IN-VITRO EVALUATION OF *TRICHODERMA HARZIANUM* AGAINST SOIL-BORNE FUNGI OF ECONOMIC IMPORTANCE

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ABSTRACT

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A total of 767 soil samples were collected from different crops viz. dhaincha (*Sesbania rostata*), wheat (*Triticum aestivum*) and marigold (*Tagetes patula*) from 42 locations of Bangladesh. Eighty four isolates of *T. harzianum* were obtained from the collected samples. Soil-borne plant pathogens *Sclerotium rolfsii, Fusarium oxysporum* and *Rhizoctonia solani* were isolated from infected vegetable seedlings or plant parts from different locations of Bangladesh. The isolates of both *T. harzianum* and soil-borne plant pathogens were purified and cultured on PDA slant or filter disc and preserved in the refrigerator at 5 ^{0}C for future use. The isolates of *T. harzianum* were tested to assess their antagonistic activities against soil-borne fungi *S. rolfsii, R. solani* and *F. oxysporum* following dual culture on potato dextrose agar (PDA) in the laboratory. The *in-vitro* results indicated that *T. harzianum* was strong antagonistic against three different soil borne pathogenic fungi viz. *S. rolfsii, R. solani* and *F. oxysporum* in dual culture assay. Some isolates of *T. harzianum* viz. TM7, TM11, TM12, TM14, TNA1, TNA2, TNA3, TCN3, TDG1, TDG2, TJA1, TJA2, TJA3, TKG1, TJP2, TMP1, TMP2, TKC2, TBO2, TRM1 and TB2 were found most antagonists that remarkably reduced the radial growth of all the tested pathogens.

Key words: *Trichoderma harzianum, Sclerotium rolfsii, Fusarium oxysporum, Rhizoctonia solani*.

INTRODUCTION

Soil-borne fungal phytopathogens are the causal agents of many diseases of economic importance such as foot rot, root rot, damping-off, seedling blight and vascular wilts (Lichtenzveig *et al*. 2006). These pathogens become challenging due to their years together perpetuation in soil in various form viz.oospore, conidia, sclerotia, chlamydospores or others (Mondal *et al.* 1996 and Agrios 1997). The major soil-borne notorious pathogens, *Sclerotium rolfsii, Rhizoctonia solani, Fusarium oxysporum* etc. cause diseases of many crops of economic importance in most of the tropical and sub-tropical regions of the world (Punja 1985).

With the purpose of reducing the economic losses caused by these soil-borne diseases, generally utilization of chemical fungicides is considered as easy and attractive approach for the farmers. Due to their relatively low cost, ease of use, and effectiveness, fungicides has become the primary means to combat fungal diseases (Vinale *et al*. 2008, Sharma 2011, Dias 2012). However, intensive uses of fungicides become harmful to non-target organisms and also develop resistance in pathogens, and the possible carcinogenicity as well as hazardous to environment and growers (Vinale *et al*. 2008, Doley and Jite 2012). Besides, antagonistic microbes are an attractive, environmental friendly and economic option over chemical fungicides (El-Bramawy and El-Sarag 2012). Amongst the range of antagonists, different species of *Trichoderma* of phylum Ascomycota are most useful and frequently isolated soil fungi that exist in plant root ecosystem (Harman *et al*. 2004). They are opportunistic, avirulent plant symbionts, as well as being parasites of other plant pathogenic fungi (Harman *et al*. 2004).These filamentous fungi are very wide spread in nature, with high population densities in soils and plant litters. They are saprophytic, quick growing and easy to culture and they can produce large amount of propagules with long shelf life. These *Trichoderma* species (*T. viride, T. harzianum, T. longibrachiatum, T. hamatum, T. koningii* and *T. longibrachiatum*) are very promising against phytopathogenic fungi such as *F. oxysporum, S. rolfsii, Rhizoctonia solani, Pythium ultimum* and *Sclerotinia sclerotium* (Manczinger *et al*. 2002, Benítez *et al*. 2004, Vinale *et al*. 2008). Many *Trichoderma* species are regarded as growth promoter of plants by increasing fresh weight, height and flowering in plants while potentially inhibiting pathogen growth (Sharma 2011). Hence, *Trichoderma*

spp. are extensively exploiting and seeking attention of scientists from all over the world, and are also being commercially marketed as biopesticides, biofertilizers and soil amendments (Harman *et al*. 2004, Vinale *et al*. 2008, Sharma 2011, El-Bramawy and El-Sarag 2012). The current study was conducted *in vitro* to assess the antagonistic activity of *T. harzianum* collected from different location of Bangladesh to be useful against three economically important soil borne fungal pathogens, *S. rolfsii*, *F. oxysporum* and *R. solani* causing diseases on various crops.

