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ANTAGONISTIC POTENTIAL OF SOIL FUNGI AS BIOCONTROL AGENT AGAINST RICE PATHOGENS

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Abstract

Antagonistic potential of six soil fungi *viz*., *Aspergillus flavus* Link., *A. fumigatus* Fresen., *A. niger* Tiegh., *Penicillium* sp., *Trichoderma harzianum* Refat. and *T. viride* Pers. against eight pathogenic fungi *viz.*, *Alternaria alternata* (Fr.) Keissler, *Curvularia lunata* (Wakker) Boedijn, *Drechslera oryzae* Breda de Haan (Subramanian and Jain), *Fusarium moniliforme* Sheldon, *F. solani* (Mart.) Sacc. *Microdochium oryzae* (Hashloka and Yokogi) Sam. and Hal., *Pestalotiopsis guepinii* (Desm.) Stay. and *Sarocladium oryzae* (Sawada) W. Gams and D. Hawks of rice were evaluated. In colony interaction, the highest growth inhibition (88%) was observed owing to *T. harzianum* against *Alternaria alternata.*Volatile substances from soil fungi inhibited the radial growth of the test pathogens which varied from 8.33 to 57.36%.The highest inhibition (57.36%) was found owing to *T. harzianum* against *P. guepinii.* The inhibition of mycelial growth of the test pathogens ranged from 29.05 to 64.5% owing to non volatile substances of the soil fungi. The highest mycelial growth inhibition was observed owing to *T. harzianum* against *C. lunata. Trichoderma harzianum* may be exploited commercially to control rice pathogens.

Introduction

Rice is produced all over Bangladesh with high production intensity in some areas and plays a dominant role in providing food for the people. The average per hectare production of rice in Bangladesh is extremely low as compared to other rice growing countries of the world (Abedin *et al.*2012). Seed is a common carrier of pathogens and act as the primary source of many diseases of rice. The most destructive seed borne fungal diseases of rice are brown spot (*Bipolaris oryzae*), bakanae (*Fusarium moniliforme*), blast (*Pyricularia oryzae*), sheath blight (*Rhizoctonia solani*), sheath rot (*Sarocladium oryzae*), stem rot (*Sclerotium oryzae*), leaf scaled *(Microdochium oryzae*) and grain spot (*Curvularia lunata*) which are the main causes of rice yield reduction, quality deterioration and germination failure (Mia *et al.*1979, Shahjahan *et al.*1988, Haque *et al.*2007).

Biological control agents are widely recognized against pathogenic fungi for the management of plant diseases. Antagonists as biological control agents have now become one of the most exciting and rapidly developing areas in plant pathology because it has great potential to solve many agricultural and environmental problems (Baker and Cook 1983). Different species of *Trichoderma* are used successfully to control plant pathogens (Vinalea *et al.* 2008). Colony interaction between antagonists and pathogenic fungi are studied for the determination of antagonistic potentiality of fungi, which isolated from different habitats including rhizosphere (Skidmore and Dickinson 1976, Bashar and Rai 1994 and, Brozova 2002).

Many researches have been done on *in vitro* management of rice pathogens (Farid *et al.* 2002, Mohana *et al.* 2011, Yeasmin *et al.* 2012, Mansur *et al.* 2013, Chowdhury *et al.* 2015a) but there is no adequate information on *in vitro* management through biological control agents in Bangladesh.

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Keeping the above-mentioned facts in mind, investigation has been carried out to control rice pathogens through colony interaction and application of volatile and non-volatile substances of soil fungi.

Materials and Methods

Samples of rice grains were collected after harvesting from different districts of Bangladesh including BRRI. Samples were placed in clean brown paper bag, labeled properly and preserved at 4^0 C in refrigerator for subsequent use. The fungi were isolated from the samples following “Tissue planting method” on PDA medium (CAB 1968) and “Blotter method of ISTA”. Two hundred seeds of each sample were placed on three layers of moist blotting paper (Whatman No. 1) in Petri plates. The seeds were washed with sterile water and then surface sterilized by dipping in 10% Chlorox solution for 5 minutes. Seeds were placed in each Petri plates and incubated at 25 ± 2ºC for 5-7 days.

Pathogenic fungi isolated from selected BRRI rice varieties were grown in the PDA plates and slants for further studies and preservation (Chowdhury *et al.* 2015b and 2021). The isolated pathogenic fungi and soil fungi were identified based on morphological characteristics observed under a compound microscope following standard literature (Thom and Raper 1945, Raper and Thom 1949, Gilman 1967, Barnett and Hunter 2000, Booth 1971, Ellis 1971, 1976, Ellis and Ellis 1997 and Sutton1980).

