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BIO-CONTROL OF LENTIL WILT DISEASE BY *TRICHODERMA HARZIANUM*

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ABSTRACT

Fusarium caused vascular wilt of lentil is a serious biotic threat, sometimes lowered the yield severely as pathogen resides the field always for their wide mode of nutrition. *Trichoderma* initiated bio-control was well accepted for their beneficial arsenal rather chemical hazard. Assessment of antagonism of *T. harzianum* against *F. oxysporum* by in-vitro and ex-vitro to reduce lentil wilt was the goal of this study. In vitro antagonistic study shows that *T. harzianum* IMI 392432 restricted the growth of *F. oxysporum* most in both method of dual culture but inhibition was highest (mean PIRG- 73%) in method -2 wherein test fungi paired in two opposite poles on PDA plate at 28±2°C temperature. 100% *Trichoderma* metabolites containing culture filtrate in ThFDA media totally prevents pathogen's growth. It is assumed that antibiotic compound produced by the *T. harzianum* turns poison substrate for respective pathogen. Tricho-compost treated lentil plant shown maximum reduction of wilt incidence compared to *T. harzianum* spore suspension and other combinations in ex-vitro pot study. Wilt disease appeared most (63%) in combining treatment of conventional compost + *Fusarium* spore suspension. Population density of both test fungi in conventional compost is high and maximum population of *T. harzianum* found in conventional compost +Tricho-compost application

Keywords: Biological control, antagonism, filtrate, Tricho-compost, biomass

1. INTRODUCTION

Lentil is consumed by the people of Bangladesh in their daily diet as a source of essential amino acids for balance nutrition. The area used for lentil cultivation in Bangladesh is 1,54,655 ha and the yearly production is about 1,22,000 mt wherein calculated yield were 0.853 t/ha in 2008-09 (BBS, 2009). In Bangladesh, the decreasing trend of lentil yield year after year frustrated the growers compared to the other lentil producing countries (Hossain et al.,1999). Owing to biotic and abiotic stresses, the crop yield of lentil lies below the attainable level sometimes. The per capita consumption of pulse in Bangladesh is only 12 g/day, which is much lower than WHO recommendation of 45 g/day (Afzal et al., 1999). Pulses have played an important role in sustaining the productivity of soils in Bangladesh and generally grown without fertilizer since they can meet their nitrogen requirement by symbiotic fixation of atmospheric nitrogen in the soil (Senanayake et al., 1987; Zapata et al., 1987).

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Biotic stresses like wilt is one of the serious disease limiting lentil yield (Hamali and Hassanein, 1996), caused by *Fusarium oxysporum* f. sp. *lentis* and results 100% seedling mortality for monoculture and causative weather conditions (Begum 2003).Chlamydospore of pathogen survives in soil, crop residues and colonize in roots of major crop grown in rotation with lentil and remains viable for long (Erskine and Bayaa, 1996) and may cause complete crop failure under favorable conditions in certain areas (Chaudhary and Amerjit, 2002). The disease appears in either the early stage of crop growth (seedling) or during the reproductive stage (Stoilova and Chavdarov, 2006). Although broad spectrum fungicide, pesticides sprays offer reasonable management that results imbalanced microbial community and their diverse harmful effects to the ecosystem and on human life concern over pesticide residues. It prioritizes the perception of relying less chemical input which is practiced by using beneficial bio-antagonist. *Trichoderma* species have shown efficiency on biocontrol of plant pathogens (Harman et al., 2004) and worldwide established as potential antagonist. *Trichoderma* is a soil born versatile genus, found in everywhere and considered as key genus of Agricultural soil can be associated with plants root and rhizosphere. *Trichoderma harzianum* is reportedly opportunistic invader of a wide range of phytopathogenic fungi via mechanisms of competition, rhizosphere competence, mycoparasitism, antibiosis, induced resistance, and also act as avirulent symbiont for promoting plant growth (Howell, 2003).

Understanding the efficacy of *T. harzianum* and its culture filtrate to prevent the growth of pathogen *in vitro* was the prime initiative. *T. harzianum* was evaluated here against *F. oxysporum* as a bio-control tool to check devastating lentil wilt in pot cultivation.

