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In vitro Growth Performance of *Trichoderma* species and Antagonistic activity against Soil Borne Pathogens

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Abstract: ***The study aims to evaluate the cultural characters and mycelial growth of Trichoderma species on different solid culture media. Cultural characters and linear growth rates when calculated after three days of inoculation. Which clearly indicated that T. harzianum and T. viride are fast growing bioagents and potato dextrose agar was best for growth of the Trichoderma sp. Similarly, fresh weight and dry weight were also recorded at five different media and potato dextrose broth (PDB) was found to be excellent. The antagonistic potentiality of Trichoderma species against Fusarium oxysporum f.sp. ciceri (foc), Fusarium oxysporum f.sp. udum (fou) and P. aphanidermatum were tested, maximum inhibition (65.00%) of mycelial growth was recorded against P. aphanidermatum followed by (63.66) Fusarium oxysporum f. sp. udum (fou) and (62.00%) Fusarium oxysporum f. sp. ciceri (Foc). However T. virens (T_{vi} (CSAU)) was found to be least effective against fou, foc, P. aphanidermatum.***

Keywords: *Trichoderma* sp., Culture media, Cultural Characteristic**,** Growth performance

1. Introduction

Biological control involves the use of biological organisms to control pathogens or diseases. The microbial inoculants as bio-control agents are effective and attractive alternatives to prevent the deficiencies brought about by the exclusive reliance on chemicals (Nakkeeran et al., 2002). *Trichoderma* are free-living fungi and common in soil and root ecosystems. They are opportunistic, a virulent plant symbionts, as well as being parasites of other fungi. These filamentous fungi are very wide spread in nature, with high population densities in soils and plant litters. They are environmentally safe to control plant pathogen compared to any other pesticides. Farmers can easily use antagonistic fungi commercially to increase their yield of crop and decrease the using cost of pesticides (Nusrat, et al., 2013). Members of *Trichoderma* particularly *Trichoderma harzianum* and *Trichoderma viride* are promising biological control agents against plant diseases. A wide range of media are used for growing fungi. Most mycologists develop preferences for certain types of media based on experience and peculiarities of the type of fungi that are routinely grown. Media will affect colony morphology and color, whether particular structures are formed or not, and may affect whether the fungus will even grow in culture.

2. Materials and Methods

2.1 Isolation of Trichoderma

Table 1 lists the different isolates and their details that have been used in this study. All the isolates were isolated on PDA medium according to the protocol described by Johnson and Curl (1972) and isolates were identified up to species level based on phenotypic characters like colony colour and growth; size and shape of conidiophore, phialides and conidia. The cultures were identified using the available literature (Samuels et al., 1998) and also confirmed by ITCC, Division of Plant Pathology IARI, New Delhi-12

Table 1: Identification of potential strains of *Trichoderma sp.*

Strain No.	*Name of Bioagent*	*ITCC Acc. No*	*GenBank Accession No.*	*Strain code*	*Source*	*GPS Location*
T1	*T. harzianum*	6796	KC800922	*Th* azad	CSA Kanpur Nagar	Latitude: 25° 8′ 34.821″ Longitude: 81° 59′ 2.979″
T2	*T. viride*	8315	JX119211	01PP	Hardoi	Latitude: 27° 23′ 40.729″ Longitude: 80° 7′ 47.751″
T3	*T. asperellum*	8940	KC800921	T_{asp}/ CSAU	CSA Kanpur Nagar	Latitude: 25° 8′ 34.821″ Longitude: 81° 59′ 2.979″
T4	*T. koningii*	5201	KC800923	T_K (CSAU)	CSA Kanpur Nagar	Latitude: 26° 29′ 33.384″ Longitude: 80° 18′ 6.518″
T5	*T. atroviride*	7445	KC 008065	71 L	Hardoi	Latitude: 26° 29′ 28.323″ Longitude: 80° 18′ 26.361″
T6	*T. longibrachiatum*	7437	JX978542	21 PP	Kaushambi	Latitude: 26° 34′ 27.61″ Longitude: 79° 18′ 24.623″
T7	*T. virens*	4177	KC800924	T_{vi} (CSAU)	CSA Kanpur Nagar	Latitude: 25° 21′ 39.794″ Longitude: 81° 24′ 11.414″