MATERIALS AND METHOD

Four different experiments were conducted in the laboratory of Plant Pathology Division, Bangladesh Agricultural Research Institute, Gazipur during the years from 2011 to 2014.

Isolation of *Trichoderma harzianum* from roots

Rhizosphere soil and roots of healthy plant of dhaincha (*Sesbania rostrata*), marigold (*Tagetes patula*) and wheat (*Triticum aestivum*) grown in the field was collected from different location of Bangladesh. Roots were shaken gently to remove soil particles and washed thoroughly with tap water followed by surface sterilization with sodium hypochlorite solution (0.2%) and was rinsed with sterilized distilled water 3 times. Then, the cut pieces were transferred to 9 cm Petri dishes containing 10-15 ml acid potato-dextrose agar (APDA). The Petri dishes were incubated 4 days in the dark at 25±2 ^{0}C. After 4 days of incubation, plates were observed under microscope for *Trichoderma* colony. The identified isolates *T. harzianum* were purified by hyphal tip culture in water ager (WA) and preserved in the test tubes containing PDA at 25 ^{0}C.

Isolation of *Trichoderma harzianum* from soil

In case of soil, isolation was made through Dilution Plate Technique (Akhtar 1966). One gram of composite soil sample collected from the field was taken in a test tube containing 9 ml of sterilized water to make dilution as 1:10 and was thoroughly mixed with the PDA medium. Similarly a series of dilution process were continued until the samples were diluted to 1:10000. An aliquot (1ml) of each sample was placed in a Petri dishes containing PDA. The Petri dishes were incubated and allowed 5 days for growth at room temperature (25±2 ^{0}C). The growing mycoflora was observed under microscope and identified them following Rifai and Webster (1969) and Barnett and Hunter (1998). The identified *T. harzianum* was purified by hyphal tip culture in water agar (WA) and single spore isolation technique and preserved in the PDA slant and filter disc at 5 ^{0}C in the refrigerator for future use

Antagonistic effect of *T. harzianum* against pathogenic fungi

Antifungal activity of all the isolates of *T. harzianum* against the target fungal pathogens *S. rolfsii*, *F. oxysporum* and *R. solani* were assessed *in vitro* conditions following the dual culture technique of Rini and Sulochana (2007). Both *T. harzianum* and pathogenic fungi mycelial blocks (5mm) were cut from the periphery of 3-days old culture and were placed on PDA plate, facing opposite to each other. The distance between discs was approximately 6cm. Petri dishes were incubated at 27±2 °C for 3 days. The experiment was set up following Completely Randomized Design with five replications. Colony diameter was recorded after 48 hours of incubation for *S. rolfsii*, *F. oxysporum* and *R. solani*, and 72 hours of incubation for *F. oxysporum* and *R. solani*. The colony diameter in each Petri dish was measured at three places and average was calculated. Percent inhibition of growth was calculated using the following the formula as suggested by Sunder *et al*. (1995).

$$\text{Percent inhibition} = \frac{Y - Z}{Y} X100$$

Where Y = Mycelial growth of pathogen alone (control) Z =Mycelial growth of pathogen along with antagonist.

RESULTS AND DISCUSSION

A total of 84 *T. harzianum* isolates were obtained from 767 soil and root samples (Table 1).

All *T. harzianum* isolates remarkably reduced the radial growth of *S. rolfsii*, *F. oxysporum* and *R. solani* on PDA in dual culture techniques (Table 2, 3 and 4). The *T. harzianum* isolates reduced 19.42-42.72%, 17.68-47.85% and 15.51-43.82% radial growth of *S. rolfsii*, *F. oxysporum* and *R. solani*, respectively at 48 hrs of incubation. Besides, at 72 hrs incubation *T. harzianum* isolates reduced 18.56-41.24% and 29.60-53.68% redial growth of *F. oxysporum* and *R. solani*, respectively (Table 3 and 4). Most of the *T. harzianum* isolates showed better performance in reducing the growth of *S. rolfsii*, *F. oxysporum* and *R. solani* that indicated the antagonistic effect of *Trichoderma* on the radial growth of the targeted pathogens. The *T. harzianum* isolate TNA3 suppressed 42.72% radial growth of *S. rolfsii* at 48 hrs followed by TM14, TJA2, TJA3, TNA1, TNA3, TKG1, TDG1, TJA1, TM7, TDG2, TJP2, TM2, TM3, TM11 and TM12 ranging from 34.62% to 40.66%. However, TCN1, TM1, TM8 and TM6 isolates reduced 19.42, 19.96, 20.88 and 21.79%, respectively of the growth of *S. rolfsii*. In case of *F. oxysporum*, *T. harzianum* isolate