2. MATERIALS AND METHODS

2.1 Culture of pathogen *F. oxysporum* and antagonist fungus *T. harzianum*

Wilted lentil plants were collected from an infested field to laboratory. After washing and sterilization, the infected roots were transferred to sterile Potato Dextrose Agar plate supplemented with streptomycin sulfate to prevent bacterial growth and incubated at 28±2°C for 7 days. Appeared fungal mycelium surrounding on root pieces transferred to fresh plate soon and perpetuated the culture to obtain pure culture. The disease-causing agent then allowed to sporulate at 12 h photoperiod regime need for identification. The pathogen was primarily identified based on morphological characteristics such as type, shape and color of sexual or asexual spore assisted by ultra-microscope. The pathogenicity of the fungus *F. oxysporum* f. sp. *lentis* was confirmed using lentil cv. BARI Masur-7, under pot culture conditions in net house at the field of Agronomy and Agricultural extension, in the University of Rajshahi.

Three Isolated strain of *T. harzianum* IMI 392432, IMI 392433, IMI 392434 (Rahman et al., 2011) were collected from Microbiology & Biotechnology laboratory in the Department of Botany, University of Rajshahi. *T. harzianum* isolates cultured on Potato Dextrose Agar medium prepared following Anonymous standard procedure. Single spore culture of *T. harzianum* continued to get pure culture and stored for further use. Pure stock cultures were used to make

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conidial suspensions for soil inoculation of antagonist and plant pathogen were prepared following the procedure of Erskine and Bayaa (1996), El-Hassan (2004).

2.2 Antagonistic activity of *T. harzianum* strains in dual plate

Two techniques were followed to implement dual culture technique (Dhingra and Sinclair, 1995) of test fungi. In Method-1, 5 mm diam. mycelial disc of test antagonist (*T. harzianum* IMI 392432, 392433 & 392434*)* taken from 3 days old culture and was placed to pair against same sized mycelial disc of *F. oxysporum* about 30mm from periphery on PDA plate of 90mm diam. The pathogen and antagonist disc were place at equal distances from the periphery of the Petri plate. The plates were incubated at 25±2°C in light condition for the days need to overlap pathogen colony. In Method-2, 5mm- diam. mycelial plug of antagonist fungi was placed and paired with *F. oxysporum* at the opposite end in PDA plate or in peripheral boundary. The plates were incubated at 28 ±2°C in light condition for the days need to overlap pathogen colony. A single plug of same sized antagonistic and test fungi alone was placed on PDA plate that served as control in both methods. Radial growth of *F. oxysporum* was measured by Edington et al (1971) formula. $I = \{\frac{(C2-C1)}{C2} \times 100$

Where I=percentage inhibition of radial mycelial growth, C_2=Radial growth of pathogen in control, C_1= Radial growth of pathogen in test plate.

The numbers of days taken by *Trichoderma* isolates to overlap the pathogen colony completely were recorded as second assessment. The isolate taken the shortest number of days was counted for signifying good antagonistic properties and select for next investigation.

2.3 Antifungal activity of *T. harzianum* culture filtrate

The presence and role of antifungal metabolites secreted by *T. harzianum* (IMI 392432) in liquid culture filtrate and their effect on pathogen (*F. oxysporum*) for reduction of biomass weight and mycelial growth was tested here. In this regards *T. harzianum* cultured primarily in 250ml culture flask containing 100ml Potato Dextrose Broth (PDB) medium and incubation was done in rotary shaker at 150 rpm with 25˚C temperature by 24 hours rotation for 30 days. Fungal culture was filtered through Whatman No.1 filter paper and collect the broth. The fine Fungal mats were then harvested by centrifugation of culture broth at 4100×g for 10 minutes. Discarding the pellet, supernatant broth further filtered using a sterile What man micro GD/X syringe filter (Whatman International Ltd., New Jersey, USA) with a pore size of 0.22 μm. Fungus free filtrate confirmed finally by culturing them on PDA media and store in refrigerator at 4°C temperature for use. Two types of filtrate media were prepared for antifungal study (i) ThFDA (*T. harzianum* Filtrate Dextrose Agar) medium were prepared using four concentrations of filtrates (25%, 50%, 75%, 100%) supplemented with 2% dextrose and 2% agar (wt/vol). (ii) ThFDB (*T. harzianum* Filtrate Dextrose Broth) were prepared using same concentration of

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filtrates supplemented with only 2% dextrose (wt/vol). The both filtrate media were autoclaved at 121°C for 10 min. ThFDA media dispense in Petri-plates and 5mm diam. disc of three days old *F. oxysporum* culture place at the centre of medium. PDA plates with no filtrate seeded with a plug from old *F. oxysporum* served as the control. The plates were incubated in a growth cabinet at 25±2°C temperature. After 7 days, the growth inhibition was analyzed by measuring the radial growth of the *F. oxysporum* colony following Edington et al (1971) formula mentioned before.