2.2 Preparation of different Media

Various culture media such as Potato Dextrose Agar (PDA), Trichoderma specific medium (TSM), Maltose Extract Agar (MEA), Czapek's Dox Agar and Rose Bengal Agar (RBA) were used for cultural observation. These media were prepared in the recommended protocol, pH maintained, sterilized at 121° C for 20 minutes, and then poured in sterile petridishes for further use. A 5mm block of the 3 days old pure culture of *Trichoderma* sp. were placed upside down at the center of each plate. The inoculated petridishes were kept in the growth chamber at 23±2ºC temperature. All the works were done under aseptic condition and average colony diameter in millimeters was calculated and recorded.

2.3 Preparation of Standardized Inoculums

Spore suspensions were prepared by adding 15 ml of sterile distilled water according to Singh *et al.*, (2013). For the measurement of fresh weight and dry weight of *Trichoderma* sp. were grown in conical flasks. The inoculated flasks were kept in the growth chamber at 27±2°C for 7 days. For the measurement of fresh weight and dry weight of *Trichoderma* sp., the resultant active growing cultures were aseptically washed three times with sterilized distilled water to remove remaining media. The filtrates were discarded and the fungal biomass on the filter paper was air dried at room temperature for 24 hours. Then the fresh weight (mg/100ml) was measured with electric balance. The fungal biomass of all treatments were oven dried at 30°C temperature for 72 hours. Then the oven dried fungal biomass was weighted (mg/100ml) to have the dry weight.

2.4 In vitro confrontation assays

In vitro antagonistic activity of *Trichoderma* spp. against test phytopathogen *Fusarium oxysporum* f sp. *ciceri, Fusarium oxysporum* f sp. *udum* and *pythium aphanidermatum* were determined by dual culture technique described by Singh *et. al.,* (2013). Three replications were kept for each treatment (Table 3). Observations on colony growth were recorded and percent inhibition was measured by using the following formula:- $I=\frac{(C-T)}{C}\times 100$

3. Results and Discussion

3.1 Growth Rate of *Trichoderma* sp. on Different Culture Media

Mustafa and co-workers (2009) studied the growth of *Trichoderma* spp. on five semi synthetic media including PDA and found PDA as the best medium. Das and co-workers (1997) also studied growth of *Trichoderma* spp. on PDA and four other natural media and found wheat bran as the best. Nusrat et al., (2013) reported that Potato dextrose agar was more effective for average linear growth rate of *T. harzianum* and produce maximum biomass of *T. harzianum* in potato dextrose broth. Singh et al., 2011 reported that potato dextrose agar media was excellent for growth and sporulation of *Trichoderma atroviride* and its shelf life study in different carrier based formulation. Shahid et al., 2011 also reported that PDA media was best for growth and sporulation of *Trichoderma longibrachiatum*. After 3 days of inoculation, Average Linear Growth Rate of *Trichoderma* sp. on different tested culture media varied significantly (Figure 3). The data revealed that maximum mycelial growth was obtained in PDA (4.9 mm for *T. harzianum*, 4.7 mm for *T. viride*, 4.4 mm for *T. asperellum*, 4.2 mm for *T. longibrachiatum*, 4.3 mm for *T. atroviride,* 4.0 mm for *T. koningii* and 3.7 mm for *T. virens*) followed by TSM (4.2 mm for *T. harzianum*, 3.4 mm for *T. viride*, 3.4 mm for *T. asperellum*, 3.3 mm for *T. longibrachiatum*, 3.1 mm for *T. atroviride,* 3.4 mm for *T. koningii* and 3.1 mm for *T. virens*) and lowest in rose bengal agar medium. Although CDA, MEA shows moderate growth rate during the experiments (Elad et al., 1981) and also differed significantly (Table 2).

