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Bioefficacy of *Trichoderma* isolates against soil-borne pathogens

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The study of morphology and bioefficacy of *Trichoderma* was undertaken to select the effective isolates against soil-borne pathogens. Fifty one (51) isolates (23 isolates of *Trichoderma virens* and 28 isolates of *Trichoderma harzianum*) were morphologically characterised based on the growth characteristics on PDA medium, the size and shape of phialides and conidia.These isolates were screened for bioefficacy against soil borne plant pathogens (*Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsii*) based on their percent inhibition observed during dual culture, volatile and non-volatile methods. Eight *T. virens* isolates and 12 *T. harzianum* isolates were proven to be potential isolates against the soil-borne pathogens tested. No correlation was found between bioefficacy and morphology in both species isolates.

Key words: *Trichoderma virens*, *Trichoderma harzianum*, morphology, dual culture, biocontrol and bioefficacy.

INTRODUCTION

Trichoderma spp. is cosmopolitan and abundant fungi in soil in a wide range of ecosystems and climatic zones. They are characterized by rapid growth, capability of utilizing diverse substrates and resistance to noxious chemicals (Klein and Eveleigh, 1998). Their economic importance includes their role as primary decomposers, producers of antibiotics and enzymes as well as biocontrol agents against a wide range of plant pathogens (Hjeljord and Tronsmo, 1998; Kubicek and Penttila, 1998; Rossman, 1996; Sivasithamparam and Gisalberti, 1998). *Trichoderma* spp. may inhibit the phytopathogenic fungi either by inducing resistance and plant defence reactions or by direct confrontation through mycoparasitism and antibiosis as well as competition (Howell, 1998, 2003; Papavizas, 1985; Verma et al., 2007).

In the direct interaction between *Trichoderma* spp. and the phytopathogenic fungi, mycoparasitism is one of the mechanisms observed in which the antagonist coils around the hyphae of the pathogen, develops hook-like structures known as appressoria coupled with production of lytic enzymes and then penetrates the pathogen hyphae (Chet, 1987; Kubicek et al., 2001). Coiling of the phytopathogenic fungal hyphae by *Trichoderma* spp. is one of the parameters used to characterize the myco-parasitism (Howell, 2003; Rocha-Ramirez et al., 2002). *Trichoderma* spp. produces a plethora of secondary metabolites showing anti microbial activity (Vinale et al., 2008). The chemical composition of secondary metabo-lites depends on the strains and classified as volatile (water-soluble) or non-volatile (water-insoluble) com-pounds (Ghisalberti and Sivasithamparam, 1991).

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The knowledge of mechanisms of interaction of *Trichoderma* spp. with phytopathogenic fungi and plant host is of utmost importance to enhance the practical application of these beneficial microorganisms. *Trichoderma* spp. is among the microorganisms most frequently used as antagonists against soil-borne pathogens (Hjeljord and Tronsmo, 1998 and Hyakumachi et al., 1996). Soil-borne phytopathogens are known worldwide for causing root diseases in diverse cultures (Ogoshi, 1996).

Taxonomy of *Trichoderma* is currently based largely on morphological characters such as mycelia growth, phialides shape and size and conidial shape and size. However, most species descriptions are based on examination of a limited number of strains where the morphological differences are clear but these differences become less clear as more strains are studied. This result suggests that there are no enough morphological and cultural characters to reliably define species level (Samuels et al., 2013).

The isolates were identified using morphological characters. *In vitro* bioefficacy tests (dual culture, volatile and non-volatile methods) were performed, against soil-borne pathogens to understand the ability of these isolates to produce water-soluble metabolites or volatile inhibitors. This approach is useful in selecting some potential isolates of *Trichoderma* spp. against soil-borne pathogens.

MATERIALS AND METHODS

Morphological characterisation of *Trichoderma* isolates

The cultural characteristics of 51 isolates of *Trichoderma* spp. were studied in potato dextrose agar (PDA). The identification was performed using an interactive key for strain identification (Rifai, 1969; Domsch et al., 1980; Bissett, 1991 a, b; Samuels et al., 2013) based on the growth characters on PDA along with microscopic observations of the isolates. Conidiophores branching and apex of the conidiophore disposition, shape and size of the phialides and conidia size and shape were recorded. The photographs were taken under 100x magnification (phialides size and shape and conidial size and shape) and under 10x (conidiophore branching) magnification were measured in micrometer by using ImageJ software.

Bioefficacy of *Trichoderma* isolates against soil borne pathogens

Soil-borne pathogens

Soil-borne plant pathogens (*Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsii*) were obtained from Indian Type Culture Collection, Division of Plant Pathology, Indian Agricultural Research Institute (IARI) and identified based on the morphological characters (Rangaswami, 1958) and maintained in the PDA slants by repeated subculturing throughout the study.

Trichoderma isolates

Bioefficacy of *Trichoderma* isolates against three soil-borne plant pathogens, *viz., F. oxysporum, R. solani,* and *S. rolfsii* were evaluated using dual culture technique and production of volatile and non-volatile antibiotics (Dennis and Webster, 1971a, b).

Dual culture method

Trichoderma isolates were tested for their potential to antagonize *in vitro* against three soil-borne pathogens (*F. oxysporum, R. solani,* and *S. rolfsii*) using dual culture method. The test fungus and *Trichoderma* isolates were grown on PDA at 28±2°C for a week. A disc of 5 mm of the target fungus cut from periphery of the mycelium was transferred to Petri plate with PDA. *Trichoderma* was transferred aseptically to the same plate. Each plate received two discs, one of *Trichoderma* mycelium and other of the test pathogen, placed 7 cm away from each other. The plates were incubated at 28 ± 2^0 C and observed after eight days for growth of antagonist and test fungus, index of antagonism as percent growth inhibition of test pathogens was calculated (Morton and Stroube, 1955) (Figures 3 and 4).

Volatile method

The volatile test was carried out to observe the production of volatile inhibitors by *Trichoderma* isolates. The upper lid of PDA plates was inoculated with agar 5 mm disc of *Trichoderma* isolates and the lower lid was inoculated with soil-borne pathogens simultaneously. The two lids were taped together with adhesive tape (Dennis and Webster, 1971b) and incubated at 28±2°C for eight days. The growth of soil-borne pathogens was recorded after 72 h. In the control, soil-borne pathogens were cultured in the same way but without *Trichoderma* isolates in the bottom plate (Dennis and Webster, 1971a) (Figures 3 and 4).