Six antagonistic soil fungi *viz. Aspergillus flavus* Link, *A. fumigatus* Fresen., *A. niger* Tiegh., *Penicillium* sp., *Trichoderma harzianum* Refat and *T. viride* Pers. were isolated from the rhizosphere of the several healthy rice crop fields according to the method described by Romana *et al*. (2015). They were selected to test their antagonistic potential against the pathogenic fungi following dual culture technique described by Bashar and Rai (1994). Five mm blocks of each test pathogen and selected soil fungus were placed 3 cm apart on PDA medium in paired combination. Three replications were maintained in each case. The inoculated plates were incubated at 25 ± 2ºC temperature for 7 days. The colony growth of the pathogen was measured at both sides, that is towards and opposing each other from their central loci. The radial growth was measured after 3, 5 and 7 days. In dual culture, assessment of colony interactions grading was done based on intermingling and inhibition zone which were determined by the model of Skidmore and Dickinson (1976). Per cent inhibition of the growth of the test fungi due to the presence of antagonists were also calculated as follow:

$$I = \frac{r1 - r2}{r1} \times 100$$

Where, I = per cent growth inhibition

r_1 = the radial growth of the test fungus towards the opposite side

r_2 = the radius of the test fungus towards the soil fungus

Data were collected as inhibition percentage of the radial growth of the test pathogen in mm in each replication.

Effects of volatile and non- volatile metabolites of the selected soil fungi against the test pathogens were also calculated following the methods described by Bashar and Rai (1994). The per cent growth inhibition of radial growth of test pathogen was calculated by the formula given above. The results were evaluated by analysis of variance by using STAR statistical program.

Results and Discussion

Antagonistic potential of selected six soil fungi against the eight tested pathogens *viz.*, *Alternaria alternata* (Fr.) Keissler, *Curvularia lunata* (Wakker) Boedijn, *Drechslera oryzae* Breda de Haan (Subramanian and Jain), *Fusarium moniliforme* Sheldon, *F. solani* (Mart.) Sacc. *Microdochium oryzae* (Hashloka and Yokogi) Sam. and Hal., *Pestalotiopsis guepinii* (Desm.) Stay. and *Sarocladium oryzae* (Sawada) W. Gams and D. Hawks are presented in Table 1. In this study antagonistic relationship ranged from grade 2 to 4. However, grade 3 was found most commonly encountered type of colony interaction which was followed by grades 2 and 4. *Trichoderma harzianum* showed grade 4 interaction against all the test pathogens except *A. alternata.* It was followed by *Aspergillus niger* which is similar with the observation of Prince *et al.* (2011) and Akter *et al.* (2014). Prince *et al.* (2011) observed grade 4 interaction between *T. harzianum* and *Colletotrichum falcatum.* Akter *et al.* (2014) also reported grade 4 interaction between *T. harzianum* against *Colletotrichum* sp., *Curvularia lunata, Fusarium moniliforme*, *F. oxysporum, F. semitectum* and *Phomopsis,* individually.

Table 1. Antagonistic potential of soil fungi against the test pathogens of rice.

Name of fungi	Test pathogens							
	Aa	Cl	Do	Fm	Fs	Mo	Pg	So
				Grades of colony interactions				
Aspergillus flavus	3	2	3	3	3	3	3	2
A. fumigatus	2	2	2	2	3	2	2	2
A. niger	3	4	4	2	2	3	3	2
Penicillium sp.	3	3	3	3	3	2	3	2
Trichoderma harzianum	2	4	4	4	4	4	4	4
T. viride	2	3	4	3	3	3	2	3
				% inhibition of test pathogens				
Aspergillus flavus	50.10 d	50.78 d	65.6 c	46.00 d	60.25 b	60.00 b	55.00 b	47.36 cd
A. fumigatus	45.25 e	42.52 f	50.00 d	42.00 e	53.20 c	52.00 c	51.25 c	46.00 d
A. niger	70.66 b	73.87 c	70.25 b	66.66 b	46.66 d	62.00 a	55.15 b	50.00 c
Penicillium sp.	50.25 de	45.53 e	42.15 e	**35.50 f**	48.66 d	54.25 d	45.00 d	50.00 c
Trichoderma harzianum	**88.00 a**	74.55 b	75.25 a	55.25 c	86.00 a	60.25 b	55.25 b	82.00 a
T. viride	60.25 c	80.00 a	75.50 a	76.00 a	55.25 c	50.15 c	75.00 a	56.00 b
CV %	1.98	0.98	1.41	1.91	1.05	1.55	1.02	1.35

