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Efficacy of Trichoderma isolates as biocontrol agent against *Alternaria solani*

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Abstract
Species of genus *Trichoderma* are able to improve growth of the plant and commonly used as bio fungicides typically against soil borne phytopathogenic mycoflora. The present research work was conducted with the aims to determine biomass production of *Trichoderma* species on different media, chitinase activity on chitin medium, seed germination test with *Trichoderma* on tomato seeds and their antagonistic behavior using dual culture assay against *Alternaria solani* on different compost including carbon rich compost, nitrogen rich compost and nutrient enriched compost were tested. The two species of *Trichoderma* isolated from rhizosphere of citrus, wheat and tomato of different localities of district Sargodha, Punjab, Pakistan that were identified by using ITS1 and ITS4 primers. Three strain of *T. harzianum* HM, HK, HC and one strain of *T. asperellum* TH were evaluated and all strains produced maximum mycelial biomass on Yeast Peptone Dextrose Agar (YPDA) media and Potato Dextrose Agar (PDA) while green conidia production was seen on Oatmeal Agar (OA) media. Water Agar (WA) found to be least effective for biomass production. Rapid tomato seed germination and inhibition % of *Alternaria solani* in dual culture assay observed maximum by *T. harzianum* HK as compared to all strains while carbon rich compost inoculated *Trichoderma* strains found to be most effective rather than nitrogen rich compost and nutrient enriched compost.

Keywords: *Trichoderma* species, molecular characterization, nutrient media, Chitinase, seed germination, antagonism, compost

1. Introduction
Alternaria solani is a soil bone pathogenic fungus causing early blight (foliage disease), collar rot (basal stem of seedlings) stem lesions and fruit rot of tomato (*Lycopersicon esculentum* Mill.) and early blight is most destructive disease causes complete defoliation leads to severe yield losses (35-78%) in tomato [22, 7] while collar root causes 20-40% seedling losses in the field. Management of early blight of tomato before and after crop maturity is mainly through resistant cultivars as well as chemical pesticides that are damaging the environment [15, 9]. Most valuable and environmental friendly method for control of early blight of tomato is the use of resistant varieties/cultivars when they are available [7]. On the other hand, breeding for development of resistant cultivar can be complicated when no dominant gene is identified [30]. In addition, pathogens new races going to be overcome host resistant. These all factors promote the research in biocontrol that is independent from recent concern related to environmental protection [13].
Most of the data in literature concerning to biological control refers to *Trichoderma* that has been recognized successful and effective biocontrol agent of various pathogens as well as diseases for long period of time [19, 17]. Species of *Trichoderma* interact with roots and their soil surroundings where they release lytic enzymes and many other components leads to plant systemic resistant against abiotic stress [24]. A successful ecological feature of this genus *Trichoderma* is mycoparasitism and efficient defensive approach induced in plant [25]. The species of *Trichoderma* are used as biocontrol of various plant pathogens including foliar and soil plant parasites and also offer eco-friendly approach for suppression of pathogen growth and plant diseases [21, 5].
Non-chemical approaches such as organic amendment and suppressive compost having long term benefits against the management of plant diseases are available but need to be commercialized [3, 1, 2]. Organic matter amendment improves the soil productivity by nutrient and water retention [29]. Hence, organic matters amendment can enhance the natural biocontrol activity against soil-borne pathogens and diseases [11, 18]. The present research work was conducted with the aims to determine biomass production of *Trichoderma* species on different media, chitinase activity on chitin medium, seed germination test with *Trichoderma* on tomato seeds and their antagonistic behavior using dual culture assay against *Alternaria solani* on different compost including carbon rich compost, nitrogen rich compost and nutrient enriched compost were tested.

2. Material and Methods
2.1 Isolation of *Trichoderma* and pathogen
All isolates were collected from rhizosphere of citrus, wheat and tomato of different localities of district Sargodha, Punjab, Pakistan. Isolation of *Trichoderma* species and *Alternaria solani* was done on potato dextrose agar (PDA) media from different soil samples and tomato soil respectively followed by soil dilution method. *Trichoderma* isolates were identified by molecular characterization and pathogen confirmed by pathogenicity test.

2.1.1 Molecular characterization of *Trichoderma* species
Genomic DNA was extracted by using the CTAB method as described by Sambrook and Russell (2001). PCR analysis

was performed for ITS region by using ITS1 and ITS4 primers. The gene fragment was sequenced and analyzed. The Phylogenetic analysis was performed by using MEGA version 5.2 with WAG model.

