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Screening of *Trichoderma* species for virulence efficacy on seven most predominant phytopathogens

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***In vitro* studies on the efficacy of *Trichoderma* species against phytopathogens revealed the antagonistic potential of eight different species of *Trichoderma* isolated from the rhizosphere soils of varied locations of Uttar Pradesh and were evaluated *in vitro* against the most widely occurring soil inhabiting plant pathogens viz., *Fusarium oxysporum* f. sp. *ciceri*, *Alternaria solani*, *Phytopathora infestans, Pythium aphanidermatum, Sclerotium rolfsii, Bipolaris sorokiyana* and, *Rhizoctonia solani* and identify the most potential and effective strains *Trichoderma* with high antagonistic activity. Eight species of *Trichoderma* highly inhibited the growth of the seven test phytopathogens by producing volatile compounds showing variability in antagonistic potential of different *Trichoderma* spp. against the different pathogens tested. The test antagonists grow faster than the pathogen and produced inhibition zones. These antagonistic interactions influence the incidence and severity of the disease caused by the pathogen. The present communication describes the impact of different *Trichoderma spp*. on growth inhibition of plant pathogens under *in vitro* conditions. The data revealed that *Trichoderma reesei* (Tr(CSAU*)* showed the maximum inhibition percentage of mycelial growth was recorded as against *Scelerotium rolfsii* (69.14%) followed by *Bipolaris sorokiniana* (80.33%), *Alternaria brasiciae* (83.3%), while, in case of remaining plant pathogens such as *Pythium aphanidermatum*, *Fusarium oxysporum* f. sp. *ciceri*, *Trichoderma harzianum (Th.azad)* were reduced the highest radial mycelium growth of *Pythium aphanidermatum*, *Fusarium oxysporum* f. sp. *ciceri* (85 and 80%) respectively. Apart from *Trichoderma reesei* and *Trichoderma harzianum* (*Th.azad*), *Trichoderma viride* (01PP) also inhibited the mycelial growth of *Phytophthora infestans* (80.83%) and *Rhizoctonia solani (*70.42%*).*

Key words: Antagonism, *Trichoderma*, phytopathogens.

INTRODUCTION

Plants are a major source of food, fodder, medicines and many other useful products for humans. Diseases are the important biotic causes for low crop yield and poor quality seed. Pathogens being soil and seed borne, possess a great problem in disease management. Soil borne diseases are difficult to control and seed treatment with fungicides does not protect the crop for longer periods. Continuous use of the same fungicide against the same pathogen results in the development of fungicide resistant strains of the pathogen (Shanmugam and Varma, 1998; Kumar and Dubey, 2001; Mamgain et al., 2013). Moreover, chemical measures may establish imbalances in the

microbiological community for the activity of beneficial organisms which otherwise improve the crop health. The demand for alternative to chemical control of plant pathogens has become stronger owing to the concerns about the safety and environmental aspects of chemicals. However, biological control offers the chance to improve crop production within the existing resources, besides avoiding the problem of pesticide resistance (Dekker, 1976; Khan et al., 2014). The genus *Trichoderma* is common filamentous imperfect fungi (*Deuteromycetes*), the most common saprophyte in the rhizospehere and foun in almost all soils. Characterization for the antagonistic potential of *Trichoderma* spp. is the first step in utilizing the full potential of *Trichoderma* spp. for specific applications. *In vitro* screening of different pathogens is an effective and rapid method for identifying species with antagonistic potential. *Trichoderma*, a filamentous soil borne mycoparasitic fungus, has been shown to be effective against many soil borne plant pathogens (Papavizas, 1985; Pan et al., 2001; Jash and Pan, 2004) as they have more than one mechanism of action. Therefore, the study was conducted to evaluate the antagonistic activity of eight different *Trichoderma* species viz., *Trichoderma viride*, *T. harzianum*, *T. reesei*, *T. atroviride, T. asperellum, T. koningii, T. longibrachiatum* and *T. virens*, in inhibiting the growth of some most widely occurring soil inhabiting plant pathogens viz., *Fusarium oxysporum* f. sp. *ciceri*, *Alternaria solani*, *Pythium aphanidermatum, Phytopathora infastans*, *sclerotium rolfsii , bipolaris sorokiayana Rhizoctonia solani* and identify *Trichoderma* spp. with a high antagonistic potential. Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods. *Trichoderma* spp. is now the most common fungal biological control agents that have been comprehensively researched and deployed throughout the world. Several fungal cell wall degrading enzymes, amongst them chitinase and glucanase, which seem to play an important role in the antagonistic action of *Trichoderma* against a wide range of fungal plant pathogens. The present study aimed to find out the efficiency of *Trichoderma* spp. against some phytopathogens.