Table 2: Growth rate of *Trichoderma* sp. on different culture media

Trichoderma sp.	*PDA*	*TSM*	*CDA*	*MEA*	*RBA*
T. harzianum (T. Azad	5.1	4.4	3.7	3.4	2.4
T. viride (01PP)	5.0	4.2	3.3	3.1	2.2
T. asperellum (T-asp)/ CSAU	4.8	4.0	3.1	2.3	1.8
T. longibrachiatum (21PP)	4.9	4.2	3.3	2.2	1.9
T. atroviride (71 L)	4.2	3.5	2.7	2.1	1.9
T. koningii (T.k/CSAU)	4.0	3.2	2.4	1.8	1.6
T. virens (T.vi/CSAU)	3.9	3.0	2.1	1.7	1.5

3.2 Measurement of fresh weight and dry weight

Nusrat et al. (2013) reported that maximum biomass of *T. harzianum* was produced in potato dextrose broth and the minimum was produced in water broth. The data revealed that maximum fresh and dry weight was obtained in PDB followed by TSM, Czapek-Dox and MEB (Harman et al., 1991) but lowest fresh and dry weight was found in RB-broth (Table 3).

Table 3: Fresh weight and dry mycelia weight of *Trichoderma* sp. on different broth media

***Trichoderma* sp.**	**PD broth**		**TS broth**		**CD broth**		**ME broth**		**RB broth**	
	F	**D**	**F**	**D**	**F**	**D**	**F**	**D**	**F**	**D**
T. harzianum (T. Azad	5.34	1.42	5.19	1.35	4.98	1.30	4.36	1.03	3.72	0.58
T. viride (01PP)	5.28	1.35	5.03	1.29	4.32	1.22	4.06	0.98	3.45	0.51
T. asperellum (T-asp)/CSAU	5.09	1.27	4.89	1.26	4.05	1.20	3.88	0.95	3.09	0.47
T. longibrachiatum (21PP)	4.90	1.24	4.13	1.21	3.89	1.18	3.26	0.84	3.00	0.42
T. atroviride (71 L)	3.88	1.23	4.02	1.20	3.62	1.17	2.07	0.79	2.87	0.38
T. koningii (T.k/CSAU)	3.67	1.21	3.34	1.17	3.14	1.13	2.00	0.72	2.98	0.40
T. virens (T.vi/CSAU)	3.07	1.18	2.87	1.10	2.88	1.08	2.02	0.68	1.79	0.35
CD (P<_ 0.05)	0.431	0.022	0.632	0.024	0.737	0.027	0.876	0.023	0.987	0.038
CV (%)	2.983	1.353	2.58	1.195	2.64	1.431	3.021	1.187	3.220	2.302

F- Fresh weight, D- Dry weight

3.3 Confrontation Assays

The isolates of *Trichoderma viz. T. harzianum, T. viride, T. asperellum, T. longibrachiatum, T. atroviride, T. koningii and T. virens* were used to evaluate the antagonistic potential, dual culture technique was carried out with three legume pathogens *viz. F. oxysporum* f. sp. *ciceri*, *F. oxysporum* f. sp. *udum* and *P. aphanidermatum*. Effect of different species of *Trichoderma* with respect to suppression of mycelial growth of the three test pathogens were recorded.

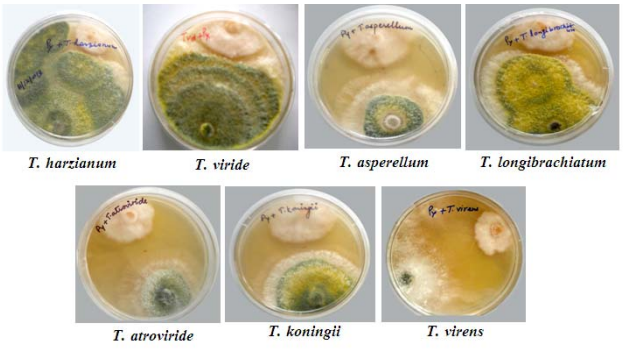

Figure 1: A- Plate confrontation test of *Trichoderma* sp. against *Pythium aphanidermatum*