ThFDB were dispense in 250ml conical flask at the rate of 100ml and 2 plugs of 5mm diam. mycelial disc of *F. oxysporum* seeded in flask which inoculated with rotary shaker (150 rpm) at 25±2°C temperature for 10 days. Sterilized PDB media seeded with same plug of *F. oxysporum* culture similarly as control to compare the filtrate effect. Biomass growth of pathogen in ThFDB and control media, assessed by harvesting with centrifugation at 4100×g for 10 minutes. Fresh weight of test pathogen measured after 12-hour air drying at room temperature and dried in oven at 50°c for 6 hours to get dry weight for each.

2.5. Pathogenecity test of *F. oxysporum* in pot culture

Earthen pot (size: 26×16.5 cm) were filled up with soil mixture of loam and sand (2:1 vol/vol) with the treatments of Tricho-compost (2.7×10^6CFU/g) and conventional compost (3:1). Different combination of pathogen and antagonist spore suspension too applied; *F. oxysporum* at the rate of 100 ml (2.0×10^6 CFU/ml) and *T. harzianum* IMI 392332 at the rate of 60 ml (4.8×10^6CFU/ml). Ten surface-sterilized (with house hold bleach for 10 min.) lentil seeds of BARI Masur-7; a highly susceptible variety collected from Bangladesh Agriculture Development Corporation (BADC), Rajshahi. were evenly sown in each pot and placed on a net house. Chemical fertilizer (NPK-1:2:1 applied on pot at 15 days interval and carefully watered by hand 2–3 days later.

The treatments employed in this experiment are as follows
T_1: Tricho-compost only; T_2: Conventional compost only

T_3: Chemical fertilizer only; T_4: Tricho-compost+ *F. oxysporum* spore suspension;

T_5: Conventional compost+ *T. harzianum* spore suspension;

T_6: Conventional compost +*T. harzianum* and *F. oxysporum* spore suspension;

T_7: Con. compost +Tricho-compost

Biocontrol activity in pot culture was measured as wilt symptoms produced by the pathogen on treated plants or checked with time. The disease incidence was recorded starting from the 7^{th} day after inoculation and continued for nine weeks using a 1-9 scale (Bayaa et al., 1995). 1: no symptoms, 3: yellowing of the basal leaves only, 5: yellowing of 50% of the foliage, 7:

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complete yellowing of the foliage, flaccidity of the top leaves along with partial drying, 9: whole plant or part of the plant wilted or dried.

Soil sample of each treatment has passed through serial dilution technique for the detection of pathogen and antagonist fungi individually as CFU in maximum wilted condition in lentil pot cultivation. 10 g air dried soil sample (after 15 days) dissolve in 100ml distilled water and shake-well in a rotary shaker for 30 minutes to get stock sample.1 ml of stock sample diluted serially till 10^2 dilution factor as per recommended for fungus cell count. 1ml of dilute solution in both cases spread over PDA plate and *Trichoderma* Selective Medium (TSM) reported by Elad et al. (1981) and incubated at 25±2°C for three days to enumerate the fungus cell. Finally, the counted fungus population was expressed using following formula as per dry weight of soil.

$$CFU = \frac{No\ of\ colonies\ on\ agar\ plate \times Dilution\ factor}{Amount\ of\ culture\ used\ to\ make\ a\ plate}$$

2.6. Experimental Design and Data Analysis

Completely Randomized Design (CRD) with five replications were followed as experimental design in growth measurement of fungus from culture media as well as pot grown plant and data were analyzed statistically using MSTAT-C computer program and means were compared following Duncan's Multiple Range Test (DMRT).

