Control of Seed - Borne Fungi of Rice by Aspergillus and Trichoderma

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Abstract

Seed-borne fungi cause enormous losses in rice production. Six antagonistic fungi comprising three species of Trichoderma viz. T. viridae, T. harzianum, T. hamatum, and three species of Aspergillus viz. A. flavus, A. niger, A. terreus, were used against five important rice pathogenic fungi (i.e. Bipolaris oryzae, Curvularia lunata, Fusarium moniliformae, Sarocladiumoryzae and Trichocoins padwickii) for the purpose of biological control. The highest radial growth inhibition exhibited by A. niger and T. harzianum against Sarocladium oryzae (48.07%) and Trichocoins padwickii (65.40%) respectively. A. terreus produced distinct inhibition zone against all five rice pathogens in dual culture. The use of culture filtrates of antagonist successfully reduced growth of rice pathogenic fungi. Among the three concentrations (5%, 10%, and 20%) used, maximum radial growth inhibition was recorded in case of highest concentration (20%) of culture filtrate. Maximum inhibition recorded against Trichoconis padwickii, which was 68.58% and 85.35% by A. niger and T. harzianum respectively. Among the six antagonists, T. harzianum was found to be best in radial growth inhibition and Sarocladium oryzae was the susceptible one than any other tested fungi.

Key words

Seed-borne fungi, Aspergillus, Trichoderma, Biological control, Dual culture, Culture filtrate, radial growth, inhibition zone.

I. INTRODUCTION

Once chemical control of plant disease management was popular in reducing crop loss. But now an environmental awareness discourages use of chemical agents for management of crop diseases. In this context, environment friendly biological control can be alternative component for the controlling of plant diseases. Workers both at home and abroad are engaged in the evaluation of biological antagonists against different pathogens [1], [12]. *Trichoderma* spp. was found to be effective in controlling pathogenicity of *Sclerotium rolfsi* which caused damping off and seedling blight of rice [4]. Scientists tried to evaluate *Trichoderma* spp. and *Aspergillus* spp. against seed-borne fungi of different crop [5], [14]. Bioformulations of *Aspergillus* and *Trichoderma* also successfully control seed-borne pathogens [16], [17]. It can be used as substitute for chemical fungicide for the control of plant pathogenic fungi. Keeping the above mentioned view in consideration, the research was done to control the seed-borne fungi of rice by using *Aspergillus* and *Trichoderma*.

II. MATERIALS AND METHODS

Seed-borne fungi were isolated during assay of mycoflora associated with rice seeds identified following standard procedures [11], [7]. Three *Trichoderma species* viz. *T. viridae, T. harzianum, T. hamatum,* and three *Aspergillus species* viz. *A. flavus, A. niger, A. terreus,* were used against the isolated rice pathogenic fungi for the purpose of biological control. *T. viridae* and *T. harzianum* (TdI & TdII) were collected from Bangabandhu Sheikh Mujibur Rahman Agricultural University. Salna, Gazipur while *T.hamatum* (TdIII) was isolated from Jahangirnagar University, Savar, Dhaka. Three *Aspergillus* spp. [*i.e. A. flavus* (AspI), *A. niger* (AspII), *A. terreus* (AspIII)] were isolated by the author as contaminant from the PDA plate during isolation work of the rice pathogens. The *in vitro* inhibition of radial growth of the isolated rice pathogens were assessed following two methods, a) By dual culture method and b) By the culture filtrates of the antagonist.

A) Dual Culture Method

Percentage of growth inhibition and colony interaction of the rice pathogens and the antagonist were evaluated in dual culture on PDA medium (pH-5.5) at room temperature. Potential antagonist's culture blocks of 5 mm. diameter cut from the margin of 8 days old cultures of both test pathogen and antagonists were placed opposite to each other on PDA in 90 mm. glass Petri-plates. The distance between inoculums blocks were 50 mm. The inoculated plates were incubated at room temperature (28±2°C). The colony growth of the pathogen was measured at both sides i.e. towards and opposing each other from their central loci. The radial growth of

pathogen measured after 5 days of incubation at both sides *i.e*. towards and opposing each other from their central loci. Intermingled or inhibition zone was also measured at the same period. The assessment of interaction was made following the model of Skidmore and Dickinson [19]. The parameters used for the assessment of colony interaction were the width of inhibition zone, intermingled zone and percent inhibition of radial growth, i.e., 100 x $(r_1-r_2)/r_1$, where, r_1 denotes the radial growth of the rice pathogen towards the opposite side and r_2 denotes the radius of the pathogen towards the antagonist to fungus [8].