2.2 Pathogenicity test

Fresh harvested tomato fruits of same size from greenhouse brought into the fungal plant laboratory of College of Agriculture, Sargodha and washed with tap water. Washed fruits 2-3 times surface sterilized for 1-2 mints with 2% sodium hypocholoride (NaOH) and rinsed twice with distilled water. Fruits were dried in hood or laminar flow chamber and inoculated with 5mm PDA disk of *Alternaria solani* incubated at 25±2c^{o} for symptoms development.

2.3 Effect of nutrient media on *Trichoderma*

The mycelial growth, biomass and spore production of three isolates of *T. harzianum* HM, HK, HC and one isolate of *T. asperellum* TH were investigated on different nutrient media includes Oat Meal Aagr (OA), Potato Dextrose Agar (PDA), Water Agar (WA) and Yeast Peptone Dextrose Agar (YPDA) media. An autoclaved single cellophane sheet as size of petri plate was placed on solidified media plate and single block of 5mm each isolate was positioned at center of Petri plates containing different nutrient media. All treatment replicated three times and plates were incubated at 25±2ºC. Biomass production was determined by mycelial harvesting from cellophane of each replicate.

2.4 Chitinase activity test

Chitinase activity of *Trichoderma* isolates was determined on chitinase medium comprising of following ingredients (per litter) 3g of ammonium sulphate [$(NH_4)_2SO_4$)], 1g of citric acid ($C_6H_8O_7$), 0.3g of magnesium sulphate ($MgSO_4.7H_2O$), 2g of monopotassuim sulphate (KH_2PO_4), 0.15g bromocresol purple, 200 µl tween 80 and 4.5g colloidal chitin (Agrawal and Kotasthane, 2012). Colloidal chitin prepared from crab shells flakes followed the method of Murthy and Bleakley (2012). Medium pH was adjusted at 4.7 by using NaOH or HCL and then autoclaved for 15 min at 121ºC. Autoclaved medium was poured into petri plates and allowed to solidify. Single plug (5mm) of each isolate was positioned at center of Petri plates and incubated at 25±2ºC for formation of colored zone.

2.5 Dual culture antagonism test

The *Trichoderma* isolates including *T. harzianum* HM, HK, HC and *T. asperellum* TH were evaluated *in vitro* against *Alternaria solani* by using dual culture assay in two different ways in 90 mm Petri plate with the use of 7 days old culture 5mm plug of antagonist and pathogen. 1) Each plug of antagonistic and pathogenic fungi placed at equivalent distance from periphery. 2) One plug of pathogen placed at the centre of Petri plate and 3 plugs of antagonistic fungi placed in a triangle form from equal distance of pathogen. Inoculated PDA plates incubated in an incubator at 25±2ºC and percentage inhibition was calculated by using following formula;

$$\text{Percentage Inhibition (\%)} = \frac{R_1 - R_2}{R_1} \times 100$$

Where R_1 is the pathogen radial growth in control petri plate, R_2 is the pathogen radial growth in *Trichoderma* inoculated petri plate.

2.6 Seed germination test

Tomato seeds were obtained from fresh and healthy Yaqui cultivar and allowed to dry for 30 minutes on blotter paper. Aqueous solution of *Trichoderma* isolates conidial suspension was prepared and spore calculation done by using haemocytometer at $1x10^7$ spores per ml. Seeds were treated with 1% NaOH and rinsed thrice with water. Dry seeds were coated with *Trichoderma* isolates spore suspension supplemented with 2% starch (w/v) (as an adhesive) for 2-3 minutes while seeds were dipped in water serve as control. The 100 number of seeds were placed on double layer of moist filter paper in petri plate replicated thrice for germination incubated at 25±2ºC and observed every 24 hours. Seed germination percentage calculated by using following formula:

$$\text{Seed Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

2.7 Pot Experiment

Pot experiment was conducted with three replicates to compare three different composts viz carbon rich compost, nitrogen rich compost and nutrient enriched compost comprises of sulphur and zinc that mixed at the ratio of 1:10 (1kg compost: 10kg soil) with soil for performance evaluation of most competent isolates of *Trichoderma harzianum* and *T. asperellum* against *Alternaria solani* causes early blight of tomato under *in vivo* conditions. The three days old seedlings of tomato with control and *Trichoderma* treated were sown in surface sterilized plastic bag filled with autoclaved compost mixed with soil. Pots were treated with conidial suspension of *Trichoderma* isolates containing $1x10^7$ spores per 10 ml counted by using haemocytometer while control treated with pathogen *Alternaria solani*. After 2.5 weeks of inoculation plant height, number of leaves, shoot fresh weight, shoot dry weight, and root fresh weight as well as root dry weight recorded on carbon rich compost, nitrogen rich compost and nutrient enriched compost.