3.RESULT AND DISCUSSION

3.1 Percent Inhibition of Radial Growth (PIRG) of *F. oxysporum* by *T. harzianum*

3.1.1 Dual culture test

In dual culture test, each of tested *T. harzianum* strain limited the colony growth of the pathogen. Among these three-individual strains, the best antagonistic potential was displayed by *T. harzianum* IMI 392432. This strain limited the *F. oxysporum* colony as 24.9 mm and 21.18 mm diam. respectively in method-1 and method -2. The percent inhibition of radial growth (PIRG) was 61.48 % and 72.33 % in method-1 and method-2 respectively. This strain overlapped the test fungi by 7-8 days and covered the whole plate in shortest duration. The lowest percentage of pathogen inhibition by antagonistic isolates *T. harzianum* IMI 392434 was found in method-1 that is 53.02 % but PIRG was much higher that is 66.88 % in method-2. It took maximum time to overlap the pathogen fungi that is 11 days and 14 days respectively in method-1 and 2 which shows low scale of antagonism. *T. harzianum* IMI 392433 shown medium inhibition and took 8 days in method -1 and 10 days in method-2 to cover the whole plate and the percentage of its inhibition was 56.36 % and 68.35 % consecutively in method-1 and method-2 (Table-6.1).

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Table 3.1 Antagonism of *T. harzianum* against *F. oxysporum* by dual culture test

Culture method	*T. harzianum* strains	Mean PIRG of *F. oxysporum*	Time for overgrowth on pathogen	Scale of Antagonism
Methods-1	IMI 392432	61.480±0.993b	7 days	Very high
	IMI 392433	56.360±0.993a	8 days	High
	IMI 392434	53.023±0.993a	11 days	Low
Methods-2	IMI 392432	72.330±0.996b	8 days	Very high
	IMI 392433	68.356±0.996a	10 days	High
	IMI 392434	66.883±0.996a	14days	Low

**** In a column, data are the mean values with standard error having different letters within four different culture media differ significantly as per DMRT***

Mycoparasitism by hyphal interaction of antagonist fungi with test pathogen is one of the basic method to assess microbial inhibition. Percentage inhibition of radial growth (PIRG) and colony overlapping pattern is considered as a scale of mycelial inhibition. Result demonstrated that super PIRG of *F. oxysporum* have noted by the strain of *T. harzianum* IMI 392432 in both methods applied for dual culture was used in subsequent step.

Rahman et al. (2009) also found *T. harzianum* IMI 392432 isolates which was the best antagonist against *Ceratocystis paradoxa* as it inhibited the pathogen most at 80.82 % PIRG. Dharmaputra et al. (1994) examine two strain of *T. harzianum* and *T. viride* against three isolates of *Ganoderma* from oil palms and found *T. harzianum* isolates (B10-1) as best performer to inhibit the pathogen most. The zone of inhibition was clear and radial growth inhibited most in method-2 wherein test fungi placed on the margin of culture plate and incubated at 28°C. This method is recommended for highest inhibition and it is easy to take measurement as they grew in margin towards the centre.

