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.

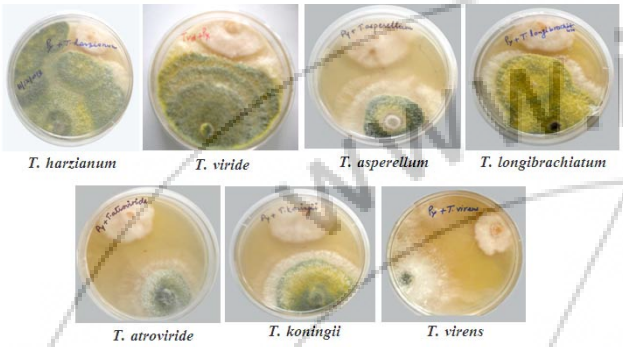

Figure 1: A- Plate confrontation test of *Trichoderma* sp. against *Pythium aphanidermatum*

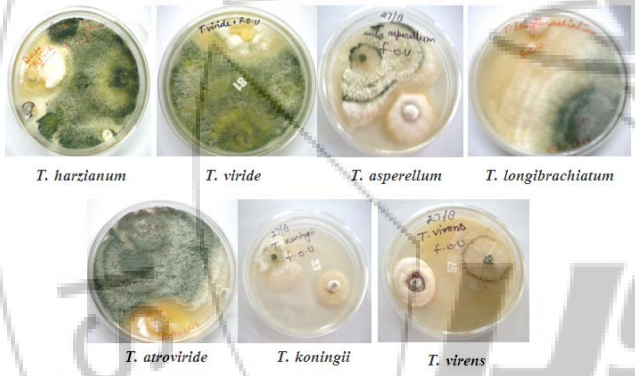

Figure 2: B-Plate confrontation test of *Trichoderma* sp. against *Fusarium oxysporum* f. sp. *udum*

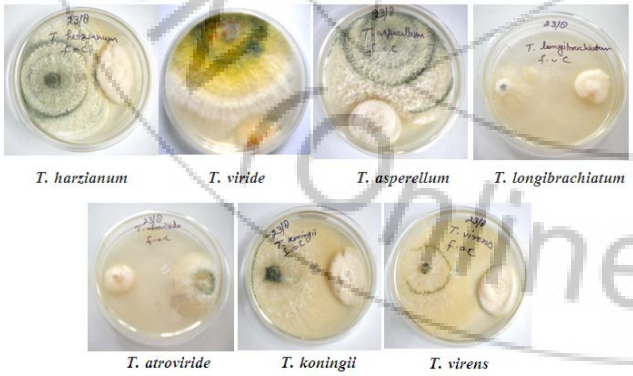

Figure 3: C-Plate confrontation test of *Trichoderma* sp. against *Fusarium oxysporum* f. sp. *ciceri*

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*).

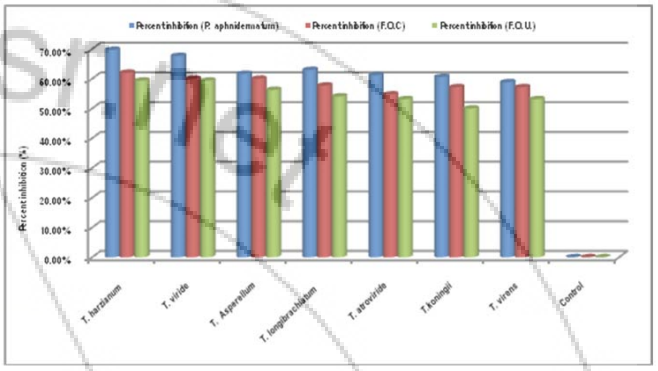

Figure 4: Efficacy of *Trichoderma* sp. against fungal pathogens

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