2.8 Statistical analysis

The readings of experimental results were obtained and data analyzed with the help of statistical software R by using least significant test (LSD). The results which have $P<0.05$ were considered as significant.

3. Results

3.1 Isolation and molecular identification of *Trichoderma*

All four strains of *Trichoderma* selected among 15 isolates based upon their morphological differences were isolated and molecular characterized as *T. asperellum* (TH) and *T. harzianum* (TM, TK and TC). Genomic DNA was extracted by using CTAB method from a single pure colony of one isolate of the fungus and PCR analysis was performed for ITS region. A single fragment of 550bp length was amplified and sequenced. The sequence analysis and BLAST results showed very close similarity with the

isolates of *T. asperellum* and *T. harzianum* (Figure: 1). Maximum likelihood phylogenetic tree was constructed with MEGA5 program to present the relationship of isolates from Pakistan and other regions of the world.

3.2 Pathogenicity test

The Pathogenicity test that was carried out on Yaqui cultivar inoculated with *Alternaria solani* and produces the symptoms (Figure: 2). The symptoms were similar in appearance to dark brown lesions with concentric rings that occurs on leaves while mature lesions covered by velvety black mass of spores that only be visible under light microscope. The microscopic characteristics revealed that *Alternaria solani* have brown and septate haphae as well as conidiosphore produced zigzag appearance. Conidiophores bear branched and simple conidia which have longitudinal and transverse septations. The conidium end nearest to conidiophores is round and tapper towards apex.

3.3 Effect of nutrient media on *Trichoderma* speices

Four different nutrient media includes oat meal agar (OA), potato dextrose agar (PDA), water agar (WA) and yeast peptone agar (YPDA) were used for the biomass yield of *Trichoderma* species. All nutrient media showed significant results except water agar media and YPDA found to be most effective in respect to mycelial yield while OA is best for green conidia production for *Trichoderma* species. PDA found to be most successful nutrient media for mycelial and conidial production. *Trichoderma harzianum* HK showed best growth rate on all nutrient medium followed by *T. harzianum* HM, *T. asperellum* TH and *T. harzianum* HC (Table: 1, Figure: 3).

3.4 Chitinase activity test

Chitinase activity of *Trichoderma* species was tested on chitinase medium supplemented with colloidal chitin contains bromocresol purple (indicator dye) adjusted at pH 4.7. *Trichoderma* species resulted change in indicator dye (bromocresol purple) and shift towards alkaline pH. A clear purple zone (shifted from yellow color) was seen after 3 days of incubation at 25±2. The colloidal chitin medium classified into categories includes low, medium, high and no chitinase activity. *T. harzianum* HK showed maximum chitinase activity followed by *T. harzianum* HM, *T. asperellum* TH and *T. harzianum* HC (Figure: 4).

3.5 Dual culture antagonism test

According to findings obtained in dual culture technique, the best inhibition of *Alternaria solani* was examined in *T. harzianum* HK (45-68%) while *T. harzianum* HM (43-66%) also found to effective antagonist against *Alternaria solani*. *T. asperellum* TH inhibited 45-56% growth of *Alternaria solani* and *T. harzianum* HC found to be least effect as compared to *T. harzianum* HK, *T. harzianum* HM and *T. asperellum* TH. Dual culture technique 2 was effective as compare to techniques 1 because *Alternaria solani* inhibited by higher inoculum of *Trichoderma* that was in equal in technique 1 (Table: 2, Figure, 5).

3.6 Seed germination Test

The seed germination speed and its percentage were measured that is common indicator of seed viability. So, this value is too little sensitive for detection of reduction in seed quality that effects performance of seed under field conditions. The seed germination speed was measured after 24, 48 and 72 hours. *Trichoderma* treated seed had more germination speed as compared to untreated seeds. After 72 hours, *Trichoderma* treated seeds have 100% germination while untreated was 68% and *T. harzianum* HK showed 100% germination after 48 hours that found to be most effective in seed germination and its development (Table: 3).