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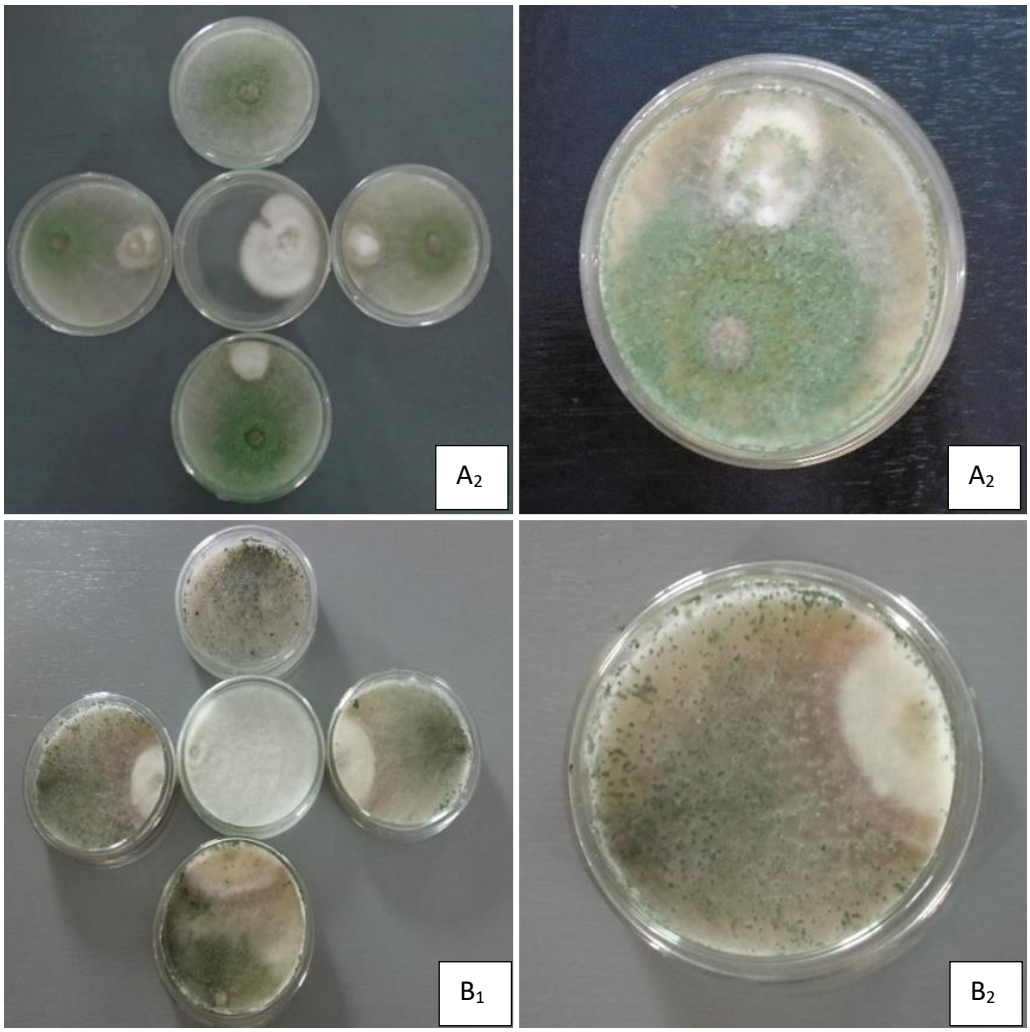

Figure 3.1 Inhibited *F. oxysporum* by *T. harzianum* strains in dual plate: A1, A2 = method-1 and B1, B2 = method-2 (In A1, B1 - left, bottom and right petri-plate for *T.h.* IMI 392432, 33, 34 paired with pathogen, top for only *Trichoderma* and middle for only *Fusarium*; A2, B2 = enlarge view of overlapped pathogen in respective method)

3.1.2 Culture filtrate assay

In culture filtrate assay, liquid culture of the best isolates of *T. harzianum* IMI 392432 (in response of dual culture test) was prepared at three concentrations (25%, 50%, 75%, 100%) which used to amend the PDA media is termed as ThFDA to observe the growth of *F. oxysporum.* The study shows 25% LCF (liquid Culture Filtrate) not inhibited the pathogen too

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much and found 40 mm mycelial growth of *F. oxysporum*. 50% LCF inhibit the test fungi moderately and pathogen produce only 35 mm mycelial diameter of colony. Highest PIMG (Percent Inhibition of mycelial Growth) was 85% observed in 75% LCF of *T. harzianum* that allowed *F. oxysporum* to grow and proliferate only 12 mm mycelial diameter. No growth of *F. oxysporum* was found in 100% LCF of *T. harzianum* thus full concentration exert total inhibition of test fungi among other filtrate percentage. So, 100% PIRG generated against 100% filtrate concentration containing *Trichoderma* metabolites. As the thermostability of *Trichoderma* filtrate compound was not affected by autoclaving at 121°C for10 min. was used for next step of biomass inhibition.

