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In vitro Evaluation of *Trichoderma viride* and *Trichoderma harzianum* 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* and *Trichoderma harzianum* were tested against *Fusarium oxyspoum* strains under *in vitro* conditions. The results revealed that *Trichoderma harzianum* showed maximum inhibition 77.77% over *Fusarium oxysporum* strain (E), whereas in *Trichoderma viride* showed maximum inhibition 71.00% over *Fusarium oxysporum* strain (E) in dual culture plate technique. The studies proved that *Trichoderma* spp. have the potential to control *Fusarium oxysporum* strains under *in vitro* to the extent of 77.77% by dual culture plate technique therefore they can be used as effective Biocontrol agents against *Fusarium oxysporum* phytopathogens.

Keywords: *Fusarium oxysporum, Trichoderma viride, Trichoderma harzianum,* inhibition, dual culture plate technique

Introduction

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) [9]. Like various other plant pathogens, *Fusarium oxysporum* has several specialized forms, known as formae specialis that infect a variety of hosts causing various diseases. *Fusarium oxysporum* fungus enters the plant through the roots and pervades throughout the plant through the vascular system.*Fusarium* wilt disease most expansion is in warm regions of world and in acid soils salt soil and on plant debris that this is because of existence of resistant spores (chlamydospores) (Inglis and Kawchuk 2002) [6]. There are many biological control strategies for the control of soil-borne diseases. Among the potential biocontrol agents in the rhizosphere, several strains of the genus *Trichoderma* are reported to be effective in controlling a variety of fungal plant diseases (Meuzies, 1993; Chet and Inbar, 1994) [8]. *Trichoderma* species are known to be an efficient biocontrol agent against a range of disease in many economically important crops (Carvalho *et al.*, 2014) [2].

Trichoderma is a filamentous fungus which have attracted the attention because of their multi prong action against various plant pathogens (Harmam *et al.*, 2004) [5]. 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 defence 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 chemicals are available for the control of different soil borne diseases and have maintained strong paradigm but from the past few years, use of bio agents in disease control has gained a significant momentum due its certain beneficial effects and eco-friendly nature as compared to the chemicals that cause environmental degradation including pollution of surface and ground water and also soil besides increasing pest resurgence. Researches in bio control against soil borne plant pathogens were initiated during 1930s. bio control agents have been gaining as a potential and effective also as a plant health promoter due to its fascinating mechanisms like the production of antifungal metabolites, competition for space and nutrients, mycoparasitism, induction of defence responses and promotion of salinity and drought tolerance.

Materials and Methods

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 bio agents against *Fusarium oxysporum* strains by dual culture plate technique:

The above mentioned fungal bio-agents were evaluated *in vitro* for their antagonistic effect against *F. oxysporum* by dual culture technique (Dennis and Webster, 1971a) [4] 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* and *Trichoderma harzianum* were 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) [1].

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

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

Results and Discussion

In vitro evaluation of bio agents against *Fusarium oxysporum* strains by dual culture plate technique.

The antagonistic activity of *Trichoderma viride* and *Trichoderma harzianum* were screened *in vitro* against *Fusarium oxysporum* strains by dual culture plate technique on PDA media for 7days.Two antagonists *Trichoderma viride* and *Trichoderma harzianum* were tested against five strains.The result of *Trichoderma harzianum* wass howed maximum inhibition 77.77% over *Fusarium oxysporum* strain (E) and minimum inhibition 54.16% was shown by *Fusarium oxysporum* strain (A) (Mean = 68.16%), whereas *Trichoderma viride* was showed by maximum inhibition 71.00% over *Fusarium oxysporum* strain (E) and minimum inhibition 62.50% over *Fusarium oxysporum* strain (Mean =66.92%).

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

Fusarium oxysporum strains	*Trichoderma viride*		*Trichoderma harzianum*	
	Mycelial growth in (mm).	Percent (%) inhibition over control.	Mycelial growth in (mm).	Percent (%) inhibition over control.
A	32.1	64.33	41.25	54.16
B	27.5	69.44	30	66.66
C	29.4	67.33	26	71.11
D	33.75	62.50	25.90	71.14
E	26.1	71	20	77.77
Control	90	-	90	-