TJA3 reduced 47.85% radial growth followed by TNA3, TM14, TJP1, TKG1, TMP1, TJP2, TJA2, TM7, TJA1, TBO1, TM6, TNA2 and TDG2 where TMP3 reduced only 17.68% redial growth at 48 hrs incubation. The *T. harzianum* isolate TBO3 suppressed 45.02% vegetative growth of *R. solani* at 48 hrs incubation followed by TM14, TJA3, TKC1, TNA3, TMP1, TRM1, TB1 and TJA2 where TM1, TM2, TM3 and TM6 isolates reduced 15.51, 17.30, 17.98 and 18.43% redial growth, respectively. Therefore, among the isolates of *T. harzianum* TM7, TM11, TM12, TM14, TNA1, TNA2, TNA3, TCN3, TDG1, TDG2, TJA1, TJA2, TJA3, TKG1, TJP2, TMP1,TMP2, TKC2, TBO2, TRM1 and TB2 were found effective for suppressing the radial growth of pathogenic fungi *S. rolfsii, R. solani* and *F. oxysporum* in *in-vitro* study.

The majority of the *T. harzianum* isolates collected from different location of the country exhibited variable antagonistic activity against the S. *rolfsii, R. solani* and *F. oxysporum* in dual cultures. The antagonistic activity of *Trichoderma* against S. *rolfsii* was reported by many authors (Sultana *et al*. 2012, Shaigan *et al*. 2008, Yaqub and Shahzad 2005, Khattabi *et al*. 2004, Howel 2003, Yogendra and Singh 2002, Virupaksha *et al*. 1997). The fungus *F. oxysporum* was also reported to be suppressed by *Trichoderma* (Devi *et al*. 2013, Meraj and Nandkar 2012, Sharma 2011, John *et al.*2010, Ramezani 2010, Nikam *et al*. 2007, Kaur *et al*. 2003). Similarly the antagonistic activity of *Trichoderma* against *R. solani* was also reported by many investigators (Tapwal *et al*. 2014, Seema and Devaki 2012, Prasad and Kumar 2011). The inhibition of radial growth of two interacting organisms i.e. pathogenic fungi and *T. harzianum* in dual culture might be attributed to non-volatile and volatile inhibitory substance e.g. harzianic acids, tricholin, viridian, glisoprenins, massoilactone gliovir and heptelidic acid etc. released by one or both organisms in the growth medium that probably hinder the pathogen colonization (Rini and Sulochana 2007). Mishra *et al.* (2011) and Amin *et al.* (2010) observed the inhibitory activity of volatile and non-volatile compounds of *Trichoderma* species on the growth of *Rhizoctonia, Fusarium, Alternaria, Colletotrichum* etc. Comparatively fast growth of *T. harzianum* than the pathogenic fungi might be indication of competition efficiencies of antagonists for space and nutrients (Devi *et al*. 2013). Wide variation in the antagonism of a population of *T. harzianum* was reported by Davet (1986). Similarly, Tapwal *et al*. (2011) and Prasad and Kumar (2011) reported that *Trichoderma* as potential antagonist against the phytopathogen like *R. solani* and *F. oxysporum* in dual culture technique. Nagamma and Nagaraja (2015) also reported that *T. harzianum* effectively inhibited the mycelial growth of *S. rolfsii* under *in-vitro* conditions. Fakhrunnisa *et al*. (2006) found *T. harzianum* to be highly effective against *F. oxysporum.* The present study concluded that *T. harzianum* was the best biocontrol agent against economic important soil borne fungal pathogens *S. rolfsii, R. solani* and *F. oxysporum.*

Table 1. Collection of soil samples from different locations of Bangladesh and isolation of *Trichoderma harzianum*