Non-volatile method

The non-volatile test was carried out to find the production of water soluble inhibitors by the *Trichoderma* isolates against soil-borne pathogens (Dennis and Webster, 1971b). The isolates of *Trichoderma* culture filtrate concentration 7.5 and 15% (v/v) was inoculated in 100 ml sterile potato dextrose broth in 250 ml conical flasks. Inoculated flasks were incubated at 28±2°C for 15 days. The culture was filtered through Whatman No.42 filter papers and filtrate was collected in a sterile flask. The culture filtrate was added to molten PDA medium to obtain a final concentration of 10% (v/v). The medium was poured into the Petri plates at 15 ml/plate and 5 mm discs of pathogens were inoculated after solidification. Control plates were maintained without amending the culture filtrate. Petri plates were sealed with parafilm tape and incubated at 28±2°C for 8 days. Radial growth of soil-borne pathogens was recorded (Figures 3 and 4) and percent inhibition was calculated as per formulae adopted by Garcia (1991) as:

$$IRG\ (\%) = 100\ [(R1-R2) / R1]$$

where R1 is the farthest radial distance grown by the pathogen in the direction of the antagonist (control) while R2 represents the distance grown on a line between inoculation positions of the pathogen and the antagonist.

Grouping of *T. virens* and *T. harzianum* isolates based on percent inhibition against soil-borne pathogens.

To select potential isolates of *Trichoderma* species effective against soil-borne pathogens, grouping has been done based on percent

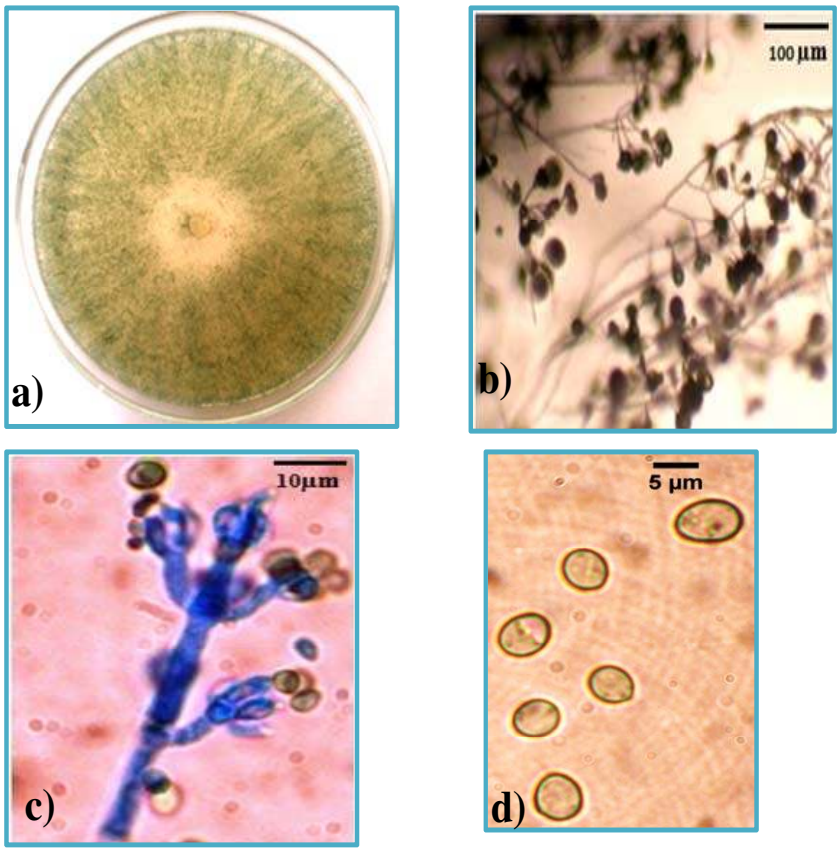

Figure 1. Morphological characters of *Trichoderma virens* isolates. a) Mycelial characters on PDA; b) Conidiophores branching in culture tube; c) Phialides; d) Conidia.

inhibition (modified Bell's scale method). Group 1: (>75-100%), Group 2: (50-75%) and Group 3: (<50%) considered as high, moderate and low potential isolates, respectively (Tables 5 and 6).

RESULTS

Morphological identification of *Trichoderma* isolates

Morphological characters such as growth characteristics, phialides disposition, shape and size and conidial shape and size of *Trichoderma* isolates were studied through microscopy and 23 isolates were confirmed as *T. virens* (dark coloured, conidiation effuse, covering the entire plate to green flat pustules concentrated near the margin and 28 isolates *T. harzianum* (pea coloured loosely aggregated flat pustules spread throughout the plate was observed in most of the isolates. Colourless to dark brown colour was observed at the reverse side of the plate) based on key given by Giddens et al. (1958), Rifai (1969) and Bissett (1984, 1991a, b) (Figures 1 and 2)

Concerning *T. virens*, the highest cultural growth was observed in the isolate V-19 (82.50 mm) and lowest in the isolateV-8 (47.50 mm). The length, width at the middle and width at the base of phialides in isolates of *T. virens* studied were from 5.80 (V-10) to 11.21(V-22) µm, 1.40 (V-20) to 3.07 (V-19) µm and 0.99 (V-20) to 2.38 (V-19) µm, respectively. Phialides shape was ampulliform in all *T. virens* isolates studied. Length and width of conidia of isolates were significantly varied ranging from 5.00 (V-1) to 6.59 (V-6) µm and 4.04 (V-1) to 5.26 (V-6) µm, respectively. The length/width (L/W) ratio of conidia ranged from 1.08 (V-22) to 1.31 (V-7) µm. Conidial shape of was obvoid to broadly ellipsoidal in all the *T. virens* isolates (Table 1).

In *T. harzianum* isolates the highest colony growth was obtained with the isolate H-10 (80 mm) and lowest with the isolate H-7 (43.50 mm). The length, width at the middle and width at the base of phialides in the isolates studied ranged from 7.87 (H-18) to 14.52 (H-22) µm and 2.25 (H-11) to 4.23 (H-25) µm and 1.54 (H-9) to 3.27 (H-5) µm, respectively. Phialides shape was ampulliform and laginiform. Length and width of conidia of isolates significantly varied from 3.50 (H-13) to 4.73 (H-17) µm and 2.76 (H-1) to 3.64 (H-20) µm, respectively. The length/width (L/W) ratio of conidia ranged from 1.12 (H-6) to 1.41 (H-5) µm. Conidial shape of *T. harzianum* was obvoid to globose (Table 2).

