. *Trichoderma* is a filamentous fungus which has attracted the attention because of their multi prong action against various plant pathogens (Harmam *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 *Trichoderma spp.* generally grows in its natural habit on plant root surface and therefore it controls root diseases in particular.

Many pathogenic microorganisms have developed resistance against chemical fungicides. This seriously hinders the management of diseases of crops and agricultural plants. Considering the deleterious effects of synthetic fungicides on life supporting systems, there is an urgent need for alternative agents for the management of pathogenic microorganisms. Biological control is still in its research phase with few studies reported for bacterial wilt (Messiha *et al*, 2007) [8]. Disease control is currently based on heavy uses of many neuro toxic fungicides, which are damaging the environment and /or pose a threat to public health via food residues, ground water contamination or accidental exposure. The problem caused by fungicides and their residues have amplified the need for effective, biodegradable fungicides with greater selectivity alternative strategies have included the investigation for new type of fungicides, and the re- evaluation and use of bio control agents for disease control.

Materials and Methods

Collection of pathogenic and antagonistic micro - organisms

The Bio-control agent used in this study i.e. *Trichoderma viride a*nd five strains of pathogenic *Fusarium oxysporum* were obtained from microbial culture collection bank (MCCB), Department of Industrial Microbiology, Jacob Institute of Biotechnology and Bioengineering.

List of *Fusarium oxysporum* strains

Fusarium oxysporum strains	Source of isolates
MCCB 0068	From soil (A)
MCCB 0356	From rhizospheric soil of chickpea (B)
MCCB 0364	From rhizospheric soil of lentil crop (C)
MCCB 0412	From rhizospheric soil of moong bean (D)
MCCB 0455	From rhizospheric soil of chickpea (E)

In vitro evaluation of *Trichoderma viride* against *Fusarium oxysporum* strains by dual culture plate technique

The above mentioned fungal bio-agent was evaluated *in vitro* for their antagonistic effect against *F. oxysporum* by dual culture technique (Dennis and Webster, 1971a) [4, 5] on PDA medium.

15ml of PDA medium was poured into sterile Petri plate and allowed for solidification. Seven days old 5 mm disc of *F. oxysporum* strains were cut with a sterile cork borer and placed near the periphery on one side of PDA plate. A plate without antagonist was maintained as control. The inoculated plates were incubated at 28 ˚C for seven days. Each treatment was two replicated.

The antagonistic activity of *Trichoderma viride* was screened *in vitro* against *Fusarium oxysporum* spp. by dual culture plate technique. The antagonistic efficacy against test pathogens was evaluated on PDA medium. Both pathogen and antagonists were grown on sterilized PDA plates separately for 7 days. For testing antagonism in dual culture method, a mycelial disk of 5 mm in diameter of antagonist were excised from the edge of an actively growing 7 old culture plate and inoculated opposite to the pathogenic fungi in the same plate 1cm away from the edge inoculated similarly. For each treatment two replicates were maintained and incubated at 26 ± 2^{0}C. The test pathogen was inoculated in the middle of the plate in duplicates these paired cultures of antagonist and test pathogen were placed equidistant from the periphery so that they would get equal opportunity for their growth. After the incubation period, the radial growth of *Fusarium oxysporum* strains. In control, as well as in treatment plate was measured and the per cent inhibition was calculated using the formula (Rehman *et al.,* 2013) [9].

$$I = \frac{(C - T)}{C} \times 100$$

Where,
I = per cent inhibition
C = Growth of the pathogen in control plate (mm)
T = Growth of the pathogen in dual culture plate (mm)

In vitro evaluation of *Trichoderma viride* against *Fusarium oxysporum* strains by (sealing agar plate method)