B) Culture Filtrates Method

To assess the effect of culture filtrate, the antagonist fungi were grown on PDA medium. Three mycelial agar discs, each of 5 mm diameter, of an individual antagonist, were cut from the actively growing margins of 5 day old culture and were inoculated into a 250 ml conical flask containing 100 ml potato dextrose broth medium. After 10 days of incubation at (30±2°C) the culture of an antagonist was filtered first through a filter paper and then centrifuged at 3000 rpm for 20 minutes and finally filter through a micropore filter paper under vacuum pressure to obtain cell free culture filtrate. Culture filtrate of an antagonist containing its non-volatile metabolites was tested in three concentrations (5, 10 and 20 percent) against each rice pathogen. Culture filtrate of a particular concentration was obtained by supplementing it with required amount of sterilized PDA medium. Each Petri-plate was inoculated centrally with a 5mm. mycelial agar disc cut from the margin of actively growing culture of a rice pathogen. All the plates were incubated at room temperature (28±2°C). The radial growth of the colonies was measured after 5 days of incubation. The percent growth inhibition of a rice pathogen was calculated by using the following formula:

$$I = \frac{C-T}{C} \times 100$$

Where,
I, denote percent growth inhibition
C, denotes growth in control
T, denotes growth in treatment.

III. RESULTS AND DISCUSSIONS

The results on the colony interaction between the rice pathogens and the fungal antagonists have been presented in Tables 1 to 5, following the model of Skidmore and Dikinson [19]. The commonest type of colony interaction in most cases was Bi (G3) for all the pathogens tested. *Aspergillus terreus* showed D (G_5) type of colony interaction against all the five test pathogens. Bii (G_2) type of colony interaction was observed for Trichoderma hamatum against all the five pathogens except Sarocladium oryzae.

Radial growth of Sarocladium oryzae was inhibited to a great extent by A. niger (AspII) which was 48.07% and the lowest was 10.59% against Curvularia lunata. The range of inhibition caused by A. flavus (AspI) was maximum 33.41% to minimum 13.33% in Sarocladium oryzae and Trichocoins padwickii respectively. A. terreus (AspIII) showed the highest radial growth inhibition in Sarocladium oryzae (37.12%) and the lowest in Trichocoins padwickii (10.63%). Various degree of inhibitory effect was noticed depending on the test fungi and antagonists used.

T. harzianum (TdII) showed the highest inhibition of radial growth against all the pathogens tested. Inhibition was highest in Trichocoins padwickii (65.40%), followed by Fusarium moniliforme (65.03%), Sarocladium oryzae (56.99%), Bipolaris oryzae (42.48%) and Curvularia lunata (36.35%) by this antagonist. Least inhibitory effect was shown by T. hamatum against all the fungi tested and the range of inhibition was 33.39% (in Trichocoins padwickii) to 7.53% (in Curvularia lunata). The antagonist species competed successfully with the pathogens for space and nutrients. The radial growth of the pathogen was much lesser in the Trichoderma inoculated plates compared to the untreated ones. This finding supports the observation of earlier reporters [10]. Among the six antagonists used T. harzianum (TdII) was found to be best in radial growth inhibition and Sarocladium oryzae was the susceptible one than any other tested fungi.