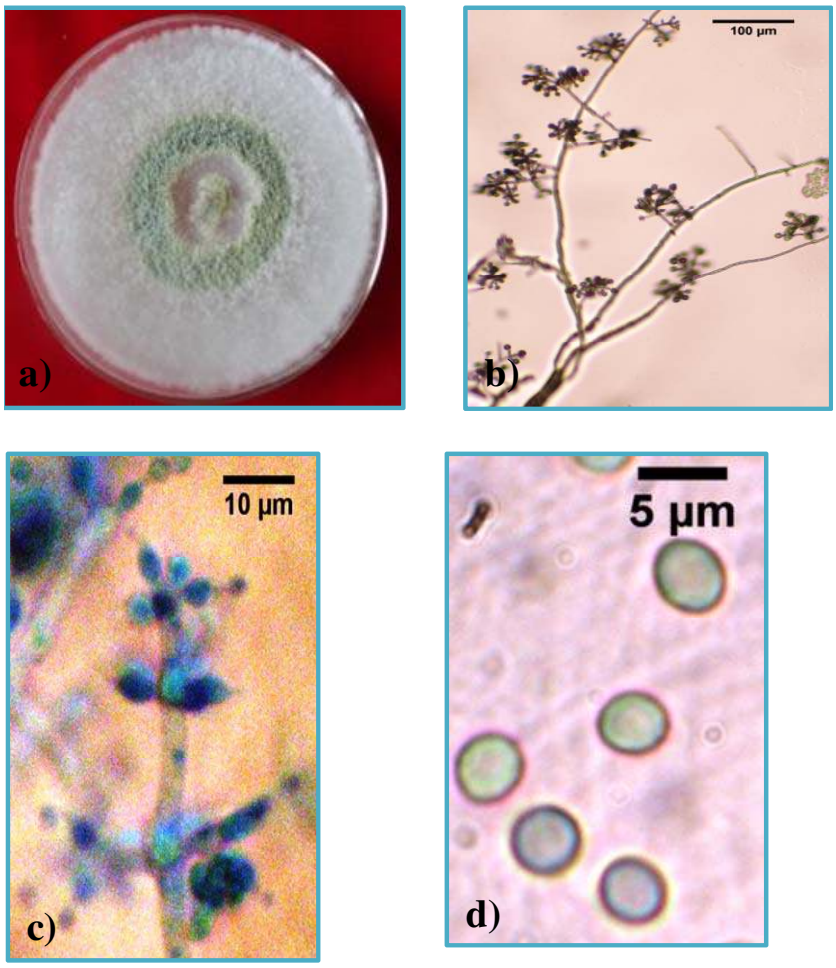

Figure 2. Morphological characters of *Trichoderma harzianum* isolates. a) Mycelial characters on PDA; b) Conidiophores branching in culture tube; c) Phialides; d) Conidia.

Bioefficacy of *Trichoderma* species isolates against soil borne pathogens

Effect of Trichoderma virens on radial growth of the soil-pathogens

Dual culture method: To select the effective bio-agents of *Trichoderma* isolates against soil-borne plant pathogens *viz., F. oxysporum, R. solani* and *S. rolfsii* (Figure 4), dual culture technique was used. All isolates of *T. virens* inhibited the mycelial growth of the soil-borne plant pathogen significantly over control. Among 23 isolates of *T. virens,* isolate V-9 inhibited the growth of *F. oxysporum* up to 82.31% which was significantly superior over all other isolates, while the isolates V-12 (53.88%) and V-5 (54.85%) showed the lowest inhibition. Isolate V-21 showed the highest percent inhibition of *R. solani* growth (81.76%) as compared to other isolates studied, while the isolate V-4 (42.93%) and V-18 (50.03%) showed the lowest inhibition. Isolate V-8 showed the highest percent inhibition of *S. rolfsii* growth (87.39%) which was significantly superior to all other isolates, while the isolates V-12 (50.23%) and V-6 (51.47%) showed the lowest inhibition (Figure 3 and Table 3).

Volatile method: Among 23 *T. virens* isolates tested for their effect of antifungal volatile metabolites production against the soil-borne plant pathogens, isolate V-7 (77.71%) was the most effective on mycelial growth of *F. oxysporum* followed by isolates V-16 (69.21%). The isolates V-4 (35.79%) and V-6 (41.05%) were the least effective against *F. oxysporum*. Isolate V-17 recorded maximum growth inhibition (76.98%) against *Rhizoctonia solani* followed by the isolates V-12 (74.84%). The isolates V-13 (52.53%) and V-15 (55.71%) were the least effective. Isolate V-8 recorded maximum growth inhibition (88.93%) against *S. rolfsii* followed by the V-22 (85.78 %). The isolates V-11 (37.82%) and V-2 (41%) were the least effective (Table 3 and Figure 3).

Table 1. Morphological characters used for the identification of *T. virens* isolates.

Strain	Culture growth on PDA (mm) at 26±2°C 3 days	Phialides				Conidia				References
		Shape	Size(µm)			Shape	Size (µm)			
			Length	Width at middle	Width at base		Length (L)	Width (W)	L/W ratio	
V-1	70.00	Ampulliform	9.714	2.078	1.504	Obvoid to broadly ellipsoidal	**5.006**	**4.044**	1.238	Rifai (1969) and Bisset (1991b)
V-2	63.50		7.159	1.843	1.259		5.312	4.559	1.165	
V-3	66.00		7.891	2.293	1.201		5.520	4.346	1.270	
V-4	69.00		8.132	2.991	1.823		5.506	4.600	1.197	
V-5	63.50		9.277	2.516	1.455		6.010	5.061	1.188	
V-6	52.50		6.439	1.716	1.170		**6.594**	**5.268**	1.252	
V-7	55.00		8.031	2.226	1.331		5.654	4.313	**1.311**	
V-8	**47.50**		9.089	2.473	1.555		5.578	4.618	1.208	
V-9	71.00		6.468	1.849	1.197		6.376	5.250	1.215	
V-10	63.00		**5.805**	1.973	1.263		6.058	4.805	1.261	
V-11	75.00		6.600	1.855	1.326		5.803	4.607	1.260	
V-12	70.00		7.800	2.234	1.475		6.064	4.880	1.243	
V-13	79.00		9.964	1.709	1.211		6.133	4.874	1.258	
V-14	75.00		8.835	2.280	1.661		6.459	5.004	1.291	
V-15	75.00		8.235	1.876	1.359		5.572	4.615	1.207	
V-16	73.00		7.184	1.974	1.352		6.173	4.751	1.299	
V-17	65.50		8.206	1.915	1.270		5.397	4.574	1.180	
V-18	66.00		6.426	1.490	1.389		5.465	4.290	1.274	
V-19	**82.50**		9.351	**3.079**	**2.380**		5.650	4.660	1.212	
V-20	61.50		7.445	**1.401**	**0.998**		5.817	5.156	1.128	
V-21	77.50		6.933	2.047	1.221		5.535	5.030	1.100	
V-22	79.00		**11.210**	2.521	2.311		5.368	4.958	**1.083**	
V-23	80.00		10.170	2.852	2.211		5.018	4.169	1.204	
S. Em±	0.17		1.07	0.06	0.07		0.19	0.10	0.01	
CD (p=0.05)	1.67		4.22	1.00	1.13		1.03	0.75	0.27	

Values in bold indicate highest and lowest sizes and culture growth of *T. virens* isolate.

Non-volatile method: Crude antibiotics produced from culture filtrate of the *T. virens* isolates were tested on radial growth of the soil-borne pathogens. All the isolates were found to reduce the radial growth of the pathogens over the control. Isolate V-23 (81.80%) recorded the maximum growth inhibition of *F. oxysporum* growth followed by the isolate V-19 (80.15%). The isolates V-3 (58.92%) and V-16 (59.01%) were the least effective. Isolate V-19 (71.61%) showed maximum growth inhibition of *R. solani* followed by the isolates V-23 (70.14%). The isolates V-18 (43.32%) and V- 5 (47.77%) were the least effective. Maximum growth inhibition (83.22%) was observed with *S. rolfsii* by the isolate V-9

Table 2. Morphological characters used for the identification of *T. harzianum* isolates.