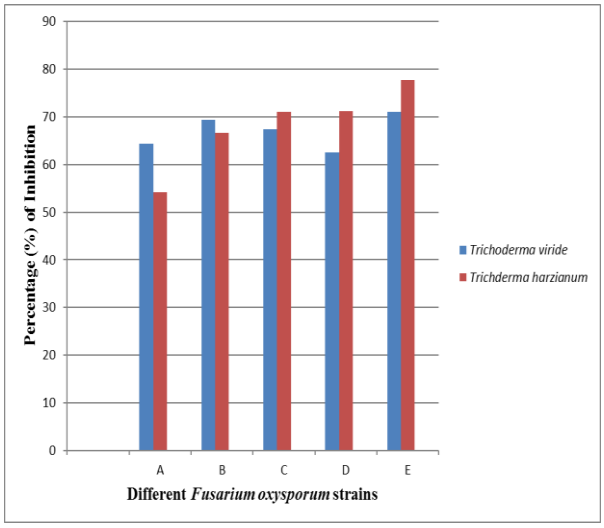

Fig 1: Percentage inhibition of Biocontrol agents on different *Fusarium oxysporum* strains by dual culture plate technique

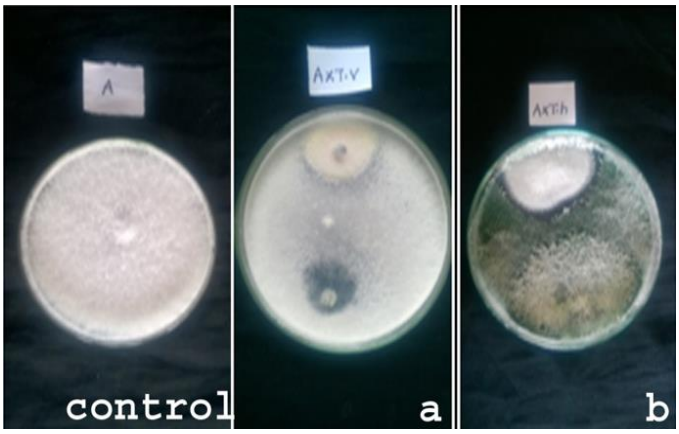

Plate 1a: Treatment of strain A with *Trichoderma viride* b. Treatment of strain A with *Trichoderma harzianum*

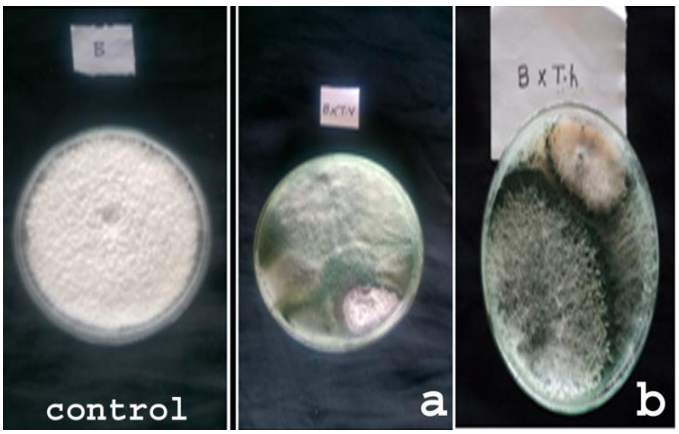

Plate 2a: Treatment of strain B with *Trichoderma viride* b. Treatment of strain B with *Trichoderma harzianum*

Plate 3a: Treatment of strain C with *Trichoderma viride* b. Treatment of strain C with *Trichoderma harzianum*

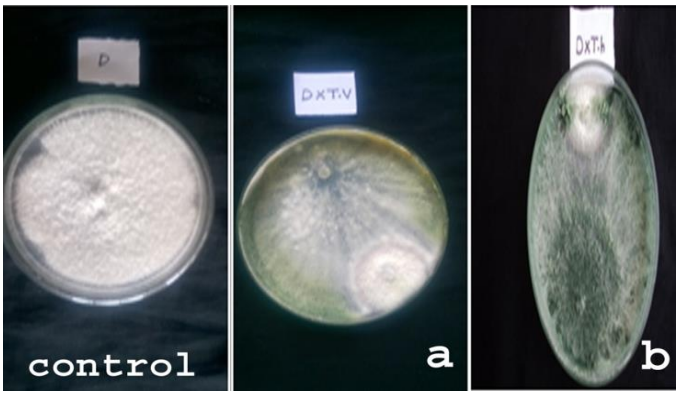

Plate 4a: Treatment of strain D with *Trichoderma viride b.* Treatment of strain D with *Trichoderma harzianum*