MATERIALS AND METHODS

Isolation and purification of Trichoderma species

The isolation of eight *Trichoderma* spp. from rhizospheric zone of soil through serial dilution plate techniques (Johnson and Curl, 1972) on modified *Trichoderma* Selective Medium (TSM) (Saha and Pan, 1997). The green coloured colonies were identified by slide culture technique and compared with taxonomic key of Rifai (1969) at genus and species level and deposit to ITCC division of plant pathology New Delhi for reconfirmation. After confirmation cultures of *Trichoderma* spp. were maintained on PDA slants and stored in the refrigerator at 4ºC for further studies.

Isolation of plant pathogens

The pathogens were isolated from disease plants showing symptoms of disease. These isolated pathogens were identified, purified and tested for pathogenicity (Tapwal et al., 2011). The hyperparasitic potential of eight *Trichoderma* species were screened *in vitro* against seven test plant pathogens viz., *Fusarium oxysporum* f. sp. *ciceri*, *Alternaria solani*, *Rhizoctonia solani, Pythium aphanidermatum, Phytopathora infastans*, *sclerotium rolfsii and bipolaris sorokiayana* by dual culture plate technique and production of volatile and non-volatile metabolite part of Petri plate with respective test pathogen on the upper lid of plate served as control. Three replicates were maintained for each treatment. The assembly was opened after 72 h and the observations were recorded by measuring colony diameter of the test pathogen (in mm) in each plate and that of the control plates.

Efficacy of *Trichoderma* spp. on growth of the pathogens by dual-culture plate method

For testing antagonism in dual culture method (Morton and Stroube, 1955), a mycelial disc (6 mm) was cut from the margins of actively growing region of seven day old cultures of *Trichoderma* spp. and inoculated at one end of the petriplates (1 cm away from the edge of the plate) with sterilized potato dextrose agar (PDA) medium and simultaneously at the opposite end of a mycelial disc (6 mm) of the test pathogens. The experiments were conducted with three replications/plates for each treatment, while control plates were inoculated only by tested fungus. Plates were then incubated at 27 ± 1°C. Observation were recorded after seven days of inoculation including area covered by the *Trichoderma* spp. (eight *Trichoderma* spp.) and the pathogen while percent of inhibition was calculated using the following formula (Vincent, 1947):

$$\text{Percent growth of inhibition} = \frac{\text{colony growth in control plate-colony growth in intersecting plates}}{\text{colony growth in control}} \times 100$$

RESULTS

Our results explain that significant success in biocontrol is achieved under *in vitro* conditions. It is evident from the data presented in Table 1 and showed in Figures 1, 2 and 3, that the *Trichoderma* spp. suppressed the radial growth of different phytopathogens significantly on potato dextrose agar medium in the dual culture. *Trichoderma* spp. isolated from the rhizosphere soils of different location of Uttar Pradesh identified and confirmed on the basis of morphological and physiological characterization and micrometry observations revealed that they belong to eight different species viz., *Trichoderma viride,*