Strain	Culture growth on PDA (mm) at 26±2°C 3 days	Phialides				Conidia				References
		Shape	Size(µm)			Shape	Size(µm)			
			Length	Width at middle	Width at base		Length (L)	Width (W)	L/W ratio	
H-1	50.00	Lagini form and ampulliform	10.896	3.496	2.435	Obvoid to Globose	3.561	**2.760**	1.290	Bisset (1991b)
H-2	64.50		11.609	3.507	2.081		3.759	3.025	1.242	
H-3	61.00		10.883	4.281	3.182		3.855	3.015	1.279	
H-4	47.50		10.549	3.136	1.965		4.220	3.169	1.332	
H-5	51.00		11.918	4.231	**3.275**		4.289	3.032	**1.415**	
H-6	51.00		12.113	2.761	2.102		3.590	3.180	**1.129**	
H-7	**43.50**		12.808	3.249	2.112		3.772	2.847	1.325	
H-8	45.00		12.804	2.458	1.786		3.720	3.232	1.151	
H-9	53.50		9.958	2.327	**1.546**		4.006	2.913	1.375	
H-10	**80.00**		13.858	3.074	1.911		4.475	3.185	1.405	
H-11	52.00		9.219	**2.259**	1.711		4.050	3.118	1.299	
H-12	48.00		11.663	3.387	2.053		4.394	3.182	1.381	
H-13	50.00		13.282	3.817	2.558		**3.502**	3.079	1.138	
H-14	66.50		14.504	3.786	2.719		4.364	3.279	1.331	
H-15	47.50		12.013	3.585	2.541		4.575	3.418	1.338	
H-16	70.00		9.756	3.719	2.533		4.058	3.212	1.263	
H-17	50.00		10.682	2.859	2.588		**4.735**	3.531	1.341	
H-18	50.50		**7.878**	3.621	2.223		4.079	3.440	1.186	
H-19	48.00		12.247	3.528	2.143		4.112	3.379	1.217	
H-20	51.00		11.953	3.438	2.094		4.152	**3.640**	1.141	
H-21	57.00		9.929	3.099	1.786		4.115	3.346	1.230	
H-22	49.00		**14.523**	3.198	3.141		3.880	3.398	1.142	
H-23	51.00		10.678	2.876	2.584		4.328	3.291	1.315	
H-24	53.00		10.839	3.194	2.161		3.885	3.330	1.167	
H-25	49.50		10.199	**4.238**	2.896		3.910	3.210	1.218	
H-26	61.00		12.078	2.743	2.592		3.649	3.032	1.203	
H-27	47.50		12.159	2.733	2.391		3.990	3.397	1.174	
H-28	60.00		13.806	3.331	2.348		4.706	3.335	1.411	
S.Em±	0.06		3.21	0.06	0.10		0.08	0.09	0.02	
C.D (p=0.05)	1.06		7.37	1.08	1.33		0.68	0.73	0.34	

Values in bold indicate highest and lowest sizes and culture growth of *T. harzianum* isolate.

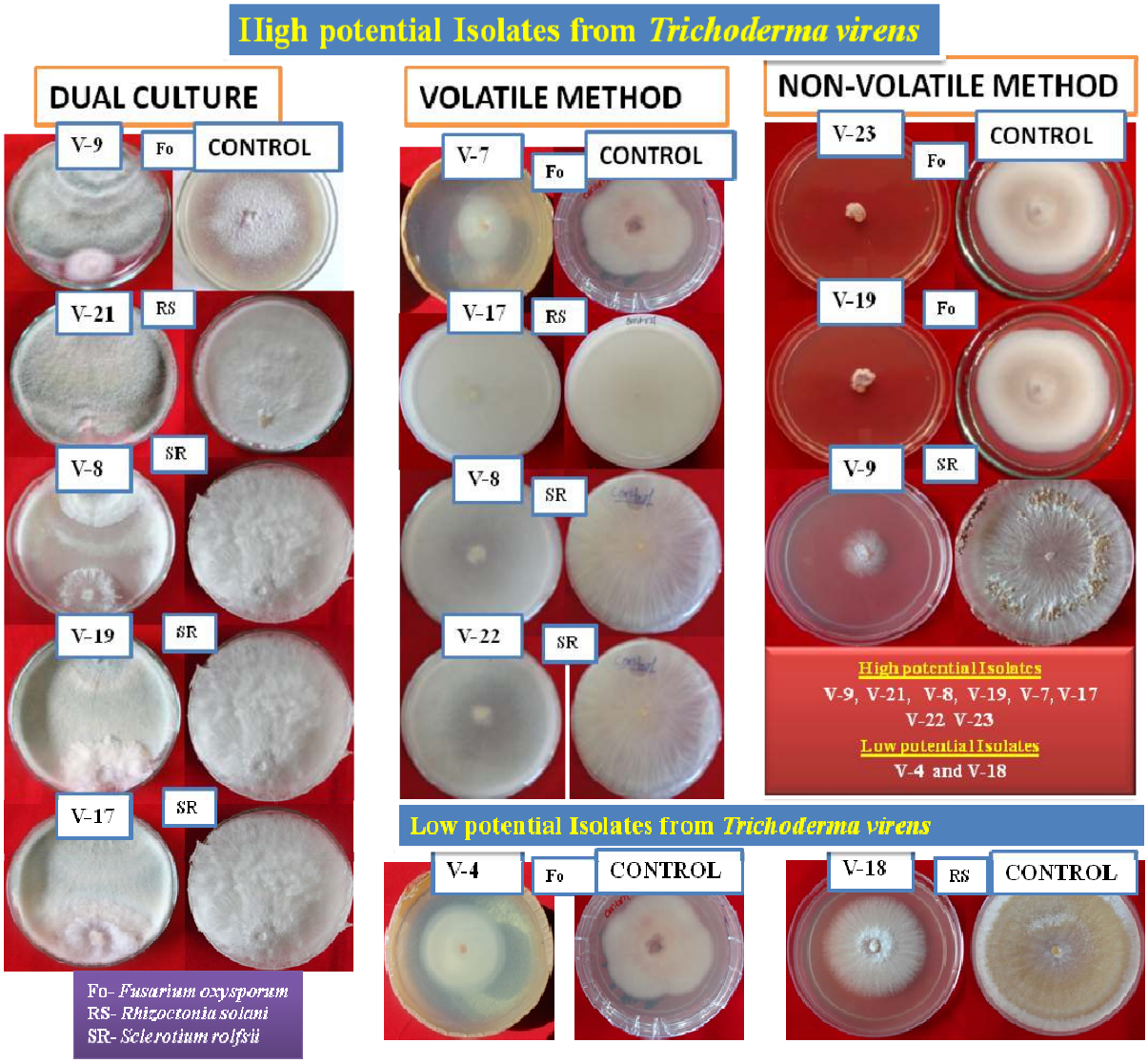

Figure 3. List of high and low potential isolates of *Trichoderma virens* selected from the bioefficacy methods.

followed by the isolate V-8 (78.36%). The isolates V-21 (51.35%) and V-18 (54.50%) were the least effective (Table 3 and Figure 3).