Production of volatile metabolites were studied by sealing agar plate method (Dennis and Webster, 1971b) [4, 5] *Trichoderma viride* was inoculated in the center of the petri plate having solidified sterilized PDA medium by placing 5 mm disk (7-day old culture) cut from the margin of the actively growing region of *Trichoderma* spp. and incubated for 4 days at 26 ± 2 ^{0}C. After that the top lid of each Petri plate was replaced with bottom part of another Petri plate with same size containing PDA medium duly inoculated with a 5 mm mycelia disks of the test pathogens after 4 days of incubation. The pairs of each plate were sealed with parfilm and incubated at 26 ± 2 ^{0}C. The PDA medium without *Trichoderma* isolate in the bottom part of the Petri plate with respective test pathogen on the upper lid of plate served as control. Two replicates were maintained for each treatment. This assemble were opened after 4 days and the observations were recorded by measuring colony diameter of the test pathogen in mm in each plate and that of control plates was measured and the per cent inhibition was calculated using the formula (Cherkupally *et al.,* 2017) [3].

$$I = \frac{(C - T)}{C} \times 100$$

Where,
I = per cent inhibition
C = Growth of the pathogen in control plate (mm)
T = Growth of the pathogen in sealed plate (mm)

Results and Discussion

In vitro evaluation of *Trichoderma viride* against *Fusarium oxysporum* strains by dual culture plate technique.

The antagonistic activity of *Trichoderma viride* was screened *in vitro* against *Fusarium oxysporum* strains by dual culture plate technique on PDA media for 7days. *Trichoderma viride* tested against five strains. The results revealed *Trichoderma viride* was shown maximum inhibition 71.00% over *Fusarium oxysporum* strain (E) and minimum inhibition 62.50% over *Fusarium oxysporum* strain (D) (Mean = 66.92%). (Plate 1), (Table 1).

Plate 1: Antagonistic efficacy of *Trichoderma viride* against *Fusarium oxysporum* strains by dual culture plate technique

Fig 1: Treatment of strain A with *Trichoderma viride*, Fig 2: Treatment of strain B with *Trichoderma viride*, Fig 3: Treatment of strain C with *Trichoderma viride*, Fig 4: Treatment of strain D with *Trichoderma viride*, Fig 5: Treatment of strain E with *Trichoderma viride*,

***In vitro* evaluation of *Trichoderma viride* against *Fusarium oxysporum* strains by sealing agar plate method.**

The antagonistic activity of *Trichoderma viride* was screened *in vitro* against *Fusarium oxysporum* strains by *Trichoderma viride* on PDA media for 8 days incubation. *Trichoderma viride* was tested against five strains of *Fusarium oxysporum* by sealing agar plate technique. The results of *Trichoderma viride* showed maximum inhibition 45.27% over *Fusarium oxysporum* strain (E) and minimum inhibition 14.72% over *Fusarium oxysporum* strain (C) (Mean = 33.21%). (Plate 2), (Table 2).

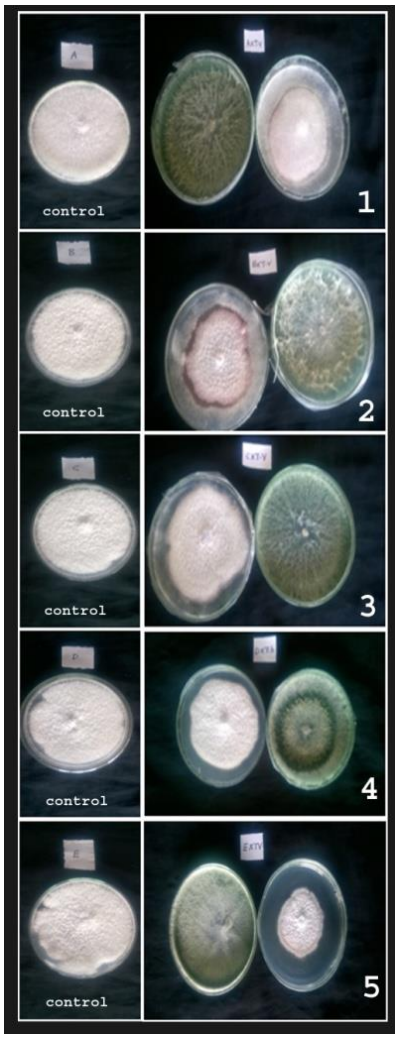

Plate 2: Antagonistic efficacy of Trichoderma viride against *Fusarium oxysporum* strains by sealing agar plate method

