Ann. Agric. Res. New Series Vol. 38 (3) : 262-269 (2017)

Mycoparasitic potential of growth promoting *Trichoderma* strains against phytopathogens

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Received : December 2016 ; Revised Accepted: May 2017

ABSTRACT

Six isolates of *Trichoderma asperellum viz.* TRS W4, TRP W8, TRS R4, TRS R8, TRP C3 and TRP C4 obtained from rhizosphere and rhizoplane of wheat, rice and chickpea were selected for their growth promoting efficacy in their native crops. These 06 isolates were tested for their biological control potential against plant pathogens *viz., Rhizoctonia solani* (isolates from rice and chickpea), *Sclerotinia sclerotiorum* (isolate from chickpea) and *Bipolaris sorokiniana* (isolate from wheat) under *in vitro* conditions. Dual culture studyrevealed that all the *Trichoderma* isolates along with Th-14, commercial *Trichoderma harzianum* strain used as positive control, completely parasitized, *i.e.* 100% mycelial parasitiation, the growth of all test plant pathogens.

Key words: *Trichoderma*, mycoparasitic potential, soil borne plant pathogens, dual culture.

Biological control of phytopathogens has been a subject of extensive research in the last few decades. However, with the increasing interest in biological control, owing to environmental and economic concerns, thousands of research experiments are going on for searching novel ways, potential enough to inhibit wide range of plant pathogens. In addition to the growth promoting potential, *Trichoderma* spp. is very well documented for its antagonistic potential of soil borne diseases, as well (Lunge *et al.*, 2012 and Junaid *et al.*, 2013) and hence been established as the most successful fungal biocontrol agent.

Faster metabolic rates, anti-microbial metabolites, and physiological conformations are key factors which chiefly contribute to the antagonism of *Trichoderma*. Also, mycoparasitism, spatial and nutrient competition, antibiosis by enzymes and secondary metabolites, and induction of plant defence system are typical biocontrol actions of this fungus (Verma *et al.*, 2007).

The present investigation is focussed towards studying the mycoparasitic potential of *Trichoderma* isolates already casting growth promoting effect in their native crops in crop specific manner.

MATERIALS AND METHODS

Isolation, purification and maintenance of *Trichoderma* isolates

Trichoderma spp. were isolated from the soil samples of rhizosphere and rhizoplane of wheat, rice and chickpea on *Trichoderma* Selective Medium (TSM), comprising K_2HPO_4 (0.9g), NH_4NO_3 (3g), $MgSO_4.7H_2O$ (0.2g), KCl (0.15g), glucose (3g), metalaxyl (Apron 35SD, 0.5g, after autoclaving), agar-agar (20g), Rose Bengal (0.08g) and sterilised distilled water (1000 ml) (Elad and Chet, 1983) through serial dilution technique (Krassilnikov, 1950). Subsequently, isolates were maintained on Potato Dextrose Agar (PDA) medium at 4°C.

Evaluation of *Trichoderma* isolates for growth promoting potential

Seeds of the wheat, rice and chickpea were bioprimed with the talc based formulations of purified *Trichoderma* isolates. Thereafter, growth promoting potential of the isolates was tested in their respective crops *in vitro* by paper towel method (ISTA, 2003), for length and fresh weight of plumule and radical. Also, the isolates were evaluated for growth enhancing efficacy under glass house conditions, for length and fresh weight of seedling shoot and root. Through a series of three rigorous screening regimes, six superior native isolates were selected from rhizosphere (TRS) and rhizoplane (TRP) of wheat (W), rice (R) and chickpea (C) *viz.* TRS W4, TRP W8, TRS R4, TRS R8, TRP C3 and TRP C4.

Finally, the selected isolates native to one crop, were cross-inoculated in other two crops and tested for their effect on above mentioned parameters *in vitro* and under glasshouse conditions. The growth promoting potential of the selected native isolates came to be either at par or significantly superior to the commercial *Trichoderma* strain Th-14, taken as positive control. But their effect of very pronounced on their native crops as compared to other crops, hence, indicating the crop specific growth promoting potential of native *Trichoderma* spp.