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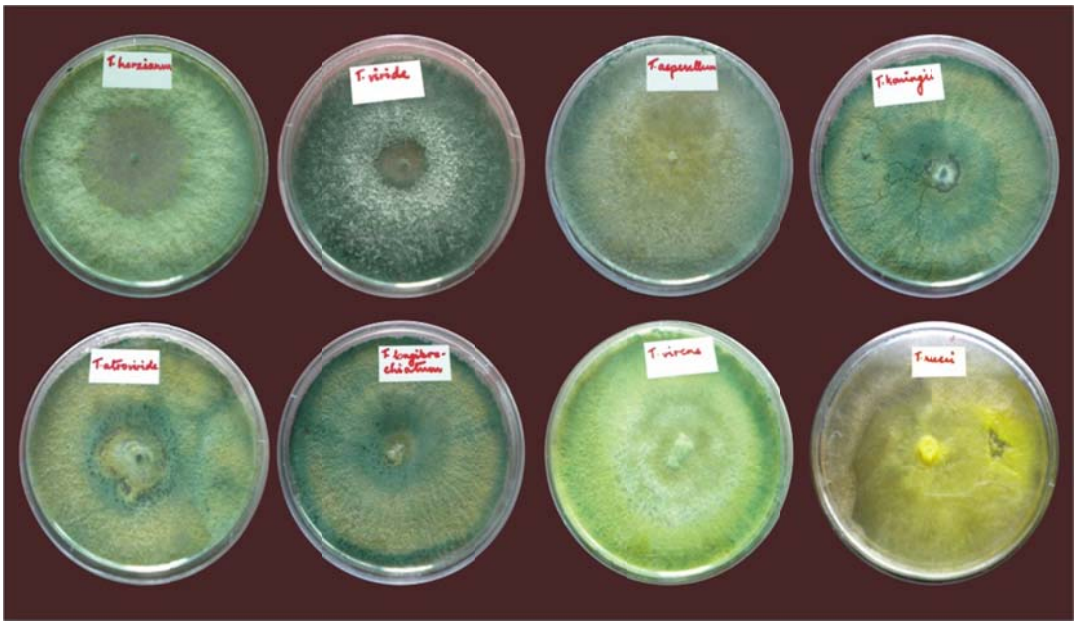

Figure 1. Eight *Trichoderma* spp. isolated from different locations of Uttar Pradesh.

Figure 2. Seven predominant plant pathogens.

T. harzianum, T. reesei, *T. atroviride, T. koningii*, *T. asperllum*, *T. virens, T. longibrachiatum* and *T. viride* was the most predominant species. The morphological and physiological characterization of these antagonistic species was accomplished on the basis of colony color, growth rate, texture, growth patterns, size of phialides and phialospores.