Among all the isolates of *T. virens* tested against three different soil-borne pathogens, highest percent mean inhibition was observed by the isolates V-9 (76.51%) and V-19 (72.38%) with *F. oxysporum.* Isolates V-19 (68.01%) and V-12 (67.37%) with *R. Solani* and isolate V-8 (84.89%) with *S. rolfsii* (Table 7).

Effects of Trichoderma harzianum on radial growth of the soil-pathogens

Dual culture method: Similarly, among 28 isolates of *T. harzianum* tested, isolate H-12 inhibited the growth of *F. oxysporum* upto 86.23% which was significantly superior over all other isolates, followed by isolates H-3 (81.35%), while the isolates H-9 (54.92%) and H-24 (60.77%) showed lowest inhibition. Isolate H-26 (84.58%) showed highest percent inhibition of *R. solani* growths as compared to other isolates studied, followed by isolates H-16 (84.50%). The isolates H-6 (55.46%) and H-15 (56.17%) showed the lowest inhibition. Isolate H-11 (85.75%) showed highest percent inhibition of *S. rolfsii* which was significantly superior to all other isolates followed by H-18 (84.42%), while the isolate H-16 (61.25%) and H-14 (64.58%) showed the lowest inhibition (Table 4 and Figure 4).

Volatile method: Isolate H-3 (83.77 %) was found to be

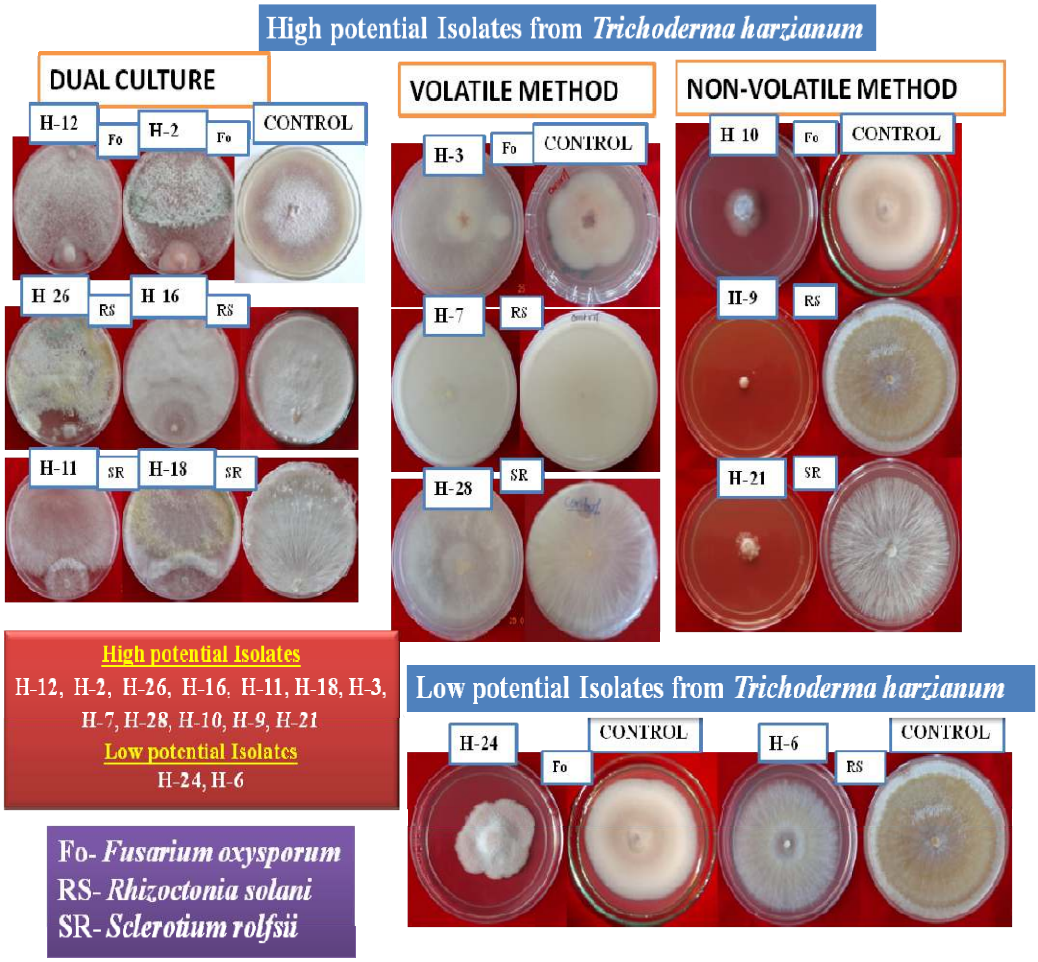

Figure 4. List of high and low potential isolates of *Trichoderma harzianum* selected from the bioefficacy methods.

more effective against *F. oxysporum* pathogen followed by the isolate H-26 (82.47%). The isolates H-6 (65.04%) and H-1 (66.29%) were the least effective. Isolate H-7 (82.63%) recorded maximum growth inhibition in *R. solani* followed by the isolates H-15 (79.48 %). The isolates H-2 (57.42%) and H-9 (59.00%) were least effective. Isolate H-28 (76.65%) recorded maximum growth inhibition against *S. rolfsii* followed by H-2 (76.59%) and the isolates H-5 (50.61%) and H-1, H-5 (50.61%) were least effective (Table 4 and Figure 4).

Non-volatile method: Isolate H-10 recorded maximum growth inhibition (81.89 %) in *F. oxysporum* followed by the isolates H-16 (78.04%) and the isolates H-24 (39.74%) and H-23 (51.92%) were least effective. Isolate H-9 (87.19%) recorded maximum growth inhibition against *R. solani* followed by the isolate H-7 (83.42%) H-6 (25.53%) and H- 24 (56.45%) were least effective. Isolate H-21 (86.04%) recorded maximum growth inhibition in *S. rolfsii* followed by the isolates H-23 (83.66%) and the isolates H-7 (63.91%) and H-8 (65.10%) are least effective (Table 4 and Figure 4).

Isolate H-3 (77.70%) showed highest percent mean inhibition of *F. oxysporum,* H-18 (76.29%) in *R. solani* and H-2 (76.35%) in *S. rolfsii* (Table 7).

Results (Tables 5 and 6) of mycelial growth percent inhibition for screening of *Trichoderma* isolates against soil-borne pathogens revealed that there was a clear difference within the isolates of *T. virens* and *T. harzianum* with respect to their percent inhibition against the pathogens tested in different methods used. It appeared that large numbers of *T. virens* and *T. harzianum* isolates fell under the category of Group 2 and very few numbers fell under category of Group 1. From the grouping, eight high potential (V-7, V-8, V-9, V-17, V-19, V-21, V-22 and V-23) and two low potential (V-4 and V-18) isolates from *T. virens* and twelve high potential (H-2, H-3, H-7, H-9, H-10, H-11, H-12, H-16, H-18, H-21, H-26 and H-28) and 2 low potential (H-6 and H-24) isolates

Table 3. Effect of *Trichoderma virens* on radial growth of soil-borne pathogens on PDA at 26± 2°C at 8 DAI.