The most promising isolates were subjected to identification by BiOLOG micro station system (Version 4.2, GEN II).

Isolation of fungal plant pathogens from rice, wheat and chickpea

The isolation of few fungal pathogens *viz. Rhizoctonia solani* (chickpea and rice strains), *Sclerotinia sclerotiorum* (chickpea) and *Bipolaris sorokiniana* (wheat) was done from the infected parts of the plants showing characteristic symptoms in their native crops.

Infected plant parts of the desired crops were collected from field and cut into small pieces of 2-3 mm in size having half healthy and half diseased portion. The small pieces were first surface sterilized with 2 per cent sodium hypochlorite (NaOCl) solution for 60-90 seconds, thereafter thoroughly washed with sterilized distilled water thrice. The pieces were placed between two layers of sterilized blotter paper to remove excess moisture. These pieces were then transferred to Petri-plates seeded with PDA medium under aseptic conditions followed by incubation at 24 ±1°C. After 48 hrs, superficial growth obtained was sub-cultured on fresh PDA-seeded Petri plates for the purification. The desired fungal pathogens were identified based on their cultural and morphological characters. The purified cultures were maintained on PDA slants and stored at 4°C in refrigerator for further studies.

***In vitro* evaluation of growth promoting *Trichoderma* isolates for their mycoparasitism**

In vitro evaluation of selected and identified growth promoting *Trichoderma* isolates (02 no. from each crop) for their mycoparasitic potential against the plant pathogens were done by dual culture technique (Morton and Stroube, 1955). Twenty ml sterilized PDA amended with chloramphenicol @ 100 mg/l. was aseptically poured in sterilized Petri-plates and allowed to solidify. Mycelial discs (5 mm) taken from the actively growing colonies of the test pathogens (7 days old culture) and *Trichoderma* isolates (3 days old culture) were placed simultaneously on the PDA-seeded Petri plates opposite to each other, 1 cm apart from the periphery. Each treatment was replicated thrice. The inoculated Petri-plates were incubated at 24±1°C. First observation was taken just after contact between the antagonist and the pathogen. Radial growth of the test pathogen was measured. This was considered as check. After contact, observations were recorded at 3, 5 and 7 days, until either the antagonist completely parasitized/overgrew on the test pathogen or the antagonist stopped growing over the pathogen. Per cent inhibition of the test pathogen was calculated by comparing the growth of the test pathogen (after parasitization) with its growth just after contact (check).

$$\text{Per cent mycelial inhibition} = C - T/C*100$$

Where,

C = radial growth (cm) of the pathogen just after contact

T = radial growth (cm) of the pathogen after parasitization by Trichoderma

Results

Identification of *Trichoderma* isolates using BiOLOG microstation system

All the crop native *Trichoderma* isolates (06) with superior growth promoting potential *viz.* TRSW4, TRPW8, TRSR4, TRSR8, TRPC3 and TRPC4 as seen in plate 1, selected after a series of rigorous screenings were identified upto species level as *Trichoderma asperellum (Trichoderma viride)* using BiOLOG microstation system (Version 4.2, GEN II).

In vitro evaluation of mycoparasitic potential of selected *Trichoderma* isolates against fungal plant pathogens

Six selected crop native *Trichoderma* isolates with superior growth promoting efficacy along

Table 1. ***In vitro*** **evaluation of mycoparasitic potential of selected *Trichoderma* isolates against *Sclerotinia sclerotiorum* causing stem rot in chickpea**

