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ANTAGONISTIC POTENTIALITY OF SOIL-BORNE FUNGI AGAINST THE MAJOR SEED-BORNE PATHOGENIC FUNGI OF RICE IN BANGLADESH

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Abstract

A total of twenty BRRI rice varieties *i.e.*, BRRI dhan 56 to BRRI dhan 75 were collected from Bangladesh Rice Research Institute (BRRI) to detect the seed borne fungi associated with seeds of selected rice varieties. Four antagonistic potentials of soil fungi comprising three species of *Aspergillus viz.*, *A. flavus* Link, *A. fumigatus* Fresenius, *A. niger* van Tieghem and species of *Trichoderma viride* Pers. were used against six important rice pathogenic fungi (*i.e. Bipolaris oryzae* (Breda de Haan) Shoemaker, *Curvularia lunata* (Wakker) Boedijn, *Fusarium equiseti* (Corda) Saccardo. *Fusarium fujikuroi* Nirenberg, *Microdochium fisheri* Hern.-Restr. and Crous. and *Nigrospora oryzae* (Berk. & Br.) for the purpose of biological control. In dual culture colony interaction out of four antagonistic fungi, *T. viride* showed the highest growth inhibition (87.15%) against *Fusarium fujikuroi.* The maximum inhibition (82.63%) of radial growth of the test pathogens were observed in case of *T. viride* owing to the volatile metabolites. On the other hand, the highest amount for the effect of non-volatile metabolites of *T. viride* on *N. oryzae* and *F. fujikuroi* at 20% concentration. The present investigation suggests that the isolates of *Trichoderma* is a biocontrol agent that can help control seed borne fungi in rice.

Introduction

Rice provides 76% of calorie and 66% of total protein requirement of daily food intake(1). In Bangladesh, the total cultivable land covers about 75%(2). Total rice production in Bangladesh was about 10.59 million tons in the year 1971 when the country's population was only about 70.88 millions. However, the country is now producing about 25.0 million tons of rice to feed her 135 million people. At present the total annual rice production in Bangladesh is about 34.71 million metric tons(3) whereas 497.8 million tons is the world's total production(4). Rice is the most important food for over two billion people in Asia and for hundreds of million in Africa and Latin America. Approximately 90% rice is produced and consumed in Asia(5). Seed-borne fungal diseases of rice are brown spot (*Drechslera oryzae* and *Bipolaris sorokiniana*), bakanae (*Fusarium moniliforme*), blast (*Pyricularia oryzae*), sheath blight (*Rizoctonia solani*), sheath rot (*Sarocladium oryzae*), stem rot (*Sclerotium oryzae),* leaf scald (*Microdochium oryzae*) and grain spot (*Curvularia lunata, Nigrospora orzae*)(6-9). A number

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of seed-borne fungal pathogens such as *Bipolaris, Curvularia, Fusarium, Microdochium, Aspergillus* and *Sarocladium* are frequently identified in rice seeds. Gopalakrishnan *et al.*[10] recorded eight genera of fungi *viz., Alternaria, Aspergillus, Curvularia, Bipolaris, Fusarium, Pestalotiopsis, Sarocladium* and *Trichoderma* associated with rice seed. *Curvularia lunata, Drechslera oryzae, Fusarium moniliforme, Pestalotiopsis guepinii* and *Sarocladium oryzae* were found to be five pathogenic fungi isolated from two rice varieties in Bangladesh[11].

Biological control is an innovative, cost effective, alternative to the use of chemical pesticides and eco-friendly approach. Biological control is slow but can be long lasting, inexpensive and harmless to living organisms and the ecosystem, it neither eliminates the pathogen nor the disease but brings them into natural balance[12]. In Bangladesh, scientists are also now giving their efforts to control seed borne pathogens by using some bio-agents like *Trichoderma* spp. Therefore, the present investigation was undertaken to find out antagonistic potentiality of seed borne pathogenic fungi associated with BRRI dhan 56 to BRRI dhan 75.