Isolate	Percent inhibition of *Fusarium oxysporum* growth				Percent inhibition of *Rhizoctonia solani* growth				Percent inhibition of *Sclerotium rolfsii* growth				Dual Mean	Volatile Mean	Non-volatile Mean	Grand Mean
	Dual	Volatile	Non-volatile	Mean	Dual	Volatile	Non-volatile	Mean	Dual	Volatile	Non-volatile	Mean				
V-1	68.62	47.43	72.61	62.89	68.33	58.57	55.21	60.70	68.04	53.57	63.60	61.74	68.33	53.19	63.81	61.78
V-2	71.65	43.42	69.67	61.58	64.77	63.08	61.19	63.01	72.55	41.00	63.80	59.12	69.66	49.17	64.89	61.24
V-3	76.46	66.71	**58.92**	67.36	61.72	59.34	56.73	59.26	62.29	54.34	69.56	62.06	66.82	60.13	61.74	62.90
V-4	66.69	**35.79**	66.54	**56.34**	**42.93**	61.54	54.43	**52.97**	73.07	46.50	68.65	62.74	60.90	47.94	63.21	57.35
V-5	54.85	65.46	75.55	65.29	60.60	66.76	47.77	58.38	54.25	62.70	71.78	62.91	56.57	64.97	65.03	62.19
V-6	68.69	41.05	72.79	60.84	73.56	70.22	49.96	64.58	51.47	60.79	73.51	61.92	64.57	57.35	65.42	62.45
V-7	64.62	**77.71**	69.85	70.73	59.40	59.34	65.64	61.46	55.36	65.04	69.15	63.18	59.79	67.36	68.21	65.12
V-8	80.38	57.76	62.13	66.76	67.61	57.14	56.68	60.48	**87.39**	**88.93**	78.36	**84.89**	78.46	67.94	65.72	70.71
V-9	**82.31**	68.55	78.68	**76.51**	79.37	60.77	53.74	64.63	55.36	80.40	**83.22**	72.99	72.35	69.91	71.88	71.38
V-10	74.54	46.05	69.49	63.36	61.79	58.57	55.17	58.51	74.90	47.27	64.91	62.36	70.41	50.63	63.19	61.41
V-11	65.77	56.45	75.55	65.92	73.56	70.33	58.16	67.35	59.97	**37.82**	71.78	56.52	66.43	54.87	68.50	63.27
V-12	**53.88**	55.07	75.74	61.56	70.58	74.84	56.68	67.37	**50.23**	73.18	75.73	66.38	58.23	67.70	69.38	65.10
V-13	68.65	57.76	69.85	65.42	75.30	**52.53**	55.21	61.01	73.07	41.74	72.69	62.50	72.34	50.68	65.92	62.98
V-14	63.73	67.00	69.67	66.80	55.26	64.51	56.73	58.83	76.01	54.34	56.20	62.18	65.00	61.95	60.87	62.61
V-15	69.58	51.32	60.66	60.52	52.93	55.71	58.20	55.61	68.63	71.56	68.65	69.61	63.71	59.53	62.50	61.92
V-16	72.62	69.21	59.01	66.95	60.56	61.65	53.74	58.65	64.58	42.54	64.71	57.28	65.92	57.80	59.15	60.96
V-17	61.85	53.95	63.60	59.80	55.84	**76.98**	65.48	66.10	82.94	71.71	71.29	75.31	66.88	67.55	66.79	67.07
V-18	68.62	57.76	74.17	66.85	50.03	67.25	**43.32**	53.53	54.31	42.51	54.50	**50.44**	57.65	55.84	57.33	56.94
V-19	71.54	65.46	80.15	**72.38**	63.51	68.90	**71.61**	**68.01**	83.40	64.85	64.21	70.82	72.82	66.40	71.99	70.40
V-20	72.62	56.45	69.67	66.25	51.77	57.91	58.24	55.97	53.07	53.54	55.91	54.17	59.15	55.97	61.27	58.80
V-21	76.54	64.08	69.49	70.04	**81.76**	61.65	57.44	66.95	64.51	64.45	**51.35**	60.10	74.27	63.39	59.43	65.70
V-22	78.62	65.46	63.60	69.23	63.51	60.11	62.66	62.09	78.24	85.78	68.65	77.56	73.46	70.45	64.97	69.63
V-23	56.85	66.71	**81.80**	68.45	54.13	58.57	70.14	60.95	73.07	70.10	58.13	67.10	61.35	65.13	70.02	65.50
Mean	69.12	58.11	69.96	65.73	62.99	62.88	57.57	61.15	66.81	59.77	66.97	64.52	66.31	60.25	64.84	63.80
SEm±	21.66	11.32	32.60		15.52	14.55	6.87		5.46	11.30	44.48					
CD (p=0.5)	18.93	13.68	23.22		16.02	15.51	10.66		9.50	13.68	27.12					

Values in bold indicate highest and lowest sizes for the different soil-borne pathogens.

from *T. harzianum* were selected as a promising isolates for the further studies. The high and low potential isolates showed highest and lowest percent inhibition respectively in the three methods used (Table 7).

DISCUSSION

The present findings suggested that the broad conidiophore, terminated by a cluster of 3-6 closely adpressed phialides, whorls of 2-5, ampulliform phialides, dull blackish green color mycelium, effuse conidiation, broadly ellipsoid to obvoid, conidia in case of *T. virens*. Similarly in case of *T. harzianum*, whorls of phialides 2-6, ampulliform to laginiform, narrower at the base,

Table 4. Effect of *Trichoderma harzianum* on radial growth of soil-borne pathogens on PDA at 26± 2°C at 8 DAI.