Trichoderma isolates	Radial growth of pathogen just after contact		Radial growth of pathogen after contact with the antagonist					
			Days after contact					
			3		5		7	
	Radial growth (cm)	Mycelial parasitization (%)	Radial growth (cm)	Mycelial parasitization (%)	Radial growth (cm)	Mycelial parasitization (%)	Radial growth (cm)	Mycelial parasitization (%)
TRP W 8	3.53	0	2.60	26.34	1.53	56.65	0	100
TRS W 4	3.50	0	2.70	22.85	1.50	57.14	0	100
TRS R 4	3.60	0	2.80	22.22	1.80	50.00	0	100
TRS R 8	3.80	0	2.70	28.95	1.67	56.05	0	100
TRP C 4	3.60	0	2.60	27.78	1.03	71.39	0	100
TRP C 3	3.50	0	2.60	25.71	1.83	47.1	0	100
Th-14	3.80	0	3.20	15.79	1.90	50.00	0	100
CD (0.05)	0.16	-	0.19	-	0.13	-	-	-
CV (%)	2.62	-	3.96	-	4.88	-	-	-

Table 2. ***In vitro*** **evaluation of mycoparasitic potential of selected *Trichoderma* isolates against *Rhizoctonia solani* causing wet root rot in chickpea**

Trichoderma isolates	Radial growth of pathogen just after contact		Radial growth of pathogen after contact with the antagonist					
			Days after contact					
			3		5		7	
	Radial growth (cm)	Mycelial parasitization (%)	Radial growth (cm)	Mycelial parasitization (%)	Radial growth (cm)	Mycelial parasitization (%)	Radial growth (cm)	Mycelial parasitization (%)
TRP W 8	3.80	0	1.70	55.26	0.60	84.21	0	100
TRS W 4	5.40	0	1.83	65.55	0.53	90.18	0	100
TRS R 4	4.20	0	1.73	58.80	0.63	85.00	0	100
TRS R 8	3.40	0	1.86	45.29	0.73	78.53	0	100
TRP C 4	3.70	0	1.80	51.35	0.50	51.35	0	100
TRP C 3	3.97	0	1.70	57.18	0.50	87.40	0	100
Th-14	3.70	0	1.80	51.35	0.57	84.59	0	100
CD (0.05)	0.16		0.15	-	0.12	-	-	-
CV (%)	2.36		4.15	-	4.01	-	-	-

with commercial strain Th-14, were tested *in vitro* for their mycoparasitic potential against *Sclerotinia sclerotiorum* (chickpea), *Rhizoctonia solani* (chickpea and rice strains),and *Bipolaris sorokiniana* (wheat). Antagonistic potential of *Trichoderma* species against different fungal phytopathogens has been reported by several researchers (Papavizas, 1985; Marco *et al.*, 2003; Sharfuddin and Mohanka, 2012; Ng *et al.*, 2015).

The results of the study showed that all the six *Trichoderma* isolates alongwith Th-14 completely parasitized, *i.e.* 100 per cent mycelial parasitization, the growth of the all fungal plant pathogens on 7 days after contact (DAC) when tested *in vitro* using dual culture plating technique. The mechanism employed by *Trichoderma* against phytopathogenic fungi in dual cultures is mycoparasitism which relieson

Table 3. *In vitro* evaluation of mycoparasitic potential of selected *Trichoderma* isolates against *Rhizoctonia solani* causing sheath blight in rice

Trichoderma isolates	Radial growth of pathogen just after contact		Radial growth of pathogen after contact with the antagonist					
			Days after contact					
			3		5		7	
	Radial growth (cm)	Mycelial parasitization (%)	Radial growth (cm)	Mycelial parasitization (%)	Radial growth (cm)	Mycelial parasitization (%)	Radial growth (cm)	Mycelial parasitization (%)
TRP W 8	4.33	0	2.37	45.26	0.60	86.14	0	100
TRS W 4	4.50	0	2.13	52.67	0.53	88.22	0	100
TRS R 4	4.67	0	2.50	46.47	0.63	86.50	0	100
TRS R 8	4.30	0	2.53	41.16	0.73	83.02	0	100
TRP C 4	4.50	0	2.20	51.11	0.50	88.89	0	100
TRP C 3	4.57	0	1.80	60.61	0.50	89.05	0	100
Th-14	4.40	0	2.63	40.23	0.47	89.31	0	100
CD (0.05)	0.18		0.18	-	0.12	-	-	-
CV (%)	2.40		4.43	-	4.21	-	-	-