Eight *Trichoderma* spp. were tested against most

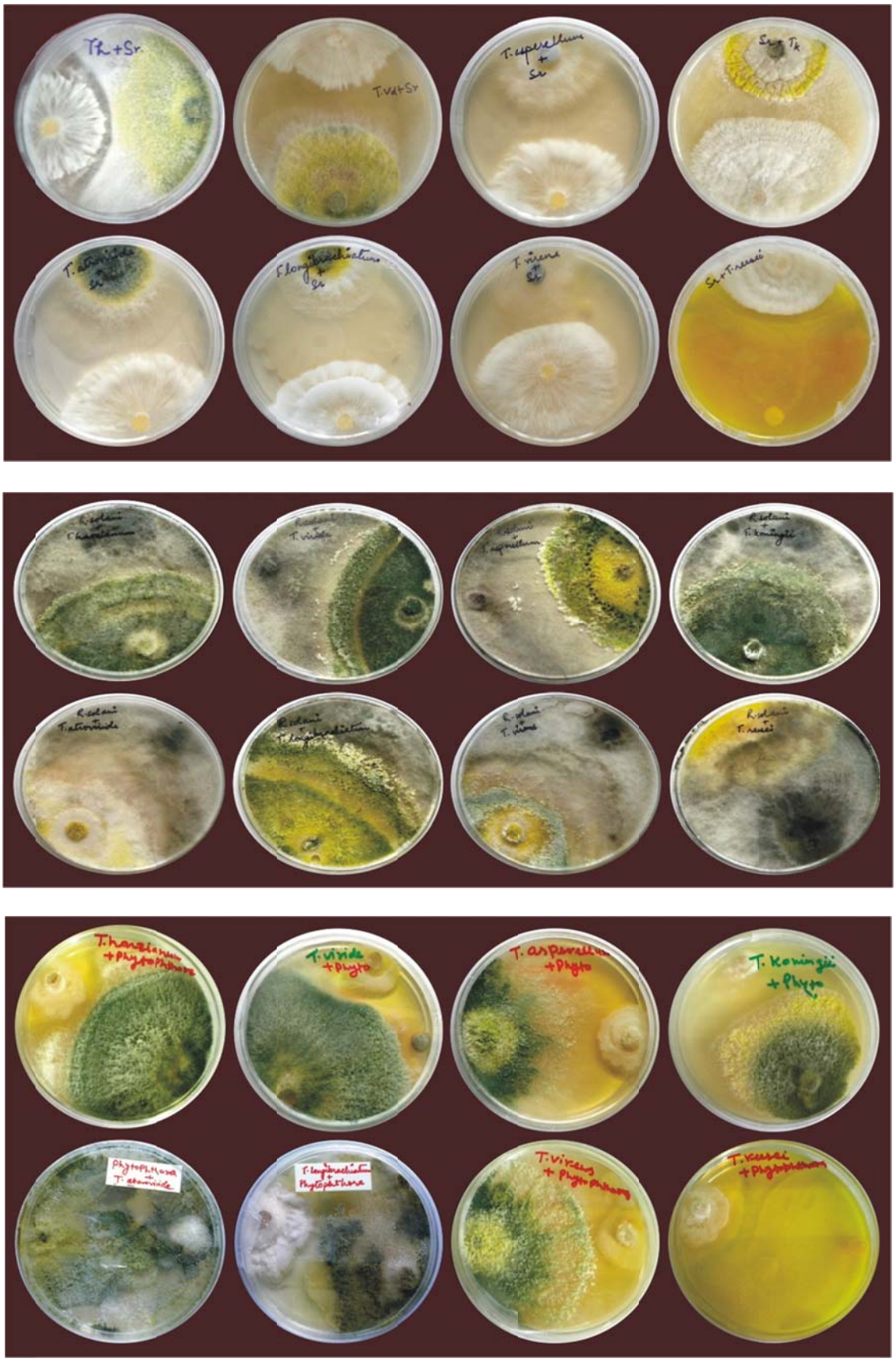

Figure 3. Confrontation test of *Trichoderma* spp. against notable plant pathogens.

Table 1. Submission of the gene sequences at NCBI database

Strain No.	Name of Bioagent	Strain code	ITCC Acc. No	NCBI GenBank Accession No.	NBAIM, Mau	Source	GPS Location
T1	*T. harzianum*	*Th* azad	6796	KC800922	TF-1271	CSA Kanpur Nagar	Latitude: 25˚ 8′ 34.821″ Longitude: 81˚ 59′ 2.979″
T2	*T. viride*	01PP	8315	JX119211	TF-1272	Hardoi	Latitude: 27˚ 23′ 40.729″ Longitude: 80˚ 7′ 47.751″
T3	*T. asperellum*	T_{asp}/CSAU	8940	KC800921	TF-1270	CSA Kanpur Nagar	Latitude: 25˚ 8′ 34.821″ Longitude: 81˚ 59′ 2.979″
T4	*T. koningii*	T_K (CSAU)	5201	KC800923	TF-1269	CSA Kanpur Nagar	Latitude: 26˚ 29′ 33.384″ Longitude: 80˚ 18′ 6.518″
T5	*T. atroviride*	71 L	7445	KC 008065	TF-1268	Hardoi	Latitude: 26˚ 29′ 28.323″ Longitude: 80˚ 18′ 26.361″
T6	*T. longibrachiatum*	21 PP	7437	JX978542	TF-1267	Kaushambi	Latitude: 26˚ 34′ 27.61″ Longitude: 79˚ 18′ 24.623″
T7	*T. virens*	T_{vi} (CSAU)	4177	KC800924	TF-1266	CSA Kanpur Nagar	Latitude: 25˚ 21′ 39.794″ Longitude: 81˚ 24′ 11.414″
T8	*T.reesei*	*T.r(CSAU)*	8372	KM999966	TF-1273	CSA farm	Latitude: 25˚ 21′ 39.794″ Longitude: 81˚ 24′ 11.414″