Aa = *Alternaria alternata*, Cl = *Curvularia lunata*, Do = *Drechslera oryzae*, Fm = *Fusarium moniliforme*, Fs = *Fusarium solani*, Mo = *Microdochium oryzae*, Pg = *Pestalotiopsis guepinii* and So = *Sarocladium oryzae.*

Grades from 1 to 5 based on Skidmore and Dickinson (1976). Grade 2: Mutual intermingling growth where the fungus is ceased and being overgrowth by the opposed fungus, Grade 3: Intermingling growth where the fungus is growing into the opposed fungus either above or below and Grade 4: Slight inhibition with a narrow demarcation line (1-2 mm). Values within the same column with a common letter (s) do not differ significantly at 5% level by LSD.

In colony interaction the radial growth inhibition of the test pathogens with the soil fungi was found to be between 35.50 to 88%. The highest growth inhibition was observed owing to *T. harzianum* against *A. alternata* which was followed by *F. solani, Sarocladium oryzae*, and *Drechslera oryzae*. The maximum inhibition of *Curvularia lunata*, *Fusarium moniliforme* and *Pestalotipsis guepinii* were 80, 76 and 75%, respectively due to *Trichoderma viride* (Table 1 and Plate 1). These results are in agreement with the findings of Prince *et al.* (2011) and Akter *et al.* (2014).

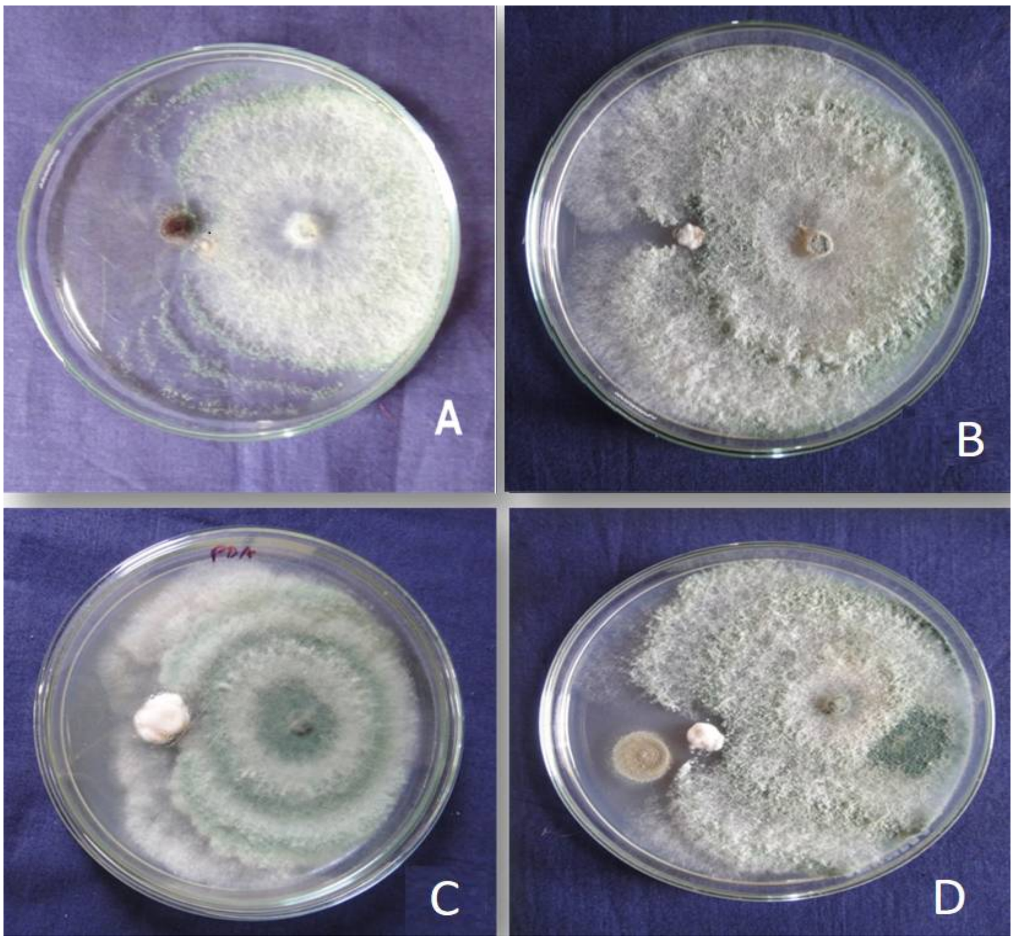

Plate 1. Colony interaction between A. *Alternaria alternata* and *Trichoderma harzianum,*B. *Fusarium solani* and *T. harzianum,* C. *Pestalotiopsis guepinii* and *T. viride,* D. *Sarocladium oryzae* and *T. harzianum.*