Isolate	Percent inhibition of *Fusarium oxysporum* growth				Percent inhibition of *Rhizoctonia solani* growth				Percent inhibition of *Sclerotium rolfsii* growth				Dual Mean	Volatile Mean	Non-volatile Mean	Grand Mean
	Dual	Volatile	Non-volatile	Mean	Dual	Volatile	Non-volatile	Mean	Dual	Volatile	Non-volatile	Mean				
H-1	74.46	66.29	55.77	65.51	70.33	72.44	69.21	70.66	72.83	50.61	70.94	64.79	72.54	63.11	65.31	66.99
H-2	80.46	72.08	59.94	70.82	57.42	**57.42**	74.34	63.06	78.00	76.59	74.46	**76.35**	71.96	68.70	69.58	70.08
H-3	81.35	**83.77**	67.98	**77.70**	72.83	77.94	62.76	71.18	76.75	71.46	79.11	75.77	76.98	77.72	69.95	74.88
H-4	74.54	79.11	58.17	70.61	65.17	70.02	62.83	66.01	79.42	62.31	68.61	70.12	73.04	70.48	63.20	68.91
H-5	78.38	67.42	63.78	69.86	67.04	73.18	61.51	67.24	74.83	50.61	72.19	65.87	73.42	63.74	65.83	67.66
H-6	74.54	**65.04**	63.78	67.79	**55.46**	65.26	**25.53**	**48.75**	75.50	66.19	66.23	69.31	68.50	65.50	51.85	61.95
H-7	70.54	76.62	63.91	70.36	61.25	**82.63**	83.42	75.77	82.00	62.31	**63.91**	69.41	71.26	73.85	70.41	71.84
H-8	79.46	69.70	70.19	73.12	71.50	62.11	74.47	69.36	72.00	59.75	65.10	65.92	74.63	63.85	69.92	69.47
H-9	**54.92**	76.73	65.87	65.84	66.50	59.00	**87.17**	70.89	72.92	68.83	74.46	71.76	64.47	68.19	75.83	69.50
H-10	76.46	69.75	**81.89**	76.03	76.67	63.80	71.84	70.77	74.83	68.89	70.89	71.54	75.99	67.48	74.87	72.78
H-11	74.46	74.46	52.24	67.05	70.17	73.95	69.21	71.11	**85.75**	62.31	69.70	72.59	76.79	70.24	63.72	70.25
H-12	**86.23**	79.00	56.09	73.77	82.08	65.33	62.83	70.08	78.08	66.19	74.51	72.93	82.13	70.17	64.48	72.26
H-13	70.58	72.08	63.94	68.87	66.33	73.18	66.71	68.74	75.50	64.91	72.08	70.83	70.80	70.06	67.58	69.48
H-14	69.00	69.81	75.96	71.59	62.58	76.33	65.46	68.12	64.58	59.75	69.81	64.71	65.39	68.63	70.41	68.14
H-15	64.62	69.70	68.11	67.47	56.17	79.48	64.08	66.57	78.71	63.63	74.46	72.27	66.50	70.94	68.88	68.77
H-16	72.54	80.19	78.04	76.93	84.50	73.25	56.45	71.40	**61.25**	59.75	67.42	**62.81**	72.76	71.06	67.30	70.38
H-17	66.69	69.70	69.87	68.75	59.92	66.95	74.47	67.11	78.08	63.60	74.35	72.01	68.23	66.75	72.90	69.29
H-18	80.38	81.49	55.77	72.55	74.17	77.87	76.84	**76.29**	84.42	73.95	69.75	76.04	79.66	77.77	67.45	74.96
H-19	62.62	74.46	54.33	63.80	65.08	65.41	69.21	66.57	81.92	64.88	75.49	74.10	69.87	68.25	66.34	68.15
H-20	70.58	74.35	48.08	64.33	61.25	68.41	60.26	63.31	78.00	53.17	70.89	67.35	69.94	65.31	59.74	65.00
H-21	78.46	79.00	71.79	76.42	62.50	76.18	81.98	73.55	74.17	61.00	**86.04**	73.73	71.71	72.06	79.94	74.57
H-22	66.69	69.81	52.24	62.91	71.58	66.14	71.84	69.86	71.58	55.80	79.00	68.80	69.95	63.92	67.69	67.19
H-23	68.62	69.70	51.92	63.41	66.42	65.26	66.58	66.09	74.17	**50.61**	83.66	69.48	69.74	61.86	67.39	66.32
H-24	60.77	71.05	**39.74**	**57.19**	65.08	68.49	56.45	63.34	72.38	61.00	69.75	67.71	66.08	66.85	55.31	62.74
H-25	78.38	72.08	65.87	72.11	62.58	65.33	66.71	64.88	72.83	64.88	74.46	70.72	71.26	67.43	69.01	69.24
H-26	70.62	82.47	59.94	71.01	**84.58**	73.03	66.58	74.73	66.42	71.39	72.08	69.96	73.87	75.63	66.20	71.90
H-27	78.46	76.73	56.09	70.43	63.83	66.80	60.20	63.61	81.29	64.88	73.32	73.16	74.53	69.47	63.20	69.07
H-28	78.38	69.70	56.09	68.06	74.08	73.18	66.71	71.32	78.75	**76.65**	72.08	75.83	77.07	73.18	64.96	71.74
Mean	72.97	73.65	61.69	69.44	67.75	69.94	66.99	68.23	75.61	63.43	72.67	70.57	72.11	69.01	67.12	69.41
SEm±	9.28	12.86	29.83		10.08	22.23	10.61		4.99	19.64	9.05					
CD(p=0.05)	12.51	14.73	22.44		13.05	19.37	13.39		9.18	18.21	12.36					

Values in bold indicate highest and lowest sizes for the different soil-borne pathogens.

whitish green to pale green color mycelium, conidiation effuse, covering the entire surface of the plate, globose to sub-globose conidia. In this study, colony morphology, phialides and conidial morphology and size could separate *Trichoderma* spp. into *T. virens* and *T. harzianum*. These findings are duly supported by earlier observations (Rifai, 1969; Domsch et al., 1980, Bissett, 1991a, b; Samuel, 1996, 2006) where they

Table 5. Grouping of *Trichoderma virens* isolates based on percent inhibition against soil-borne pathogens.

Inhibition	Group*	*Fusarium oxysporum*			*Rhizoctonia solani*			*Sclerotium rolfsii*		
		Dual culture	Volatile	Non-Volatile	Dual culture	Volatile	Non-Volatile	Dual culture	Volatile	Non-Volatile
High	Group-1 (75-100% Inhibition)	V-3, V-8, V-9, V-22	V-7	V-5, V-9, V-11, V-12, V-19, V-23	V-13, V-21	V-17	0	V-8, V-14, V-19, V-17, V-22	V-8, V-22	V-8, V-9
	Number of isolates	4	1	6	2	1	0	4	2	2
Moderate	Group-2 (50-75% Inhibition)	V-1, V-2, V-4, V-5, V-6, V-7, V-8, V-10, V-11, V-12, V-13, V-14, V-15, V-16, V-17, V-19, V-20, V-22, V-23	V-3, V-5, V-8, V-9, V-11, V-12, V-13, V-14, V-15, V-16, V-17, V-18, V-19, V-20, V-21, V-22, V-23	V-1, V-2, V-3, V-4, V-6, V-7, V-8, V-10, V-13, V-14, V-15, V-16, V-17, V-18, V-20, V-21, V-22	V-1, V-2, V-3, V-5, V-6, V-7, V-8, V-9, V-10, V-11, V-12, V-14, V-15, V-16, V-17, V-18, V-19, V-20, V-22, V-23	V-1, V-2, V-3, V-4, V-5, V-6, V-7, V-8, V-9, V-10, V-11, V-12, V-13, V-14, V-15, V-16, V-18, V-19, V-20, V-21, V-22, V-23	V-1, V-2, V-3, V-4, V-7, V-8, V-9, V-10, V-11, V-12, V-13, V-14, V-15, V-16, V-17, V-19, V-20, V-21, V-22, V-23	V-1, V-2, V-3, V-4, V-5, V-6, V-7, V-9, V-10, V-11, V-12, V-13, V-15, V-16, V-17, V-18, V-20, V-21, V-23	V-1, V-3, V-5, V-6, V-7, V-9, V-12, V-14, V-15, V-17, V-19, V-20, V-21, V-23	V-1, V-2, V-3, V-4, V-5, V-6, V-7, V-10, V-11, V-12, V-13, V-14, V-15, V-16, V-18, V-17, V-19, V-20, V-21, V-22, V-23
	Number of isolates	19	18	17	20	22	20	19	14	21
Low	Group-3 (< 50 % Inhibition)	0	V-1, V-2, V-4, V-6, and V-10	0	V-4	0	V-5, V-6, V-18	0	V-2, V-4, V-10, V-11, V-13, V-16, V-18,	0
	Number of isolates	0	5	0	1	0	3	0	7	0

characterized different species of *Trichoderma*. The few morphological characters available are variable to some degree with respect to variable climatic and geographic locations, leading to overlap among species.