Table 4. *In vitro* evaluation of mycoparasitic potential of selected *Trichoderma* isolates against *Bipolaris sorokiniana* causing spot blotch in wheat

Trichoderma isolates	Radial growth of pathogen just after contact		Radial growth of pathogen after contact with the antagonist					
			Days after contact					
			3		5		7	
	Radial growth (cm)	Mycelial parasitization (%)	Radial growth (cm)	Mycelial parasitization (%)	Radial growth (cm)	Mycelial parasitization (%)	Radial growth (cm)	Mycelial parasitization (%)
TRP W 8	2.60	0	1.50	42.30	0.43	83.46	0	100
TRS W 4	2.50	0	1.60	36.00	0.77	69.20	0	100
TRS R 4	2.83	0	2.27	19.79	0.60	78.79	0	100
TRS R 8	2.90	0	2.00	31.03	0.47	83.79	0	100
TRP C 4	2.70	0	1.60	40.74	0.47	82.59	0	100
TRP C 3	3.00	0	2.33	22.33	0.73	75.67	0	100
Th-14	2.77	0	1.47	46.93	0.73	73.64	0	100
CD (0.05)	0.17	-	0.14	-	0.11	-	-	-
CV (%)	3.63	-	4.63	-	4.21	-	-	-

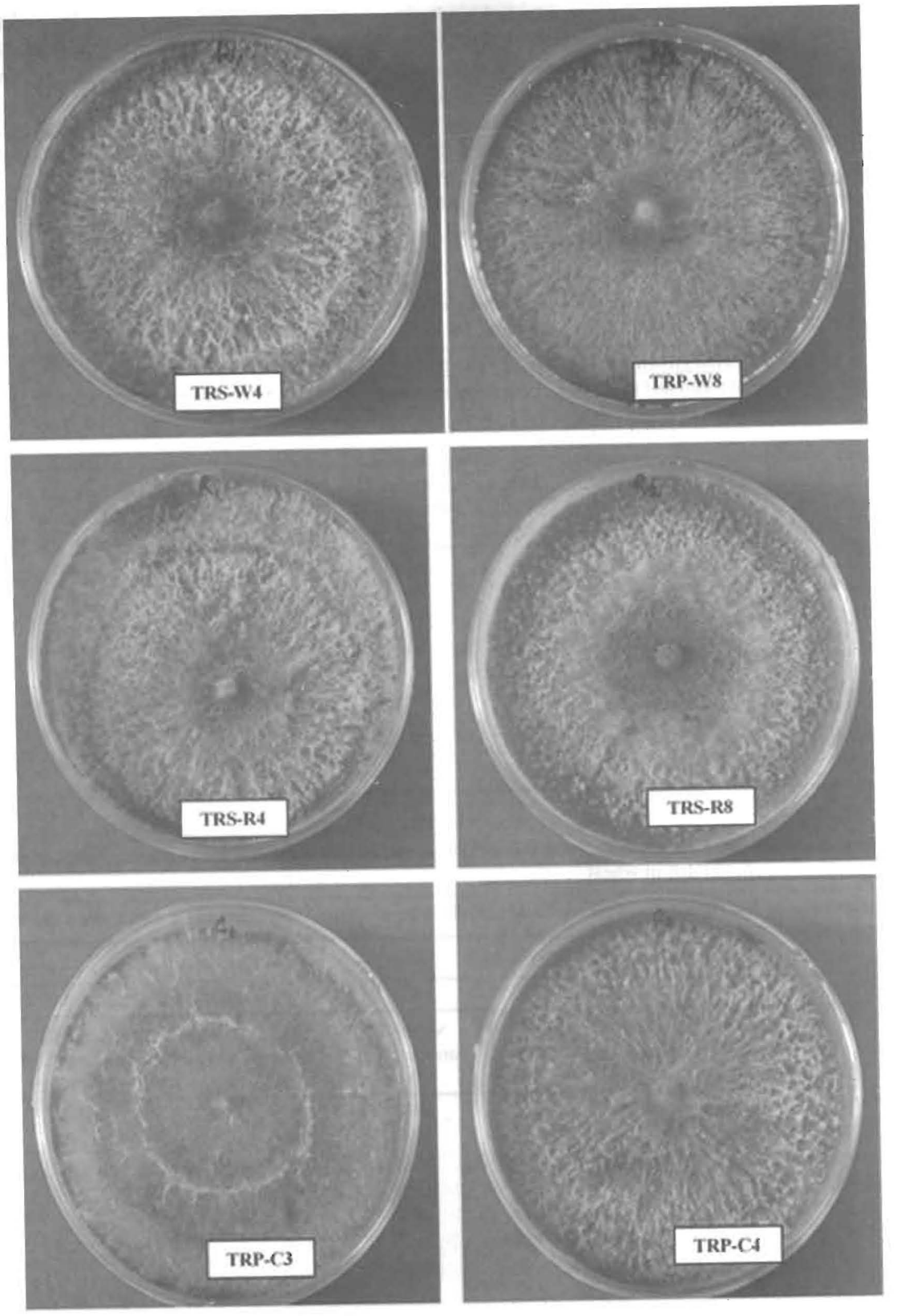

Plate 1. Selected *Trichoderma* isolates

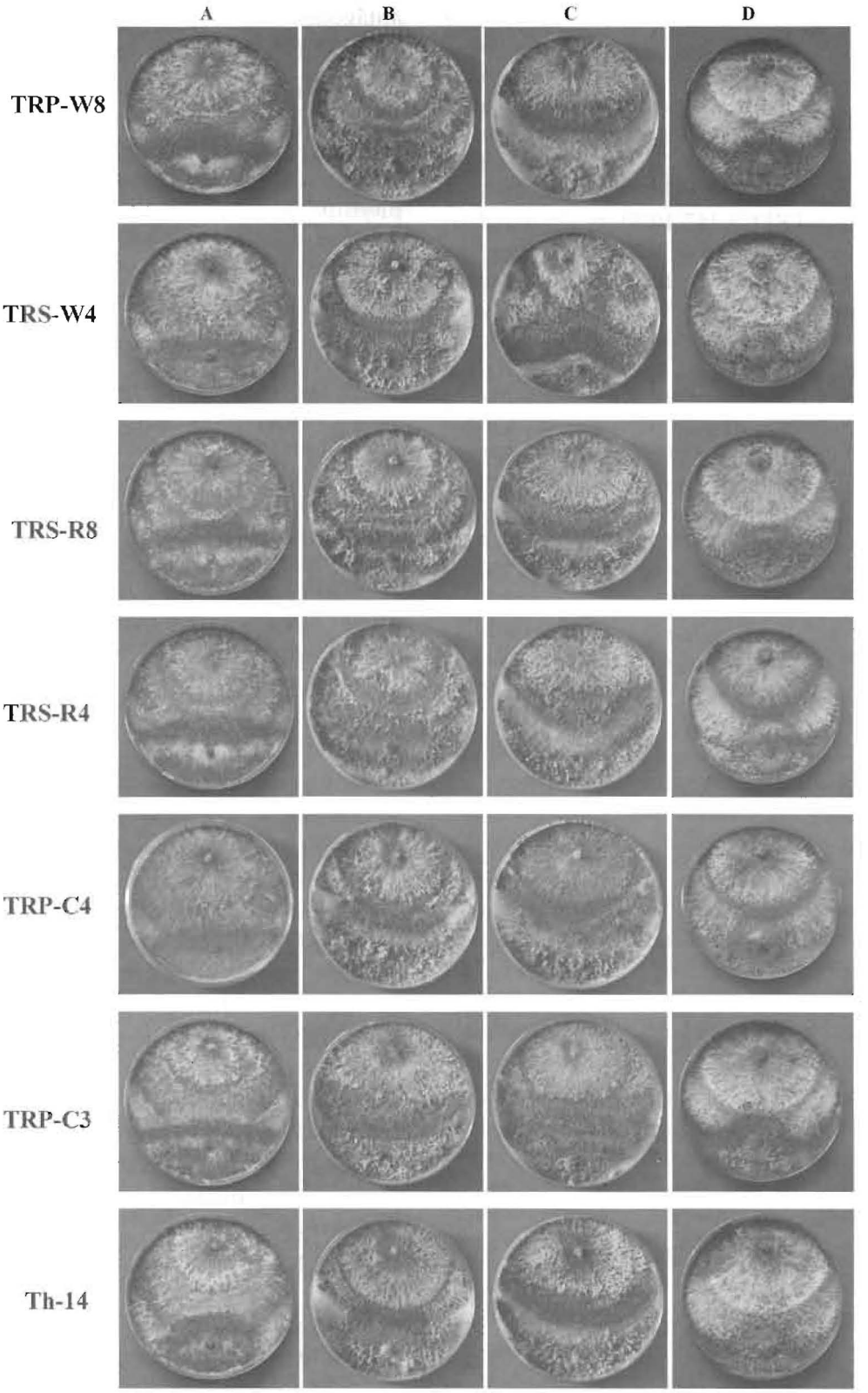