3.7 Pot Experiment

The effects of *Trichoderma* species with carbon, nitrogen and nutrient enriched (Phosphorous and zinc) compost was observed on plant height, number of leaves, shoot fresh weight, shoot dry weight, root fresh weight and dry weight after 18 days of seed potting. *Trichoderma harzianum* HK found to be most effective for root and shoot development followed by *T. harzianum* HM, *T. asperellum* and *T. harzianum* HC on all used compost. The results revealed that increase in plant height, number of leaves, shoot fresh weight, shoot dry weight, root fresh weight and dry weight in all tested treatments as compared to control while carbon rich compost found to be effect rather than nitrogen rich and nutrient enriched compost. The soil amended with different compost materials and *Trichoderma* species leads to increase in area and numbers of leaves of plant according to results and evidence shown in statistical analyzed data (Table: 4a, 4b and 4c) and Figure (6a, 6b, 6c).

4. Discussion

The present study was on *Trichoderma* species for biomass production on different nutrient media, chitinase activity and antagonist effect against *Alternaria solani* under *in vitro* and *in vivo* conditions. The choice of nutrient medium is most essential and important step in development of successful lab experiments. In our study, oat meal agar is best for *Trichoderma* conidia production while yeast peptone agar effective only for mycelial growth and PDA was best medium for both conidial and mycelial growth of *Trichoderma*. Kumar and Singh (2008) reported that generally PDA and cauliflower agar media (CAM) are best medium for growth of *Trichoderma* [16]. The growth and sporulation of *Trichoderma* species on different nutrient media was due to its inherent capability and their ecological behavior [26]. Morphological studies and growth features of *Trichoderma* was studied on various types of nutrient media Czapek agar media, potato dextrose agar, corn meal agar and oat meal agar and special nutrient agar media [12, 23]. Chitinase production is another feature of in which *Trichoderma* species produce different lytic enzymes to facilitate degradation of fungal cell wall that was tested on chitinase detection medium. Bromocresol purple (color dye) which is chromic chemical compound play an important role in this regard that causes change in color of media depend upon pH level and chitinase activity was observed visually. Agarwal and Kotasthane (2012) conducted an experiment of *Trichoderma* chitinolytic assay and reported that chitin breakdown into N-acetyle glucosamine transfer the pH towards basic (alkaline level) and change of color from yellow to purple [4].

Trichoderma species found to be most effective antagonism of foliar and soil borne pathogens. Dual culture assay was conducted against *Alternaria solani in vitro* and examined percent inhibition by biocontrol agent. According to findings of Ibarra-Medina *et al*., (2010) antagonistic isolates

can be considered as potential biocontrol agent that 70% colonized the growth of pathogen [14]. In our study, *T. harzianum* HK reduces the growth of *Alternaria solani* under *in vitro* condition from 45-68% that can be considered effective antagonist. In pot experiment under *in vivo* condition, *Trichoderma* reduces the growth of pathogen and also promoted the seed germination as well as growth of the plant. It also induced the systemic resistance in plant and facilitates plant growth [10, 31]. Compost also promoted plant growth and soil health for getting good quality fruits and vegetables. Carbon and nitrogen rich compost found to be effective for tomato growth as compared to nutrient enriched compost. If optimum ratio of carbon and nitrogen compost used for soil health then can be obtained very effective results. Chandna *et al*., (2014) reported compost very effective in photosynthetic activity of tomato and C: N ration should be maintained in compost for better performance of the crop and microbial activity [8, 6, 28].

5. Conclusion

The major challenge of the widespread use of alternatives to control plant pathogens is to meet the requirement of a low production cost. Therefore, it is necessary to develop eco-friendly approaches with no or little toxicity that control plant pathogens even when applied at low concentrations.

6. Conflict of Interests

"The authors declared no conflict of interest to publish the article".

Table 1: Effect of different nutrient media on biomass production of *Trichoderma* species

Nutrient Media	*T. asperellum* TH	*T. harzianum* HM	*T. harzianum* HK	*T. harzianum* HC	Mean
OA	149.9±0.90 ^{c}	199.3±1.09 ^{c}	231.6±1.52 ^{c}	167.6±1.04 ^{b}	187.1±1.13 ^{C}
PDA	152.3±1.13 ^{b}	213.4±0.75 ^{b}	276.3±1.72 ^{b}	130.3±0.99 ^{c}	193.1±1.14 ^{B}
WA	7.9±0.26 ^{d}	10.7±0.44 ^{d}	11.6±0.65 ^{d}	5.7±0.33 ^{d}	8.9±0.42 ^{D}
YPDA	591.4±1.31^{a}	839.0±1.37 ^{a}	962.7±1.63 ^{a}	563.9±1.25 ^{a}	739.3±1.38 ^{A}
Mean	225.4±0.9 ^{C}	315.6±0.99 ^{B}	370.5±1.38 $^{\mathbf{A}}$	216.9±0.90 $^{\mathbf{D}}$	

*Small letters are for comparison for within column comparison, Capital letters for comparison for comparison among nutrient media mean, Bold and capital letters are for comparison among species.