Table 3.2 Effect of culture filtrate of *T. harzianum* on mycelial growth of *F. oxysporum*

Concentration of *Trichoderma* culture filtrate	Mycelial growth (mm) of *F. oxysporum* on dual plate	PIMG of *F. oxysporum*
25%	40	50%
50%	30	62.5%
75%	12	85%
100%	No growth	100%
Control	80	-

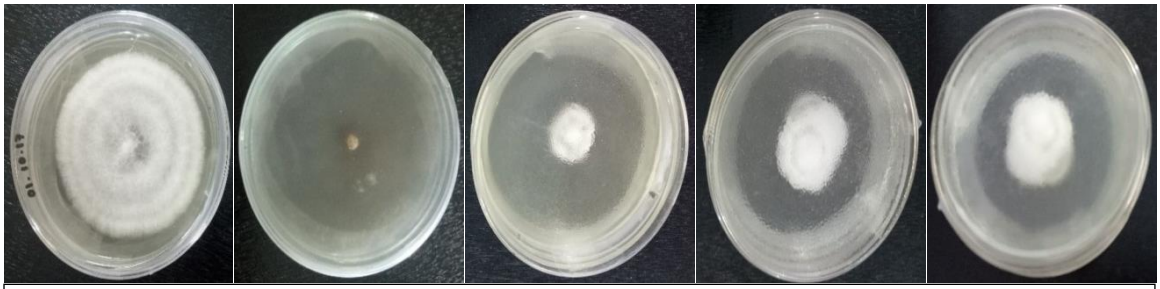

Figure 3.2 Effect of *T. harzianum* culture filtrate (%) on mycelial growth of *F. oxysporum*.

T. harzianum culture filtrate used to assess the metabolites effect on *Fusarium* growth in agar plate as the 2nd assumed mechanism of microbial antagonism is antibiosis by metabolites secretion in surrounding media. The ability of *Trichoderma* species to produce inhibitory substances against microorganism has been described by Dennis and Webster (1971) first. Effect of volatile metabolites on the growth of pathogen presented in an experiment wherein three *Trichoderma* spp. significantly inhibited the mycelial growth of the pathogen. Perveen and

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Bokhari (2012) found highest inhibition (40.91%) of the pathogen was reported by *T. viride* whereas isolates of *T. harzianum* showed considerable variation in the inhibitory effect. They conclude that cell free culture filtrate of *T. viride* incubated at 25°C showed highest growth inhibition (25.57%) followed by *T. harzianum* strain TDPs (20.59%) and T1s (17.43%) however none was fungicidal to the pathogen.

3.2. Biomass inhibition of *F. oxysporum* by *T. harzianum* culture filtrate

T. harzianum culture filtrate (100%) amended broth media were evaluated for biomass growth of *F. oxysporum* in shaked liquid culture. The maximum biomass has been found as fresh weight and dry weight are 1.573 g and 0.531 g respectively in control treatment of Potato Dextrose Broth culture. *Trichoderma* metabolite amended ThFDB produce list fresh biomass that was 0.044g. Dry biomass of *F. oxysporum* was as follows and weighted as 0.011g. Culture filtrate of *T. harzianum* was not able to prevent the *F. oxysporum* growth totally in liquid culture likewise linear growth on agar plate. Very poor growth of test pathogen indicates growth restriction of *F. oxysporum* by *T. harzianum* and suggest further study.

El-Hassan et al. (2013) demonstrate a study on the use of *T. hamatum* for biocontrol of lentil wilt disease and reported that control broth culture of *F. oxysporum* on PDA yielded 0.22 g dry biomass compared to ThFDB (*Trichoderma hamatum* filtrate dextrose broth) which produced a little amount (0.01g) of dry biomass; It was observed from their experiment that *F. oxysporum* was fail to germinate and grow after 7 days of incubation in *T. hamatum* culture filtrate. Their study indicates that the antibiotic compound produced by *T. hamatum* are not only fungistatic but also fungicidal.

Table 3.3 Effect of culture filtrate of *T. harzianum* on biomass growth of *F. oxysporum*

Culture media	Shaked culture	
	Fresh w.(g)	Dry w.(g)
PDB	1.573	0.531
ThFDB	0.044	0.011

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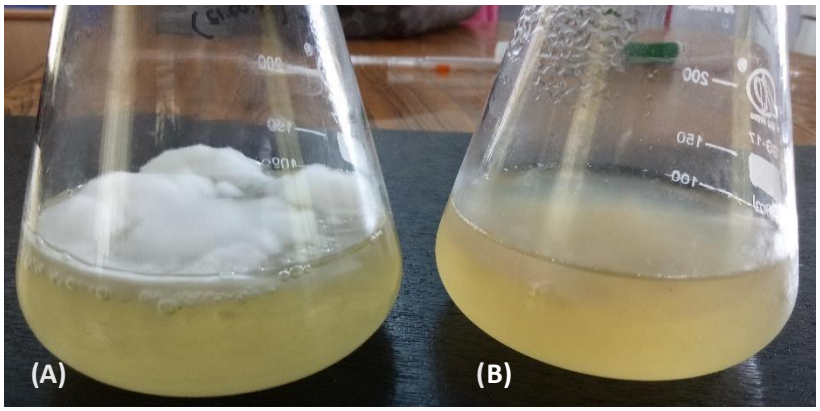

Figure 3.3 Effect of *Trichoderma* filtrate on pathogen growth; *F. oxysporum* in PDB (A) and ThFDB (B).