Plate 2. Mycoparasitic potential of selected *Trichoderma* isolates against A: *S. sclerotiorum* (chickpea), B: *R. solani* (chickpea), C: *R. solani* (rice) and D: *B. sorokiniana* (wheat) at 7 DAC

the abilities of the former to bind, recognize and produce diffusible and volatile metabolites as well as certain extracellular hydrolytic enzymes (El-katany *et al.*, 2001; Monte, 2001). However,on 5 DAC,TRPC4 was found most effective in parasitizing *S. sclerotiorum* (71.39%), followed by TRSR8 (56.65%), TRPW8 (57.14%) and TRSW4 (56.05%) and TRPC3 (47.10%) as depicted in table1, plate 2. In case of *R. solani* (chickpea isolate), TRSW4 was most effective in parasitizing the pathogen growth (90.18%) at 5 DAC, followed by TRPC3 (87.40%) by TRSR4 (85%) as depicted in table 2, plate 3. While, in case of *R. solani* (rice isolate), Th-14 was most effective in parasitizing the pathogen growth (89.31%), followed by TRPC3 (89.05%),TRPC4 (88.89%) and TRSR4 (86.54%) as depicted in table 3, plate 4. Wheat pathogen *B. sorokiniana* was most effectively parasitized by TRSR8 (83.79%), followed by TRPW8 (83.46%) and TRPC4 (82.59%) as indicated in table 4, plate 5. This is in accordance with the fact that *Trichoderma* spp. especially *T. viride* and *T. harzianum* exhibit substantial variability among strains with respect to their antagonistic activity and host range (Sivan and Chet, 1989).

The study also highlights another important point that the native *Trichoderma* isolates may exhibit growth promoting effect in crop-specific manner. On the other hand, antagonism against phytopathogens is manifested in generalized manner only. Thus, the crop-specific growth promoting *Trichoderma* isolates completely inhibited growth of all the test pathogens.

Conclusions

In the present study, all the *Trichoderma* isolates (TRP-W8, TRS-W4, TRS-R8, TRS-R4, TRP-C4 and TRP-C3) were found very effective in parasitizing (100%) the test pathogens *viz. Sclerotinia sclerotiorum* (chickpea), *Rhizoctonia solani* (chickpea and rice) and *Bipolaris sorokiniana* (wheat) within 7 days after contact. Thus, it can be concluded that *Trichoderma* isolates proves to be effective biocontrol agent and can be efficiently included in integrated disease management.

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