Table 2: Dual culture assay of *Trichoderma* with *Alternaria solani* by two different techniques

Trichoderma strains	Dual culture technique 1			Dual culture technique 2		
	Control	A.S	% Inhibition	Control	A.S	% Inhibition
T. asperellum TH	5.03±0.04 ^{a}	2.8±0.05 ^{b}	45.0±1.03 ^{a}	5.02±0.03 ^{a}	2.2±0.02 ^{b}	56.86±0.05 ^{b}
T. harzianum HM	5.01±0.05 ^{a}	2.9±0.02 ^{a}	43.13±0.05 ^{c}	5.00±0.4 ^{a}	1.73±0.04 ^{c}	66.01±0.86 ^{a}
T. harzianum HK	5.02±0.04 ^{a}	2.78±0.03 ^{c}	45.31±0.60 ^{a}	5.2±0.03 ^{a}	1.6±0.05 ^{d}	68.62±1.03 ^{a}
T. harzianum HC	5.03±0.03 ^{a}	2.84±0.04 ^{b}	44.22±0.80 ^{b}	5.1±0.02 ^{a}	3.3±0.02 ^{a}	35.29±0.5 ^{c}
Mean	5.02±0.04	2.83±0.03	44.41±0.4	5.08±0.12	2.20±0.02	41.69±0.61

* A.S: *Alternaria solani*

**Small letters are for comparison for within column comparison

Table 3: Effect of *Trichoderma* species on seed germination at different time interval

Trichoderma strains	Germination %		
	24 hr	48 hr	72 hr
Control	34.44±2.35 ^{d}	47.77±2.7 ^{d}	68.88±3.09 ^{b}
T. asperellum TH	58.88±2.22 ^{c}	68.88±1.11 ^{c}	100.0±0.00 ^{a}
T. harzianum HM	66.66±2.35 ^{b}	87.77±2.22 ^{b}	100.0±0.00 ^{a}
T. harzianum HK	68.88±1.11 ^{a}	100.0±0.00 ^{a}	-
T. harzianum HC	66.66±2.22 ^{b}	78.77±2.22 ^{b}	100.0±0.00 ^{a}

*Small letters are for comparison for within column comparison

Table 4a: Response of plant parameters on carbon rich compost emended with *Trichoderma* species

Trichoderma species	Carbon rich Compost					
	Plant Height (cm)	No. of leaves	Shoot fresh wt. (g)	Shoot dry wt. (g)	Root fresh wt. (g)	Roots dry wt. (g)
Control	5.14±0.05 ^{e}	17.66±0.33 ^{e}	1.97±0.06 ^{e}	1.15±0.05 ^{e}	0.94±0.05 ^{e}	0.54±0.02 ^{e}
T. asperellum TH	9.9±0.13 ^{c}	49.44±1.09 ^{c}	19.17±0.18 ^{c}	1.81±0.06 ^{c}	2.88±0.06 ^{c}	1.56±0.05 ^{c}
T. harzianum HM	11.48±0.08 ^{b}	64.11±1.05 ^{b}	21.54±0.18 ^{b}	2.53±0.05 ^{b}	3.65±0.06 ^{b}	2.22±0.06 ^{b}
T. harzianum HK	12.47±0.27 ^{a}	71±0.84 ^{a}	24±0.21 ^{a}	2.85±0.06 ^{a}	3.91±0.09 ^{a}	2.6±0.09 ^{a}
T. harzianum HC	8.7±0.08 ^{d}	44.66±0.62 ^{d}	18±0.04 ^{d}	1.43±0.04 ^{d}	2.37±0.04 ^{d}	1.27±0.02 ^{d}

*Small letters are for comparison for within column comparison

Table 4b: Response of plant parameters on nitrogen rich compost emended with *Trichoderma* species