3.3 Pathogenicity of *F. oxysporum* in pot culture

The study has been expanded to assess the antagonistic effect of *Trichoderma* and Tricho-compost in *ex-vitro* condition for checking wilt incidence caused for *F. oxysporum*. The highest wilt incidence found with the treatment T_5 which comprise conventional compost and *Fusarium* spore suspension and the value was 63.33%. Wilt percentage was higher (60.00%) with the treatment of T_2 which comprise only conventional compost application. Though microflora and fauna reduced some extent in curing process of compost but pathogen may be survived in conventional compost and causes more wilt disease. Next wilt occurrence was 50.00% with the treatment T_6 (Conventional compost + *Fusarium* and *Trichoderma* spore suspension). The lowest wilt percentage (20.00%) found with the treatment T_1 which comprise Tricho-compost only that reduced the wilt incidence mostly in pot soil. Super colonization of *T. harzianum* in Tricho-compost checked *F. oxysporum* maximum. The second wilt check generated by the treatment T_3 which was only chemical application interestingly. Chemical fertilizer application makes the pot soil more compact and without organic matter it is hard to colonize the microorganism may be the reason not allowing wilt pathogen to make infection to the plantlets. In treatment T_4, wilt incidence was 46.66% followed to the treatment T_7 (30.00%) which comprise conventional compost and Tricho-compost.

Akrami et al. (2011) briefed in a study that treatments of *T. harzianum*, *T. asperellum*, *T. virense* and mixture of isolates and *Trichoderma* combinations showed more biocontrol effectiveness than the other treatments. In the seed coating, the minimum and maximum *Fusarium* rot was 42.3 and 55% for these three isolates, respectively, while in soil treatment, the minimum and maximum *Fusarium* rot was 44.5 and 52.6% respectively. Khan et al. (2014) investigate the biocontrol active *Trichoderma* strain for chickpea caused by *F. oxysporum* f. sp. *ciceri* and *Rhizoctonia solani* in field conditions and found inhibited wilt incidence by 25%–56% and 39%–67% and increased the yield of chickpea by 12%–28% and 8%–24% in the 2 years, respectively.

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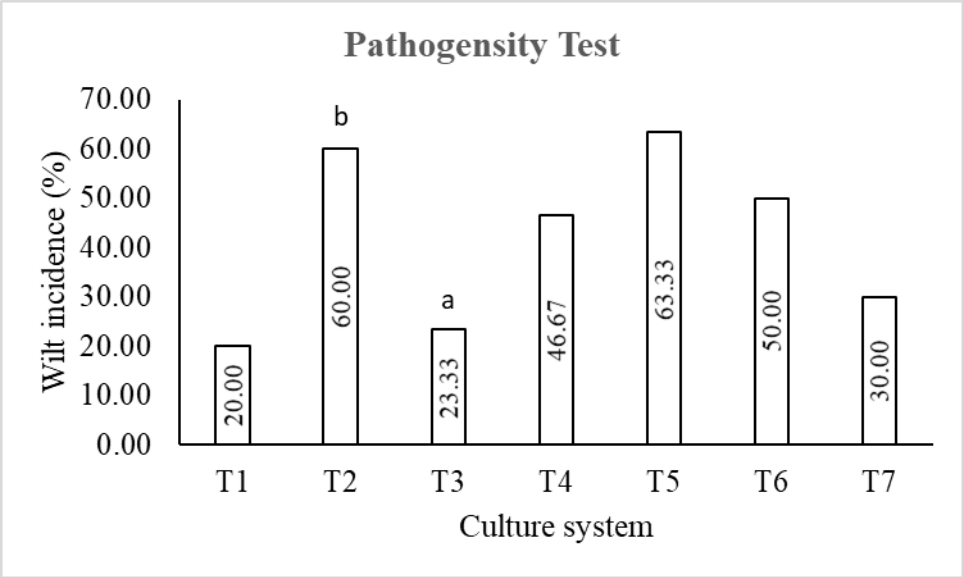