Distinct inhibition zone was recorded against all five rice pathogens when A. terreus was used. This fungal antagonist showed maximum of 15mm. inhibition zone against Curvularia lunata. The occurrence of inhibition zone between some of the antagonists and the rice pathogens could be considered as a result of the production of antibiotics, changes in pH and competition for nutrients. Mechanical obstruction to the growth and hyphal interference may also attribute to the occurrence of inhibition zone between two fungi on dual culture plates. Mutual growth of two fungi in dual cultures is also possible when both microbes show equal growth rate, equal competition and equal capacity of tolerance to toxins produced by each of them. The overgrowth is achieved when one fungal species exhibits higher growth rate, higher capacity of toxin metabolites production and more tolerance capacity against metabolites produced in comparison to other ones. This explanation was put forwarded by previous workers [20], which probably holds true to explain the results obtained in the present investigation.

Table 1: Colony Interaction Between six Fungal Antagonists and *Bipolaris oryzae* by Dual Culture Method.

Name of the fungal antagonist	Grade *	Type**	Percent inhibition of colony of the pathogen ✡	Intermingled Zone (mm)	Inhibition Zone (mm)
Aspergillus flavus	3	Bi	16.24 e (23.73)	1	-
Aspegillus niger	3	Bi	17.92 de (25.03)	2	-
Aspergillus terreus	5	D	23.14 cd (28.73)	-	3.5
Trichoderma viridae	3	Bi	36.60 b (37.23)	2	-
Trichoderma harzianum	3	Bi	42.48 a (40.69)	2.5	-
Trichoderma hamatum	2	Bii	28.66 c (32.39)	1	-
$\overline{x}$			27.34		
SE			2.39		

*= Grades from 1 (mutually intermingling growth) to 5 (mutual inhibition at a distance), based on Skidmore and Dickinson [19].
**A= Mutually intermingling growth where both fungi grew into one another without any microscopic sign of interaction.
Bi= Intermingling growth where the fungus being observed is growing into the opposed fungus either above or below its colony;
Bii= Intermingling growth where the fungus under observation has ceased growth and is being over grown by another colony;
C= Slight inhibition with a narrow demarcation line (1-2 mm);
D= Mutual inhibition at a distance more than 2 mm.
✡ Mean of three replications.
In a column values with the same letter do not differ significantly at 5% level by DMRT.
Figures in the parentheses are arcsin transformed values.

Table 2: Colony Interaction Between six Fungal Antagonists and *Curvularia lunata* by Dual Culture Method.

Name of the fungal antagonist	Grade*	Type **	Percent inhibition of colony of the Pathogen ✡	Intermingled Zone (mm)	Inhibition Zone (mm)
Aspergillus flavus	5	D	15.71 c (23.34)	-	15
Aspegillus niger	3	Bi	10.59 d (19.00)	1	-
Aspergillus terreus	5	D	34.89 a (36.21)	-	15
Trichoderma viridae	3	Bi	20.51 b (26.92)	1.5	-
Trichoderma harzianum	3	Bi	36.35 a (37.11)	2	-
Trichoderma hamatum	2	Bii	7.53 d (15.89)	1.5	-
$\overline{x}$			20.93		
SE			2.75		

Table 3: Colony Interaction Between six Fungal Antagonists and *Fusarium moniliforme* by Dual Culture Method.

Name of the fungal antagonist	Grade *	Type **	Percent inhibition of colony of the Pathogen✡	Intermingled Zone (mm)	Inhibition Zone (mm)
Aspergillus flavus	3	Bi	20.03 d (26.56)	2.5	-
Aspegillus niger	3	Bi	28.84 c (32.46)	2	-
Aspergillus terreus	5	D	22.33 d (28.18)	-	4
Trichoderma	3	Bi	38.83	1	-

viridae			b (38.53)		
Trichoderma harzianum	3	Bi	65.03 a (53.73)	1	-
Trichoderm a hamatum	2	Bii	28.58 c (32.33)	1	-
$\bar{x}$			33.94		
SE			3.69		

Table 4: Colony Interaction Between six Fungal Antagonists and *Sarocladium oryzae* by Dual Culture Method.