predominant seven phytopathogens in dual culture plates such as *Scelrotium rolfsii, Alterneria brasicae, Pythium aphanidermatum, Phytophthora infestans, Alterneria brasiciae, Bipolaris sorokiniana* and *Fusarium* oxysporum f. sp. cicero (Table 2). Among the eight *Trichoderma* spp. *Trichoderma reesei (Tr(CSAU)* shows that maximum inhibition percentage of mycelial growth of pathogen was recorded (69.14%) against *Scelerotium rolfsii* and minimum inhibition percentage was recorded (42.85%) by *Trichoderma atroviride* (71L), Similarly *Trichoderma viride* (01PP) found that maximum inhibition percentage of mycelial growth of pathogen was recorded (70.42%) against *Rhizoctonia solani* and minimum inhibition percentage was recorded (56.25%) by *Trichoderma atroviride* (71L), *Trichoderma harzianum (*Th. azad*)* revealed that maximum inhibition percentage of mycelial growth of pathogen was recorded (85.00%) against *Pythium aphanidermatum* and minimum inhibition percentage was observed (55.55%) by *Trichoderma reesei (Tr(CSAU), Trichoderma viride* (01PP) revealed that maximum inhibition percentage of mycelial growth of pathogen was recorded (80.83%) against *Phytophthora infestans* and minimum inhibition percentage was observed (66.25%) by *Trichoderma koningii (T_k(CSAU), Trichoderma reesei (*Tr(CSAU) revealed that maximum inhibition percentage of mycelial growth of pathogen was recorded (83.33%) against *Alterneria brasiciae* and minimum inhibition percentage was observed (70.37%) by *Trichoderma atroviride* (71L), *Trichoderma asperellum (*T_{asp}/CSAU) revealed that maximum inhibition percentage of mycelial growth of pathogen was recorded (73.00%) against *Bipolaris sorokiniana* and minimum inhibition percentage was observed (65.00%) by *Trichoderma atroviride* (71L), *Trichoderma harzianum (*Th. azad) revealed that maximum inhibition percentage of mycelial growth of pathogen was recorded (80.00%) against *Fusarium oxysporum* f.sp. *ciceri* and minimum inhibition percentage was observed (60.00%) by *Trichoderma reesei (Tr(CSAU).* From the above facts it was concluded that *Trichoderma harzianum* (Th. azad) shows the maximum inhibition against pytopathogens; *Pythium aphanidermatum* and *Fusarium oxysporum* f.sp. *ciceri,* bioagents *Trichoderma viride* (01PP) shows the maximum inhibition against *Rhizoctonia solani* and *Phytophthora infestans, Trichoderma atroviride* (71 L) shows the minimum inihibition against four pytopathogens namely; *Sclerotium rolfsii, Rhizoctonia solani, Alterneria brasiciae* and

Table 2. Antagonistic activity of *Trichoderma* spp. against seven *phytopathogens* by dual culture method.

Name of Bioagent	Culture No.	Source/ District	Id. No.	Average growth (mm)	% inhibition growth (mm)	Average growth (mm)	% inhibition growth (mm)	Average growth (mm)	% inhibition growth (mm)	Average growth (mm)	% inhibition growth (mm)	Average growth (mm)	% inhibition growth (mm)	Average growth (mm)	% inhibition growth (mm)	Average growth (mm)	% inhibition growth (mm)
				Sclerotium rolfsii		*Rhizoctonia solani* (Soyabean)		*Pythium aphanidermatum*		*Phytophthora infestans*		*Alterneria brasiciae*		*Bipolaris sorokiniana*		*Fusarium oxysporum f.sp. ciceri* (CSAU)	
T. harzianum	*Th* azad	CSA Kanpur Nagar	6796	34.68	50.48	25.00	68.75	13.33	85.00	21.60	72.92	16.66	81.48	26.00	65.50	45.00	80.00
T. viride	01PP	Hardoi	8315	35.00	50.00	23.66	70.42	19.00	78.88	15.33	80.83	20.00	77.70	23.00	71.25	54.00	76.00
T. asperellum	T_{asp}/CSAU	CSA Kanpur Nagar	8940	33.30	52.38	28.33	64.58	25.66	71.48	25.00	68.75	25.00	72.22	21.00	73.00	78.00	65.30
T. koningii	T_K (CSAU)	CSA Kanpur Nagar	5201	36.66	47.62	27.66	65.42	21.00	76.66	27.00	66.25	21.66	75.93	24.60	69.25	54.00	76.00
T. atroviride	71 L	Hardoi	7445	40.00	42.85	35.00	56.25	25.00	72.22	16.60	79.17	26.66	70.37	28.00	65.00	66.00	70.60
T. longibrachiatum	21 PP	Kaushambi	7437	33.33	52.38	28.00	65.00	15.66	82.60	20.0	75.00	20.50	77.22	27.60	65.50	75.00	66.60
T. virens	T_{vi} (CSAU)	CSA Kanpur Nagar	4177	33.33	52.38	31.00	61.25	17.33	80.74	23.00	71.25	18.33	70.63	23.30	70.80	60.00	73.30
T. reesei	Tr(CSAU)	CSA Kanpur Nagar	7284	21.66	69.14	26.66	66.66	40.00	55.55	15.00	80.33	15.00	83.33	22.00	72.50	90.00	60.00