Trichoderma species	Nitrogen rich Compost					
	Plant Height (cm)	No. of leaves	Shoot fresh wt. (g)	Shoot dry wt. (g)	Root fresh wt. (g)	Roots dry wt. (g)
Control	4.64±0.03 ^{e}	14.11±0.26 ^{e}	1.62±0.04 ^{e}	0.6±0.03 ^{e}	0.73±0.02 ^{e}	0.35±0.03 ^{q}
T. asperellum TH	8.01±0.06 ^{a}	42.88±0.6 ^{c}	18.23±0.15 ^{c}	1.6±0.04 ^{c}	2.7±0.02 ^{c}	1.51±0.04 ^{c}
T. harzianum HM	8.30±0.07 ^{a}	44.77±0.6 ^{b}	19.82±0.14 ^{b}	1.98±0.05 ^{b}	3.00±0.05 ^{b}	1.996±0.04 ^{b}

T. harzianum HK	8.66±0.05 [a]	50.55±1.02 [a]	21.16±0.14 [a]	2.34±0.08 [a]	3.45±0.05 [a]	2.25±0.04 [a]
T. harzianum HC	7.60±0.06 [d]	38.11±0.56 [d]	14.1±0.09 [d]	1.24±0.05 [d]	2.42±0.04 [d]	1.4±0.05 [c]

*Small letters are for comparison for within column comparison

Table 4c: Response of plant parameters on nutrient enriched compost emended with *Trichoderma* species

***Trichoderma* species**	**Nutrient Enriched Compost**					
	Plant Height (cm)	**No. of leaves**	**Shoot fresh wt. (g)**	**Shoot dry wt. (g)**	**Root fresh wt. (g)**	**Roots dry wt. (g)**
Control	4.04±0.04 [e]	10.77±0.36 [e]	1.3±0.04 [e]	0.38±0.03 [e]	0.5±0.02 [e]	0.22±0.02 [e]
T. asperellum TH	4.77±0.05 [c]	29.22±0.46 [c]	15.22±0.31 [c]	1.18±0.03 [c]	2.1±0.04 [c]	1.14±0.06 [c]
T. harzianum HM	5.23±0.04 [b]	35.11±0.42 [b]	16.85±0.38 [b]	1.37±0.03 [b]	2.45±0.03 [b]	1.45±0.05 [b]
T. harzianum HK	8.66±0.05 [a]	50.55±1.02 [a]	21.16±0.14 [a]	2.34±0.08 [a]	3.45±0.05 [a]	2.25±0.04 [a]
T. harzianum HC	7.60±0.05 [d]	38.11±0.56 [d]	14.00±0.09 [d]	1.24±0.05 [d]	2.42±0.04 [d]	1.40±0.05 [d]