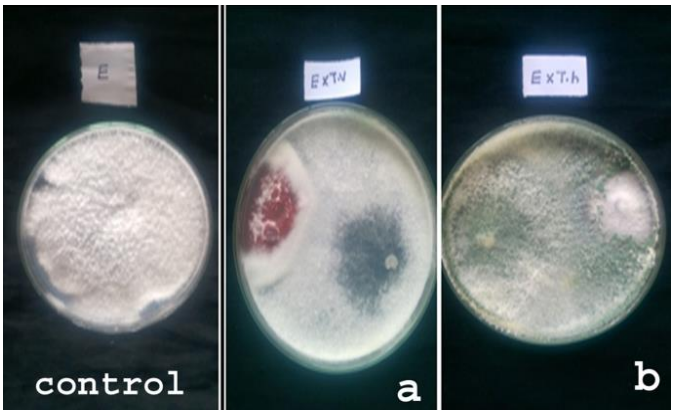

Plate 5a: Treatment of strain E with *Trichoderma viride* b. Treatment of strain E with *Trichoderma harzianum*

Trichoderma viride and *Trichoderma harzianum* were 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. harzianum* overgrown partially over the *Fusarium oxysporum* strains in 5 days, however *T. viride* has taken 7 days to overgrow. 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 both *Trichoderma species* in the Sealing agar plate method Similarly, 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 48.88% and 54.44% respectively. Reddy *et al. (*2014) [10] showed that the evaluation of *Trichoderma viride* and *Trichoderma harzianum* against *Fusarium oxysporum* by inhibition of mycelial growth 72.22% and 78.9% respectively. 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) [11] 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 77.77% by dual culture plate technique. Further it is concluded that the *T. viride* and *T. harzianum* has found to be a potential biocontrol 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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References

1. Bardia PK, Rai PK. *In vitro* and field evaluation of biocontrol agents and fungicides against wilt of cumin caused by *Fusarium oxysporum f*. Sp. Cumini. Journal of Spices and Aromatic Crops. 2007; 162:88-92.
2. Carvalho GA, Correa AS, Oliveira L, DGuedes RNC. Evidence of horizontal transmission of primary and secondary endosymbionts between maize and rice weevils (*Sitophilus zeama* is and *Sitophilus oryzae*) and the parasitoid Theocola xelegan. Journal of Stored Products Research. 2014; 59:61-69.
3. Cherkupally R, Amballa H, Reddy BN. *In vitro* antagonistic activity of *Trichoderma* species against *Fusarium oxysporum* f. sp. *Melongenae*. International Journal of Applied Agricultural Research. 2017; 12:87-95.
4. Dennis C, Webster J. Antagonist properties of species group of Trichoderma. III hypal interaction. Transactions of the British Mycological Society. 1971; 57:363-369.
5. Harman GE, Bjorkman B, Ondik K, Shovesh M. Changing paradigms on the mode of Active and uses of *Trichoderma* spp. for Biocontrol. Journal of Biopesticeds. 2008; (2):29-36.
6. Inglis GD, Kawachuk LM. Comparative degradation of oomycete, ascomycete, and basidiomycete cell walls by mycoparasitic and biocontrolfungi. Canadian Journal of Microbiology. 2002; 48:60-70.
7. Messiha NAS Van, Diepeningen AD, Farag NS, Abdallah SA, Janse JD Van, Bruggen AHC. *Stenotrophomonas maltophilia* a new potential biocontrol agent of *Ralstonia solanacearum*, causal agent of potato brown rot. European Journal of Plant Pathology. 2007; 118:211-235.
8. Meuzies JG. A strain of *Trichoderma viride* pathogenic to germination seedlings of cucumber, pepper and tomato. An International Journal edited by British Society for Plant Pathology. 1993; 10:1365-3059.
9. Ortoneda M, Guarro J, Madrid MP, Caracuel Z, Roucero MI, Mayayo E, *et al* A *Fusarium oxysporum* as a multihost model for the genetic disection of fungal virulence in plants and mammals. U S National Library of Medicine National Institute of Health. 2004; 72(3):1760-66
10. Reddy BN, Saritha KV, Hindumathi A. *In vitro* screening of antagonistic potential of seven species of *Trichoderma* against different plant pathogenic fungi. Research Journal of Biology. 2014; 2:29-36.
11. Rehman SU, Dar WA, Ganie SA, Bhat JA, Mir GH, Lawrence R, *et al*. Comparative efficacy of *Trichoderma viride* and *Trichoderma harzianum* against *Fusarium oxysporum* f sp. C*iceris* causing wilt of chickpea. African Journal of Microbiology Researc. 2013; 7(50):5731-5736.