Sl. No.	**Location**	**Number of soil samples tested**	**Number *T. harzianum* isolates obtained**
1	Gazipur	25	3
2	Mymensingh	16	2
3	Manikgonj	18	2
4	Savar, Dhaka	15	2
5	Madhupur, Tangail	25	3
6	Tangail	20	0
7	Keshoreganj	12	1
8	Jamalpur	25	2
9	Sherpur	07	1
10	Netrakona	11	2
11	Munshigonj	15	1
12	Norsingdi	16	2
13	Dinajpur	32	5
14	Debigonj, Panchaghar	25	3
15	Rangpur	25	4
16	Jhenaidah	11	3
17	Chuadanga	07	1
18	Khulna	07	2
19	Meherpur	05	0
20	Shatkhira	08	2
21	Bogra	31	5
22	Chapainawabganj	22	3
23	Rajshahi	15	1
24	Jessore	25	2
25	Ishurdi, Pabna	25	1
26	Faridpur and Madaripur	20	3
27	Barisal	35	4
28	Patuakhali	10	1
29	Joyantapur, Sylhet	26	5
30	Akbarpur, Maulvibazar	24	1
31	Comilla	14	1
32	Chittagong	24	3
33	Pahartali, Chittagong	15	2
34	Raikhali, Chittagong	12	1
35	Khagrachari	25	3
36	Rangamati	12	2
37	Bandarban	12	1
38	Noakhali	15	2
39	Barishal	20	2
40	Lalmonirhat	20	3
41	Gaibanda	20	2
42	Thakurgaon	20	1
	Total	767	84

Table 2 *In vitro* suppression of radial growth of *Sclerotium rolfsii* by *Trichoderma harzianum* isolates at dual culture

Accessions *(T.harzianum)*	Average radial growth of *S. rolfsii* at 48hrs (cm)	Growth suppression (%)	Accessions *(T.harzianum)*	Average radial growth of *S. rolfsii* at 48hrs (cm)	Growth suppression (%)
Set-I			Set-III		
TM1	4.37	19.96	TBO1	3.85	35.29
TM2	3.55	34.98	TBO2	3.92	34.12
TM3	3.59	34.25	TBO3	4.05	31.92
TM6	4.27	21.79	TMP1	3.87	34.96
TM7	3.44	36.99	TMP2	4.03	32.27
TM8	4.32	20.88	TMP3	4.07	31.60
TM9	3.80	30.40	TKC1	3.65	38.66
TM11	3.61	33.88	TKC2	4.05	31.92
TM12	3.57	34.62	TKC3	3.95	33.61
TM14	3.24	40.66	TRM1	3.75	36.98
S. rolfsii	5.46	-	TRM2	3.82	35.80
Set-II			TBB1	3.90	34.45
TNA1	3.10	39.80	TRK1	3.98	33.11
TNA2	2.95	42.72	TPT1	4.15	30.25
TNA3	3.13	39.22	TPT2	3.88	34.80
TJA1	3.20	37.86	TB1	4.25	28.57
TJA2	3.09	40.00	TB2	4.05	31.92
TJA3	3.09	40.00	TBM1	4.22	29.08
TCN1	4.15	19.42	TBM2	4.15	30.25
TCN2	3.53	31.46	*S. rolfsii*	5.95	-
TCN3	3.49	32.23			
TKG1	3.18	38.25			
TJP1	3.54	31.26			
TJP2	3.28	36.31			
TDG1	3.23	37.28			
TDG2	3.28	36.31			
S. rolfsii	5.15	-			

Table 3 *In vitro* suppression of radial growth of *Fusarium oxysporum* by *Trichoderma harzianum* isolates at dual culture

Accessions *(T. harzianum)*	Average radial growth of *F. oxysporum* at 48hrs (cm)	Growth suppression (%)	Average radial growth of *F. oxysporum* at 72hrs (cm)	Growth suppression (%)
Set-I				
TM1	2.09	33.44	3.15	30.16
TM2	2.10	33.12	3.12	30.82
TM3	1.98	36.94	3.14	30.38
TM6	1.88	40.12	2.95	34.59
TM7	1.84	41.46	2.74	39.25
TM8	2.04	35.03	2.86	36.59
TM9	2.05	34.71	2.96	34.47
TM11	1.90	39.49	2.70	40.13
TM12	1.96	37.58	2.65	41.24
TM14	1.75	44.27	2.72	39.69
Fusarium sp	3.14	-	4.51	-