*Small letters are for comparison for within column comparison

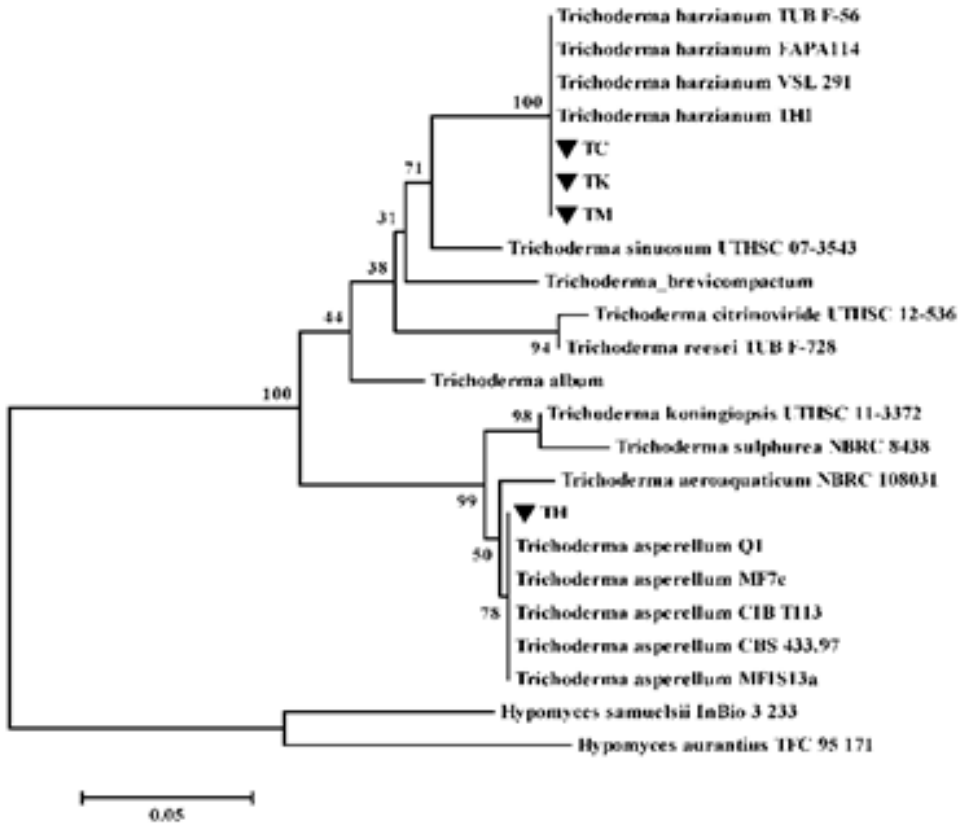

Fig 1: Phylogenetic analysis of *Trichoderma* isolates. Phylogenetic tree by using ITS locus, multiple alignments were performed by using ClustalX program. Maximum likelihood (ML) trees were constructed using the program MEGA version 5.2 with the WAG model. The scale indicates 0.1 substitutions per site

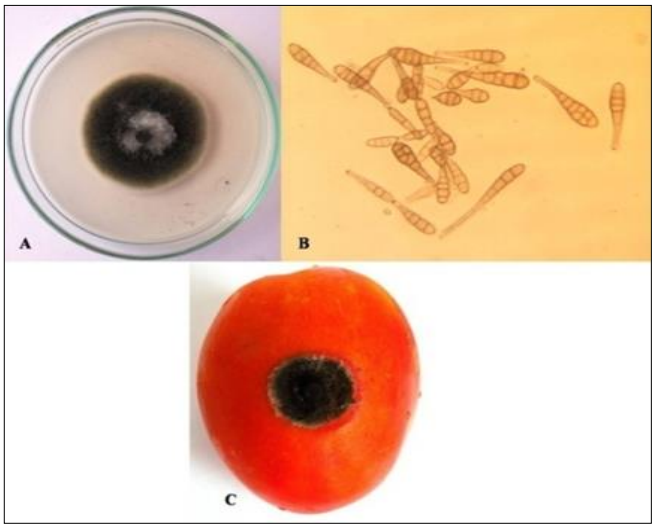

Fig 2: Pathogenecity test (A) Pure culture of *Alternaria solani* on PDA media (B) Dark brown spores of *Alternaria solani* (C) *Alternaria solani* infection on tomato fruit

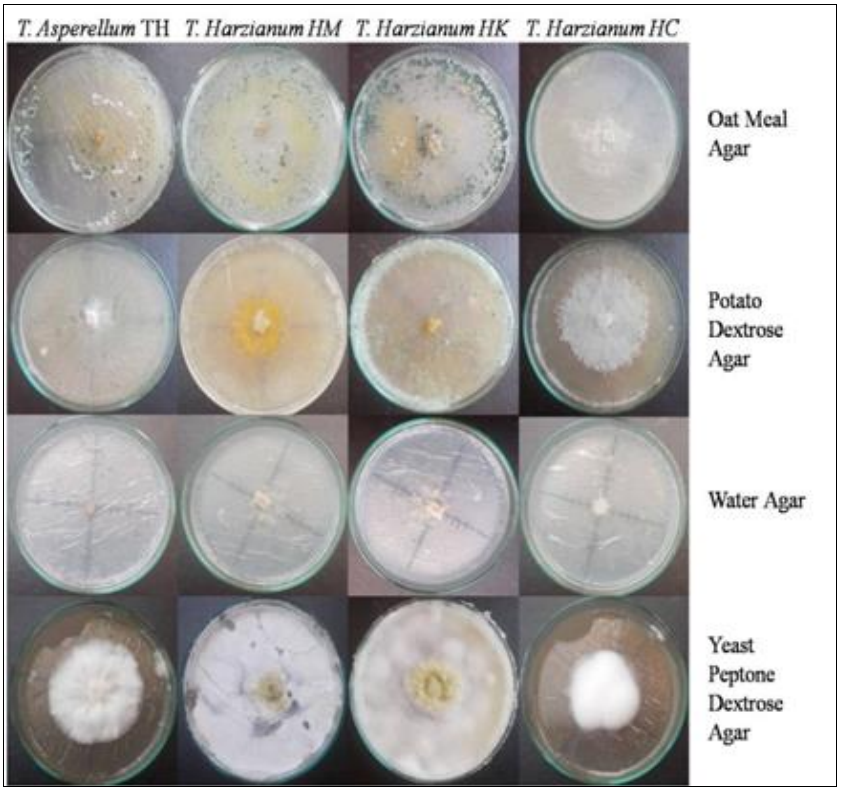

Fig 3: Effect of different nutrient media on biomass production of *Trichoderma* species