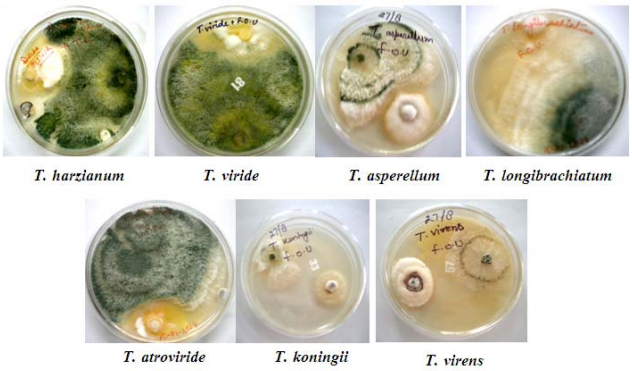

Figure 2: B-Plate confrontation test of *Trichoderma* sp. against *Fusarium oxysporum* f. sp. *udum*

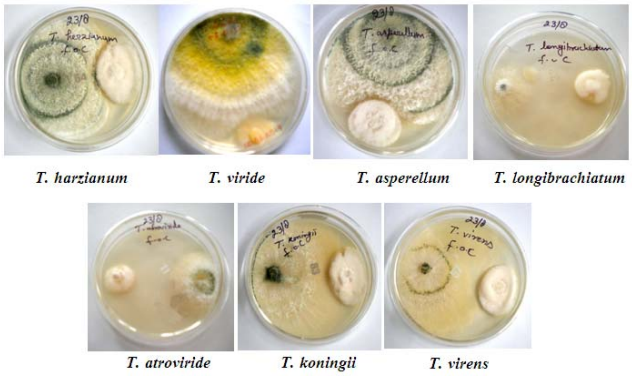

Figure 3: C-Plate confrontation test of *Trichoderma* sp. against *Fusarium oxysporum* f. sp. *ciceri*

It is evident from the data that *Trichoderma* suppressed the radial growth of *Foc, Fou and P. aphanidermatum* significantly. Highest percentage inhibition was observed in *T. harzianum* (01PP) against *P. aphanidermatum* (65.00%), followed by *Fou* (63.66%) and *Foc* (62.00%). While the least percentage inhibition was observed in *T. virens* against *Fou* (42.30) followed by Foc (43.43%) and *P. aphanidermatum* (43.75%).The results were shown in Figure.2. Joshi et al., (2010) found the antagonistic variability in different isolates of *Trichoderma* spp. collected from different places of India. The present finding was also supported by several workers (Obaiua Oti, 2007). Singh et al., (2013) also revealed that 30 isolates of *Trichoderma viride* collected from various districts of Uttar Pradesh, were found highly antagonist against three test pathogens *(F.o.u, F.o.c* and *F.o.l*).

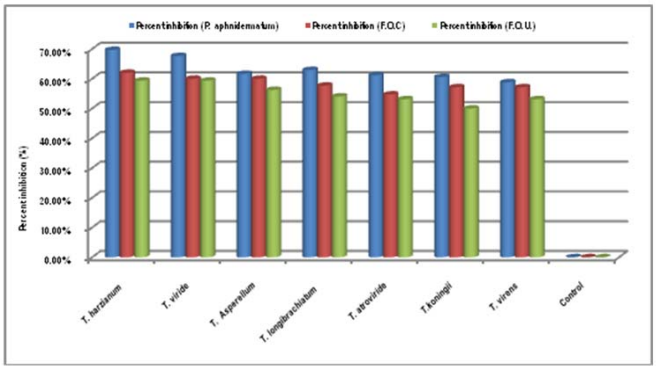

Figure 4: Efficacy of *Trichoderma* sp. against fungal pathogens

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