Name of the fungal antagonist	Grade *	Type **	Percent inhibition of colony of the Pathogen ✡	Intermingled Zone (mm)	Inhibition Zone (mm)
Aspergillus flavus	5	D	33.41 cd (35.30)		11
Aspegillus niger	3	Bi	48.07 b (43.91)	2	-
Aspergillus terreus	5	D	37.12 c (37.52)	-	10
Trichoderma viridae	3	Bi	36.40 c (37.11)	1	-
Trichoderma harzianum	3	Bi	56.99 a (49.02)	1.5	-
Trichoder ma hamatum	3	Bi	30.24 d (33.34)	1	-
$\bar{x}$			40.23		
SE			2.34		

Table 5: Colony Interaction Between six Fungal Antagonists and *Trichoconis padwickii* by Dual Culture Method.

Name of the fungal antagonist	Grade*	Type **	Percent inhibition of colony of the Pathogen✡	Intermingled Zone (mm)	Inhibition Zone (mm)
Aspergillus flavus	3	Bi	13.33 d (21.39)	2	-
Aspegillus niger	3	Bi	20.66 c (27.06)	3	-
Aspergillus terreus	5	D	10.63 d (19.00)	-	10
Trichoderma viridae	2	Bi	23.46 c (29.00)	1	-
Trichoderma harzianum	3	Bi	65.40 a (53.97)	3	-
Trichoderm a hamatum	2	Bii	33.39 b (35.30)	1	-
$\bar{x}$			27.81		
SE			4.49		

The effect of culture filtrates of six fungal antagonists (with three concentrations- 5%, 10%, and 20%) against five rice pathogens have been presented in Tables 6 and 7. In general, culture filtrates beyond 5% concentration showed significantly higher level of radial growth inhibition of the test pathogens. Among the three Aspergillus spp. culture filtrates of A. niger (AspII) at 20% concentration showed more inhibitory effect on the pathogens. The same antagonist showed highest effectiveness against Trichocoins padwickii (68.58%) followed by Fusarium moniliforme (67.52%), Curvularia lunata (61.25%), Sarocladium oryzae (45.26%) and Bipolaris oryzae (43.69%) at the same concentration. Least inhibitory effect was observed in case of A. flavus (AspI) against Bipolaris oryzae (40.21%) at 20% concentration of three Aspergillus species. Culture filtrates of T. harzianum (TdII) showed maximum inhibition of radial growth against all the five rice pathogens tested, followed by T. viride (TdI)and T. hamatum (TdIII)at the treatment with 20% concentration of the culture filtrate. The maximum inhibition of radial growth was observed by T. harzianum (TdII) against Bipolaris oryzae (88.75%) followed by Trichocoins padwickii (85.35%), Fusarium moniliforme (79.78%), Curvularia lunata (76.34%) and Sarocladium oryzae (65.59%) at 20% concentration. Least inhibition was noted in case of T. hamatum against Sarocladium oryzae (28.01%) at 20% concentration. No stimulation of the mycelial growth of the test pathogens in respect of treatments with culture filtrates was observed.

The toxin Trichodermacin produced by Trichoderma spp. in the culture filtrates has been reported to be responsible for control of the target pathogens [15]. Another report confirmed that an antifungal butanolide, harzianolide is produced by Trichoderma harzianum [3]. Species of Aspergillus is also well known for producing various kinds of active compounds including antifungal and antibacterial agents [2], [9]. By using GC-mass, IR and NMR spectroscopy, researchers isolated and identified a gamma lactones compound, viz, trans and cis 4 (3 acetoxy - 6- methoxy – 2 –hydroxyl phenyl) – 2 – methoxy butanolide produced by A. niger [16]. The production of the above mentioned chemicals are possibly responsible for the control of rice pathogens treated with culture filtrates of Aspergillus. In the present investigation maximum radial growth inhibition was recorded in case of highest concentration (20%) of culture filtrate. Maximum

inhibition recorded against Trichoconis padwickii was 68.58% and 85.35% by A. niger and T. harzianum (TdII) respectively. The inhibition of radial growth of rice pathogens have been attributed to the production of toxic substances in culture filtrates [6], nutrient impoverishment [18] and alternation of pH of the culture medium resulting from staling growth products [13].

Table 6: Inhibitory Effect of Culture Filtrates of *Aspergillus* spp. on Pathogenic Fungi of Rice.