Figure 3.4 Wilt % against six treatments of pathogen antagonist combination in lentil pot culture (T_1 = Tricho-compost only, T_2 = conventional compost only, T_3 = chemical fertilizer only, T_4 = Tricho-compost + *Fusarium* spore suspension, T_5 = Con. compost+ *Fusarium* spore suspension, T_6 = Con. compost+ *Fusarium* and *Trichoderma* spore suspension, T_7 = Con. compost +Tricho-compost).

data are the mean values with standard error having different letters within four different culture media differ significantly as per DMRT.

Table-3.4 Population density ($\times 10^2$ CFU /g soil) of antagonist, pathogen in treated pot soil

Treatments	*Trichoderma* CFU/g soil	*Fusarium* CFU/g soil
T_1	6.00×10^4	2.00×10^2
T_2	4.00×10^4	3.00×10^3
T_3	8.00×10^3	1.00×10^2
T_4	7.00×10^4	2.5×10^3
T_5	5.00×10^4	4.0×10^3
T_6	8.00×10^4	2.8×10^3
T_7	1.00×10^5	2.4×10^3

Treatments denotes; T_1 = Tricho-compost only, T_2 = conventional compost only, T_3 = chemical fertilizer only, T_4 = Tricho-compost + Fusarium spore suspension, T_5 = Con. compost+ Fusarium spore suspension, T_6 = Con. compost+ Fusarium and Trichoderma spore suspension, T_7 = Con. compost +Tricho-compost

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Maximum antagonist population (1.00×10^5 CFU /g soil) enumerated in Tricho-compost and conventional compost applied pot soil. Second highest *Trichoderma* population (8.00×10^4 CFU/g soil) counted in treating soil with conventional compost + *Fusarium* and *Trichoderma* spore suspension. Only conventional compost found with a less number of *Trichoderma* population compared to combined treatment. Though a good number of *Fusarium* found in conventional compost was also less than *Trichoderma* population. Population of *Fusarium* was highest (4.0×10^3 CFU/g soil) in T_5 which comprise conventional compost and *Fusarium* spore suspension were the reason for highest wilt incidence. Interestingly very few *Trichoderma* and *Fusarium* individual occurred in pot soil treated with only chemical indicates less microbial activity. The reliance without organic substances not facilitates the microorganism to colonize properly in compact pot soil. Super population of *Trichoderma* in combined application benefited plant most to check wilt as well as health promotion.

El-Hasan et al. (2013) evaluated *T. hamatum* in connection with biocontrol aspect of lentil wilt and explain *Trichoderma* population is co-relate with all of its beneficial arsenal. Likewise, populations of *T. hamatum* ($5x10^8$ CFU/g) in the co-inoculated treatment had significantly ($p \leq 0.05$) increased up to 9.92 Log_{10} ($9.92x10^9$ CFU/g) and slightly decreased to 7.69 Log_{10} ($5.74x10^7$ CFU/g) per gram of air-dried soil between the 10th and 40th days, respectively, after planting. When they used only the *T. hamatum* treatment, the population increased up to 8.80 Log_{10} ($6.46x10^8$ CFU/g) and decreased to 5.94 Log_{10} ($5.54x10^5$ CFU/g) per gram of soil during the same period as detected on the TSM plates. However, they found the total number of *T. hamatum* at higher colonization percentages in the combined treatment (7.69 Log_{10} CFU/g soil) with the pathogen than it was alone (5.94 Log_{10} CFU/g soil) in the vicinity of plant roots after 56 days of planting. Finally, they conclude that the antagonistic activity of *T. hamatum* was more intensive and denser on the surface of soil plates rather agar plates in the presence of *F. oxysporum* for more population.

4.CONCLUSION

T. harzianum is quite capable to hinder the growth of *F. oxysporum* in agar plate as well as soil inoculation. Therefore, treating pathogen inoculation is the cause of highest seedling wilt which minimized by *T. harzianum* at Tricho-compost application in this study.

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