Set-II				
TNA1	2.23	26.40	2.90	31.76
TNA2	1.83	39.60	2.69	36.71
TNA3	1.66	45.21	2.78	34.59
TJA1	1.78	41.25	2.73	35.76
TJA2	1.74	42.57	2.84	33.18
TJA3	1.58	47.85	2.74	35.53
TCN1	2.13	29.70	2.96	30.35
TCN2	2.24	26.07	2.90	31.76
TCN3	2.14	29.37	2.93	31.06
TJP1	1.69	44.22	2.64	37.88
TJP2	1.74	42.57	2.51	40.94
TKG1	1.73	42.90	2.93	31.06
Fusarium sp	3.03	-	4.14	
Set-III				
TDG1	2.12	35.37	3.32	31.55
TDG2	1.98	39.69	3.05	37.11
TBO1	1.95	40.55	2.98	38.56
TBO2	2.02	38.42	3.12	35.67
TBO3	2.07	36.89	3.26	32.78
TMP1	1.88	42.68	2.95	39.18
TMP2	1.97	39.94	3.12	35.67
TMP3	2.10	35.98	3.18	34.43
TKC1	2.70	17.68	3.95	18.56
TKC2	2.13	35.06	3.06	36.91
TKC3	2.07	36.89	3.24	33.20
TRM1	2.15	34.45	3.50	27.84
TRM2	2.05	37.50	3.35	30.93
TBB1	2.17	33.84	3.65	24.74
TRK1	2.25	31.40	3.75	22.68
TPT1	2.03	38.11	3.12	35.67
TPT2	2.32	29.27	3.82	21.24
TB1	2.28	30.48	3.74	22.89
TB2	2.07	36.89	3.08	36.49
TBM1	2.35	28.35	3.64	24.95
TBM2	2.15	34.45	3.25	32.99
Fusarium sp	3.28	-	4.85	-

Table 4 *In vitro* suppression of radial growth of *Rhizoctonia solani* by *Trichoderma harzianum* isolates at dual culture

Accessions (*T. harzianum)*	Average radial growth of *R. solani* at 48hrs (cm)	Growth suppression (%)	Average radial growth of *R. solani* at 72hrs (cm)	Growth suppression (%)
Set-I				
TM1	3.76	15.51	4.17	31.41
TM2	3.68	17.30	4.28	29.60
TM3	3.65	17.98	4.25	30.09
TM6	3.63	18.43	4.18	31.25
TM7	3.15	29.21	3.63	40.29
TM8	3.27	26.52	4.20	30.92
TM9	3.43	22.92	4.25	30.09
TM11	2.88	35.28	3.32	45.39
TM12	3.25	26.97	3.24	46.71
TM14	2.50	43.82	3.15	48.19
R. solani	4.45	-	6.08	-

Set-II				
TNA1	3.00	32.13	3.33	45.50
TNA2	3.07	30.54	3.30	45.99
TNA3	2.73	38.24	3.18	47.95
TJA1	3.08	30.32	3.30	45.99
TJA2	2.80	36.65	3.23	47.14
TJA3	3.00	32.13	3.47	43.21
TCN1	2.85	35.52	3.15	48.45
TCN2	3.15	28.73	3.45	43.54
TCN3	2.98	32.58	3.30	45.99
TJP1	2.95	33.26	3.30	45.99
TJP2	3.22	27.15	3.53	42.23
R. solani	4.42	-	6.11	-
Set-II				
TDG1	3.27	26.02	3.38	44.69
TDG2	3.00	32.13	3.63	40.59
TBO1	2.95	33.26	3.35	45.17
TBO2	2.72	38.46	3.08	49.59
TBO3	2.43	45.02	2.83	53.68
TMP1	2.75	37.78	3.18	52.05
TMP2	3.25	26.47	3.70	39.44
TMP3	3.03	31.45	3.43	43.86
TKG1	3.02	31.67	3.38	44.68
TKC1	2.72	38.46	3.17	48.12
TKC2	3.41	22.63	3.97	35.02
TKC3	3.08	30.32	3.40	44.35
TRM1	2.77	37.33	3.15	48.45
TRM2	3.12	29.41	3.72	39.12
TBB1	3.25	26.47	3.73	38.95
TRK1	3.10	29.86	3.65	40.26
TPT1	2.93	33.71	3.38	44.68
TPT2	2.98	32.58	3.43	43.86
TB1	2.80	36.65	3.23	47.14
TB2	2.85	35.52	3.20	47.63
TBM1	3.06	30.77	3.65	40.26
TBM2	2.98	32.58	3.43	43.86
R. solani	4.42	-	6.11	-

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