In the present finding, eight high potential and two low potential isolates from *T. virens* and 12 high potential and 2 low potential isolates from *T. harzianum* were selected as a promising isolates. The high and low potential isolates showed highest and lowest percent inhibition in the three methods used and against the three soil-borne pathogens tested. The possible explanation of this result may be due to their inherent potentiality to adapt well in introduced conditions (Papavizas, 1985; Bae and Knudsen, 2005), though it rarely occurs (Whipps, 2001). Higher growth rate ability of the selected strains are indicative of their better antagonistic potential. Mathur and Sarbhoy (1978) reported that *T. viride* and *T. harzianum* inhibited the growth of *S. rolfsii* by 88 and 86%, respectively. Mathew and Gupta (1998) showed that *T. harzianum* exhibited maximum antagonistic activity causing 58.3% inhibition of *F. oxysporum. f.* sp. *lycopersici, R. solani* and *S. rolfsii* followed by *T. hamatum*, *T. viride* and *T. virens* inhibition by 48.3, 46.1 and 44.9%, respectively. Recently, Noveriza and Quimio (2004) reported that *Trichoderma* spp. were able to cause 66.36% growth inhibition of *F. oxysporum. f.* sp. *lycopersici*, *R. solani* and *S. rolfsii* through dual culture technique and were also significantly inhibited by *Trichoderma* spp. *in vitro* (Lozoya-

Table 6. Grouping of *Trichoderma harzianum* isolates based on percent inhibition against soil-borne pathogens.

Inhibition	Groups*	*Fusarium oxysporum*			*Rhizoctonia solani*			*Sclerotium rolsfii*		
		Dual culture	**Volatile**	**Non-Volatile**	**Dual culture**	**Volatile**	**Non-Volatile**	**Dual culture**	**Volatile**	**Non-Volatile**
High	Group-1 (75-100% Inhibition)	H-2, H-3, H-4, H-5, H-8, H-10, H-12, H-18, H-21, H-25, H-27	H-3, H-4, H-7, H-9, H-12, H-16, H-18, H-21, H-26, H-27	H-10, H-14, H-16	H-12, H-16, H-26	H-3, H-7, H-14, H-15, H-18, H-21	H-7, H-9, H-18,	H-2, H-3, H-4, H-6, H-7, H-11, H-12, H-13, H-15, H-17, H-18, H-19, H-20, H-27, H-28	H-2, H-28	H-3, H-19, H-21, H-22, H-23
	Number of isolates	11	10	3	3	6	3	15	2	5
Moderate	Group-2 (50-75% Inhibition)	H-1, H-6, H-7, H-9, H-11, H-13, H-14, H-15, H-16, H-17, H-19, H-20, H-22, H-23, H-24, H-26, H-28	H-1, H-2, H-5, H-6, H-8, H-10, H-11, H-13, H-14, H-15, H-17, H-19, H-20, H-22, H-23, H-24, H-25, H-28	H-1, H-2, H-3, H-4, H-5, H-6, H-7, H-8, H-9, H-10, H -12, H-13, H-15, H-17, H-18, H-19, H-21, H-22, H-23, H-25, H-26, H-27, H-28	H-1, H-2, H-3 , H-4, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-13, H-14, H-15, H-17, H-18, H-19, H-20, H-21, H-22, H-23, H-24, H-25, H-27, H-28	H-1, H-2, H-4, H-5, H-6, H-8, H-9, H-10, H-11, H-12, H-13, H-16, H-17, H-19, H-20, H-22, H-23, H-24, H-25, H-26, H-27, H-28	H-1, H-2, H-3, H-4, H-5, H-8, H-10, H-11, H-12, H-13, H-14, H-15, H-16, H-17, H-19, H-20, H-21, H-22, H-23, H-24, H-25, H-26, H-27, H-28	H-1, H-5,H-8, H-10, H-9, H-14, H-16, H-21, H-22, H-23, H-24, H-25, H-26	H-1, H-3, H-4, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13, H-14, H-15, H-16, H-17, H-18, H-19, H-20, H-21, H-22, H-23, H-24, H-25, H-26, H-27	H-1, H-2, H-4, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13, H-14, H-15, H-16, H-17, H-18, H-20, H-24, H-25, H-26, H-27, H-28
	Number of isolates	17	18	23	25	22	24	13	26	23
Low	Group-3 (< 50 % Inhibition)	0	0	H-20, H-24	0	0	H-6	0	0	0
	Number of isolates	0	0	2	0	0	1	0	0	0

Saldana et al., 2006; Choudhary et al., 2007; Kumar and Hooda, 2007; Pan and Bhagat, 2007, 2008).

Conclusion

The objective of the present study was to investigate morphology and identification of *Trichoderma* spp. before conducting bioefficacy test *in vitro* as well as *in vivo*. Bioefficacy helps to select some promising isolates of *Trichoderma* species against soil-borne plant pathogens. Fifty one *Trichoderma* isolates obtained from Indian Type Culture Collection were morphologically characterised and identified as *T. virens* (23 isolates) and *T. harzianum* (28 isolates) on the basis of the literature reported. These isolates were tested for their bioefficacy using 3 methods (dual culture, volatile and non-volatile) against soil-borne pathogens *viz., F. oxysporum, R. solani* and *S. rolfsii.* Out of 51 isolates, 8 isolates of *T. virens* and 12 isolates of *T. harzianum* were proved as potential biocontrol agents.

Table 7. List of high and low potential isolates of *Trichoderma virens* and *T. harzianum* selected for molecular and biochemical characterization.

Name of the species	High potential	Low potential	Total	
Trichoderma virens	V-7, V-8, V-9, V-17, V-19, V-21, V-22, V-23 (8 Isolates)	V-4, V-18 (2 Isolates)	10 Isolates	24 Isolates
Trichoderma harzianum	H-2, H-3, H-7, H-9, H-10, H-11, H-12, H-16, H-18, H-21, H-26, H-28 (12 Isolates)	H-6, H-24 (2 Isolates)	14 Isolates	

Conflict of Interests

The author(s) have not declared any conflict of interests.

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