Fig 1: Treatment of strain A with *Trichoderma viride,* Fig 2: Treatment of strain B with *Trichoderma viride,* Fig 3: Treatment of strain C with *Trichoderma viride,* Fig 4: Treatment of strain D with *Trichoderma viride,* Fig 5: Treatment of strain E with *Trichoderma viride,*

Table 1: The effect of *Trichoderma viride* on mycelial growth (mm) of *Fusarium oxysporum* strains by dual culture plate technique.

***Fusarium oxysporum* strains**	***Trichoderma viride***	
	Mycelial growth in (mm).	**Percent (%) inhibition over control.**
A	32.1	64.33
B	27.5	69.44
C	29.4	67.33
D	33.75	62.50
E	26.1	71.00
Control	90	-

Table 2: The effect of *Trichoderma viride* against *Fusarium oxysporum* strains by sealing agar plate method:

***Fusarium oxysporum* strains**	***Trichoderma viride***	
	Mycelial growth in (mm).	**Percent (%) inhibition over control.**
A	54	40
B	67.25	25.27
C	76.75	14.72
D	53.25	40.83
E	49.25	45.27
Control	90	-

Trichoderma viride was tested for their efficacy against *Fusarium oxysporum* strains came into contact with the pathogen in 2 days that infers the biocontrol agent is growing rapidly in dual cultures and occupies the space. The clear zone of inhibition was observed in between antagonist and pathogen in plates indicates that *Trichoderma* spp. restrict further growth of *Fusarium oxysporum* strains. *T.viride* overgrown partially over the *Fusarium oxysporum* strains in 7 days. The fast growing antagonists caused more growth inhibition of the pathogens may be due to mycoparasitism and competition for space and nutrients. *Fusarium oxysporum* strains were comparatively less inhibited by *Trichoderma viride* in the Sealing agar plate method.

Similarly, Bardia and Rai (2007) [1] showed antagonistic effect of *Trichoderma viride* and *Trichoderma harzianum* against *Fusarium oxysporum* f. sp.cuminis by 51.15% and 58.41% inhibition of mycelial growth respectively. Rehman *et al*. (2010) [9] showed efficacy of *Trichoderma viride* and *Trichoderma harzianum* against *Fusarium oxysporum* f. sp. *Ciceris* by inhibition of mycelial growth 81% and 83.33% respectively. Cherkupally *et al*. (2017) [3] evaluated the efficacy of *Trichoderma viride* and *Trichoderma harzianum* against *Fusarium oxysporum* f. sp. *Melongenae* by inhibition of mycelial growth 78.88% and 81.11% respectively.

In agriculture, farmers depend on the use of chemical fungicides to control plant diseases caused by pathogenic fungi which constrain the yield. However, overuse of these synthetic chemicals causes hazardous to both environment and health The alternative method for replacement of chemical fungicides has led to the use of biological control agents. Microorganisms that grow in the rhizosphere are ideal for use as biocontrol agents. The studies proved that *Trichoderma* spp. have the potential to control *Fusarium oxysporum* strains under *in vitro* to the extent of 71.00% by dual culture plate technique and 45.27% by sealing agar plate method. It proves that dual culture plate technique found to be more appropriate rather than the sealing agar plate technique for *in vitro* studies, *T. viride* has found to be a potential biocontrol agent against *Fusarium oxysporum* strains. It may be therefore a promising ecofriendly bio controlling sources and cost effective for the safe agricultural practices as well as to farmers.

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