Treatments	Percent* radial growth inhibition of five major seed-borne fungal pathogen on PDA medium				
	Bipolaris oryzae	*Curvularia lunata*	*Fusarium moniliforme*	*Sarocladium oryzae*	*Trichoconis padwickii*
AspI (5%)	16.28 e (23.81)	32.60 f (34.82)	38.40 ef (38.29)	25.46 f (30.33)	31.50 f (34.14)
AspI (10%)	24.50 d (29.67)	22.28 g (28.18)	40.70 e (39.64)	33.75 d (35.55-)	33.22 f (35.18)
AspI (20%)	40.21 b (39.35)	40.87 d (39.76)	47.02 c (43.28)	42.41 b (40.63)	45.66 c (42.53)
AspII (5%)	28.25 c (32.14)	38.46 e (38.35)	43.59 d (41.32)	35.73 cd (36.69)	43.32 cd (41.15-)
AspII (10%)	43.44 a (41.21)	47.05 c (43.34)	56.49 b (48.73)	37.26 c (37.58)	39.43 de (38.88)
AspII (20%)	43.69 a (41.38)	61.25 a (51.53)	67.52 a (55.24)	45.26 a (42.30)	68.58 a (55.92)
AspIII (5%)	8.08 f (16.54)	34.44 f (35.91)	36.21 f (36.99)	30.34 e (33.40)	31.16 f (33.96)
AspIII (10%)	23.68 d (29.13)	40.45 de (39.52)	47.17 c (43.39)	35.49 cd (36.59)	39.65 e (39.06)
AspIII (20%)	42.17 ab (40.51)	53.16 b (46.83)	49.16 c (44.54)	44.38 ab (41.78)	58.42 b (49.84)

*Mean of three replications.
Note: In a column values with the same letter do not differ significantly at 5% level by DMRT. Figures in the parentheses are arcsin transformed values.
AspI= *A. flavus*, AspII= *A. niger*, AspIII= *A. terreus.*

Table 7: Inhibitory Effect of Culture Filtrates of *Trichoderma* spp. on Pathogenic Fungi of Rice.

Treatments	Percent* radial growth inhibition of five major seed-borne fungal pathogen on PDA medium				
	Bipolaris oryzae	*Curvularia lunata*	*Fusarium moniliforme*	*Sarocladium oryzae*	*Trichoconis padwickii*
TdI (5%)	33.64 e (35.43)	32.60 de (34.82)	42.30 e (40.57)	26.54 de (30.98)	39.55 f (30.98)
TdI (10%)	42.78 d (40.86)	33.51 d (35.37)	48.09 e (43.91)	30.32 c (33.40)	45.69 e (421.53)
TdI (20%)	46.34c (42.88)	37.39 c (37.70)	51.93 c (46.09)	30.52 c (33.52)	54.36 cd (47.52)
TdII (5%)	82.87 b (65.57)	33.72 d 35.49)	76.90 b (61.27)	27.36 de (30.56)	56.45 c (48.73)
TdII (10%)	86.17 a (68.19)	47.45 b (43.57)	79.97 a (63.44)	47.25 b (43.45+)	82.21 b (65.05-)
TdII (20%)	88.75 a (70.27)	76.34 a (60.94)	79.78 a 63.29)	65.59 a (54.09)	85.35 a (67.52)
TdIII (5%)	30.09 f (33.27)	30.67 e (33.65)	32.25 f (34.63)	25.34 e (30.20)	35.43 g (36.51)
TdIII (10%)	40.54 d (39.52)	34.71 d (36.09)	46.94 d (43.22)	27.70 d (31.76)	43.53 e (41.27)
TdIII (20%)	42.57 d (40.74)	46.45 b (42.99)	49.71 cd (44.83)	28.01 d (31.95)	53.49 d (47.01)

*Mean of three replications.
Note: In a column values with the same letter do not differ significantly at 5% level by DMRT. Figures in the parentheses are arcsin transformed values.
TdI= *T. viridae*, TdII= *T. harzianum*, TdIII= *T. hamatum.*

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