Volatile substances emanating from the soil fungi inhibited the radial growth of the test pathogens varied from 8.33 to 57.36%. The highest inhibition (57.36%) was recorded owing to *T. harzianum* against *P. guepinii* followed by *F. moniliforme* (46.80%), and *A. alternata* (46%) owing to *T. viride*. The per cent inhibition owing to volatile metabolites of *Trichoderma harzianum* against the mycelial growth of *C. lunata, D. oryzae, F. moniliformae, F. solani, M. oryzae, P. guepinii* and *S. oryzae* were 46, 38.85, 37.14, 25, 36.25, 57.36 and 30.33 %, respectively.These results are in agreement with the findings Romana *et al.* (2015) and Barakat *et al.* (2013). Romana *et al.* (2015) reported that highest inhibition of radial growth of *F. solani* and *F. oxysporum* was found due to *T. harzianum* followed by *T. viride* and *A. niger*, respectively. *Aspergillus flavus, A. fumigatus* and *Penicillium* sp. showed lesser degree of inhibition of radial growth of all the test pathogen (Table 2 and Plate 2a).

Table 2. Per cent inhibition of radial growth of the test pathogens owing to volatile and non-volatile substances of soil fungi.

Name of fungi	% inhibition of test pathogens owing to volatile and non volatile substances							
	Aa	Cl	Do	Fm	Fs	Mo	Pg	So
				Volatile substances				
Aspergillus flavus	25.00 d	33.33 c	33.33 ab	22.66 d	8.50 f	11.90 e	20.00 c	21.05 c
A. fumigatus	**8.33 f**	13.33 e	20.00 c	33.33 c	11.90 e	8.50 f	38.46 b	12.00 d
A. niger	40.00 b	37.50 bc	39.85 ab	46.36 a	45.25 a	25.50 d	56.60 a	25.00 b
Penicillium sp.	8.34 f	25.93 d	18.18 c	11.90 e	8.53 f	8.60 f	20.00 c	20.00 c
Trichoderma harzianum	15.00 e	45.00 a	38.85 ab	37.14 b	25.00 cd	36.25 a	**57.36 a**	30.33 a
T. viride	46.00 a	36.66 b	39.00 ab	46.80 a	21.50 b	28.75 b	56.60 a	32.31 a
CV %	5.08	4.73	4.82	2.20	3.10	2.93	3.69	5.46
				Non-volatile substances				
Aspergillus flavus	40.32 d	39.00 d	45.20 c	45.75 d	42.00 d	45.00 b	33.45 b	30.00 d
A. fumigatus	35.20 e	30.51 e	40.00 d	39.00 e	38.00 e	38.25 c	32.18 c	**29.05 d**
A. niger	55.55 b	52.50 bc	62.50 a	52.00 b	55.00 a	60.50 a	45.03 b	45.50 c
Penicillium sp.	45.00 d	50.25 c	50.40 e	50.55 c	48.25 c	48.25 d	30.50 d	43.25 c
Trichoderma harzianum	60.50 a	**64.5 a**	62.25 b	53.55 a	52.50 b	62.25 b	45.84 a	50.35 a
T. viride	45.52 c	62.25 b	61.50 b	52.25 a	55.25 a	54.50 c	36.22 b	50.00 b
CV %	1.98	0.99	1.34	1.91	1.05	1.30	1.02	1.35

Abbreviations are similar as in Table 1.

Values within the same column with a common letter (s) do not differ significantly at 5% level by LSD.