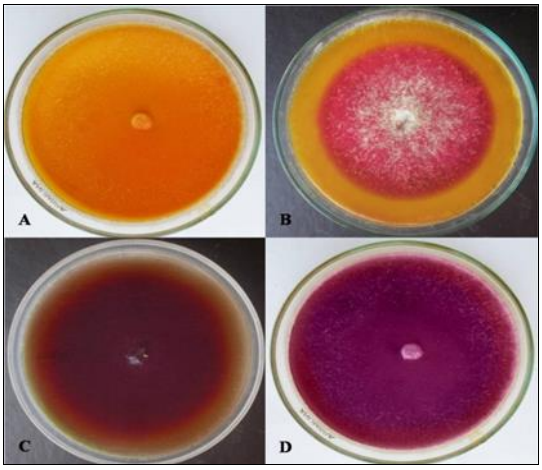

Fig 4: Chitinase activity test (A) T. harzianum HC chitinase activity (B) T. asperellum TH chitinase activity (C) T. harzianum HM chitinase activity (D) T. harzianum HK chitinase activity

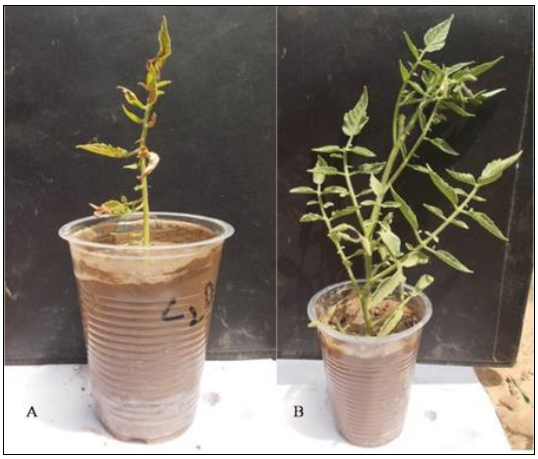

Fig 6a: Effect of carbon compost on tomato plant (A) Control (Compost amended with *Alternaria solani*) (B) Compost amended with *Trichoderma+Alternaria solani*

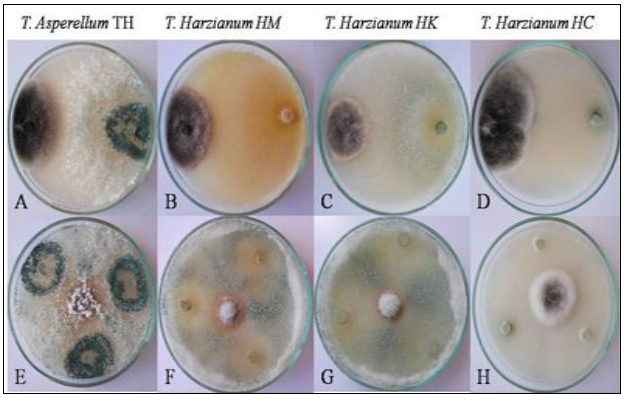

Fig 5: Dual culture test of Trichoderma species against *Alternaria solani*.Dual culture technique 1 (A-D) Dual culture technique 2 (E-F)

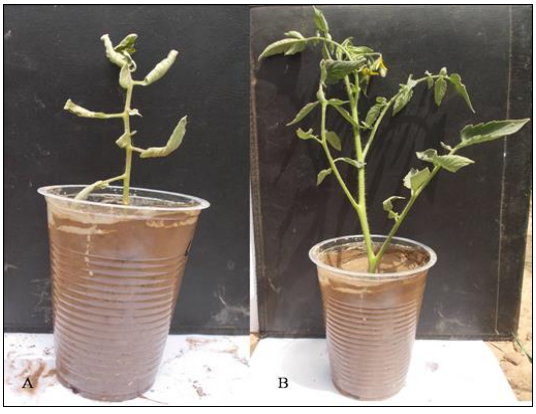

Fig 6b: Effect of nitrogen compost on tomato plant (A) Control (Compost amended with *Alternaria solani*) (B) Compost amended with *Trichoderma+Alternaria solani*

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