Bipolaris sorokiniana and finally, *Trichoderma reesei (Tr(CSAU)* shows the maximum inhibition against *Sclerotium rolfsii, Alterneria brasiciae* and minimum inhibition against *Pythium aphanidermatum, Fusarium oxysporum f.sp. cicero,* respectively.

Experimental design and statistical analysis

Statistical analysis was performed following completely randomized block design (CRBD) with three replicates in each treatment.

DISCUSSION

In the study it may be concluded that among the eight different species of *Trichoderma* exhibited different growth inhibition percentage against the tested most predominant phytopathogens with variability in the antagonistic potential. *Trichoderma reesei, Trichoderma harzianum* (*T.azad*), and also *Trichoderma viride* (01PP) showed high antagonistic potential against tested phytopathogens. Plant pathogenic fungi are a widespread problem and the use of chemicals is hardly successful (Anand and Jayarama, 2009). However, the high cost associated with the use of chemical (fungicides) to control disease caused by soil borne fungi is a limiting factor in the profitability of crop production, in this case biological control could be the best alternative. *Trichoderma* is the most commonly used fungal biological control agent and have long been known as effective antagonists against plant pathogenic fungi (Chet et al., 1981; Papavizas, 1985). Some of the species of *Trichoderma* included in the present study significantly inhibited several pathogens (Dubey, 2003).

All the *Trichoderma* spp. restricted the growth of

all the seven test phytopathogens in their own way. Thus, it is well known that all species isolated from different samples of soil are not equally antagonistic to phyto-pathogen and searching of effective and potential species to locally suit the purpose is important.

The results reported, suggests that the species of *T. harzianum, T. viride, T. reesei* and *T. atroviride* were more capable of influencing the growth of tested seven pathogens in dual culture. Similarly, isolates of different *Trichoderma* spp. to control soil borne phytopathogens have been reported to differ in their effectiveness (Rama and Krishna, 2000; Anand and Jayarama, 2009; Singh et al., 2013; Kumar et al., 2014). This result is a pioneer information that particular isolate from a particular location can be employed in bulk for treatment of disease incidence.

Conclusion

In our study it was concluded that *Trichoderma harzianum* (Th. azad) show the maximum inhibition against pytopathogens; *Pythium aphanidermatum* and *Fusarium oxysporum* f.sp. *ciceri,* bioagents *Trichoderma viride* (01PP) shows the maximum inhibition against *Rhizoctonia solani* and *Phytophthora infestans* while *Trichoderma atroviride* (71 L) shows the minimum inhibition against four pytopathogens namely; *Sclerotium rolfsii, Rhizoctonia solani, Alterneria brasiciae* and *Bipolaris sorokiniana.* Finally, *Trichoderma reesei (Tr(CSAU)* shows the maximum inihibiton against *Sclerotium rolfsii, Alterneria brasiciae* and minimum inhibition against *Pythium aphanidermatum, Fusarium oxysporum f.sp. cicero,* respectively.

Conflict of interests

The authors did not declare any conflict of interest.

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