Non-volatile substances of the soil fungi showed inhibition of mycelial growth of the test pathogens which ranged from 29.05 to 64.5%. The highest inhibition was observed owing to the culture filtrate of *T. harzianum* against *C. lunata* followed by *D. oryzae* (62.25%), *M. oryzae* (62.25%), *A. alternata* (60.50%), *F. moniliforme* (53.55%), *F. solani* (52.5%), *S. oryzae* (50.55%) and *P. guepinii* (45.84%). The lowest inhibition was observed by the culture filtrate of *A. fumigatus* against *P. guepinii* (28.18%) (Table 2). These results are in agreement with the findings of Akter *et al.* (2014) and Bashar and Chakma (2014). Akter *et al.* (2014) reported that non-volatile metabolites of *A. flavus, A. fumigatus, A. niger, T. harzianum* and *T. viride* inhibited the maximum radial growth of *C. lunata, F. moniliforme,* and *F. oxysporum.* Bashar and Chakma (2014) also reported 82% inhibition of growth of *F. oxysporum,* at 20% concentration owing to non-volatile metabolites of *T. harzianum* (Table 2 and Plate 2b).

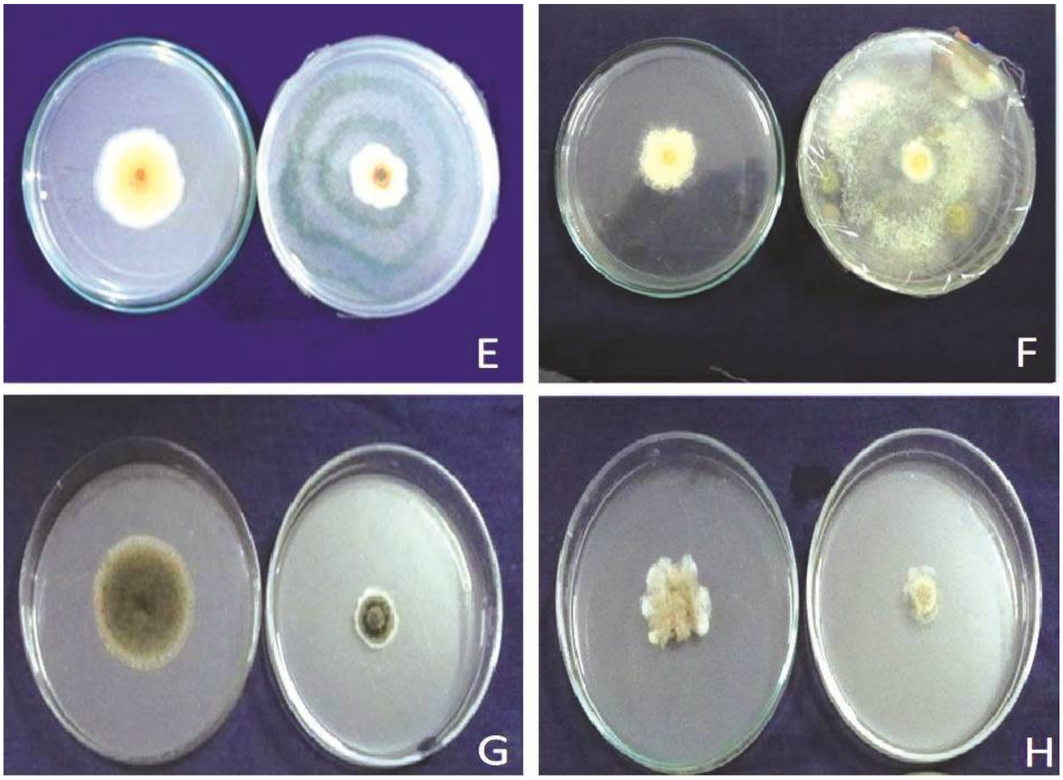

Plate 2. Per cent inhibition owing to (a) volatile substances between E. *Fusarium moniliforme* and *T. viride, F. Pestalotiopsis guepinii* and *T. harzianum* and (b) non-volatile metabolites at 10% cocentrations between G. *Curvularia lunata* and *T. harzianum,* H. *Drechslera oryzae* and *A. niger.*

Amongst the six soil fungi only *Trichoderma harzianum* showed strong antagonistic effect against all the test pathogens of rice. This effect might be due to its first growing nature, rapid sporulation and toxin producing capacity. It is known to be capable of producing antibiotics which might have suppressed the growth of the test pathogens. These findings are in consistent with the findings of Skidmore and Dickinson (1976), Adriana and Sergio (2001), Kexiang *et al.* (2002), Krupke *et al.* (2003), Shafiquzzaman *et al.* (2009), Akter *et al.*(2014), Bashar and Chakma (2014) and Romana *et al.* (2015). Considering the findings of the present experiment, *Trichoderma harzianum* could be used as a commercial bio control agent against rice pathogens.

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