Materials and Methods

Laboratory experiment was conducted at Mycology and Plant Pathology, Department of Botany, University of Dhaka, Bangladesh. Seeds of twenty BRRI released rice varieties (BRRI dhan 56 to BRRI dhan 75) were collected from Genetic Resources and Seed Division of Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur. The collected seed samples were kept in brown paper bag and stored immediately in a dry safe place in the laboratory until used for the experiments.

Fungi associated with selected rice samples were isolated with following "Tissue Planting method on PDA medium[13] and Blotter method[14]. Pathogenic potentiality of fungi isolated from selected BRRI rice varieties were grown in the PDA plates and slants for further studies and preservation[15]. Morphological studies of the fungi were drawn in detail with help of camera Lucida. Identities of the isolates were determined following the standard literatures[16-20]. Morphologically identified twenty-five fungi were selected for molecular identification. Among the 25 isolates, some isolates were unable to identify up to species level based on the morphological features only. Therefore, molecular characterization of the fungal isolates was conducted for proper identification using ITS sequence analysis[21].

Four antagonistic potentials of soil fungi *Aspergillus flavus*, *A. fumigatus*, *A. niger* and *T. viride* were selected to test their antagonistic potentials against the pathogenic fungi following dual culture technique. The width of inhibition zone, intermingled zone and per cent inhibition of radial growth were the parameters used for the assessment of the colony interaction[22]. The per cent growth inhibition of each test pathogen was calculated[23] by using the formula given below:

$$1= \frac{r1-r2}{r1} \times 100$$

where, I = Per cent growth inhibition

r_1 = denotes the radial growth of the pathogen towards the opposite side

r_2 = denotes the radial growth of the pathogen towards the antagonist.

Effects of volatile and non-volatile metabolites of the selected soil fungi against the test pathogens were also studied(24). Data were evaluated by analysis of variance (ANOVA) by using STAR statistical program and means were compared using Duncan's Multiple Range Test (DMRT).

Results and Discussion

Four antagonistic fungi were isolated from the rice field soil by serial dilution technique and were identified as *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* van Tieghem and *Trichoderma viride* Pers. ex Gray. The soil fungi were evaluated for their antagonistic potentiality against *Bipolaris oryzae* (Breda de Haan) Shoemaker, *Curvularia lunata* (Wakker) Boedijn, *Fusarium equiseti* (Corda) Saccardo. *Fusarium fujikuroi* Nirenberg, *Microdochium fisheri* Hern.-Restr. & Crous. and *Nigrospora oryzae* (Berk. & Br.) are presented in Table 1. In this study, antagonistic relationships ranged from Grade 3 to 4. However, Grade 3 (type Bi) was found to be the most commonly encountered type of colony interaction. In dual culture colony interaction *T. viride* showed Grade 3 (type Bi) interaction against all the test pathogens. The intermingled zone between the soil fungi and test pathogens was very common. The maximum intermingled zone (0.2 cm) was observed in case of *A. flavus* and *T. viride* against *B. oryzae* and *C. lunata*. *T. viride* grew over the colony of the test pathogens but in case of *A. fumigatus* and *A. niger* inhibition zone was found and it was 0.2 and 0.1, respectively (Table 1 and Fig. 1).

Table 1. Effects of dual culture colony interaction between fungal antagonists and rice pathogens

Name of Fungi		% inhibition of radial growth, intermingled and inhibition zone and type of reactions of the test pathogens			
		A. flavus	*A. fumigatus*	*A. niger*	*Trichoderma viride*
Bipolaris oryzae	% inhibition	60.00	44.44	40.00	61.67
	IMZ (cm)	0.2	-	0.2	0.3
	IHZ (cm)	-	0.2	-	-
	Grade	Bi	C	Bi	Bi
Curvularia lunata	% inhibition	42.86	45.46	37.50	63.63
	IMZ (cm)	0.2	0.2	0.15	0.3
	IHZ (cm)	-	-	-	-
	Grade	Bi	Bi	Bi	Bi
Fusarium equiseti	% inhibition	36.37	30.00	30.76	70.58
	IMZ (cm)	0.1	0.3	-	0.25
	IHZ (cm)	-	-	0.1	-
	Grade	Bi	C	Bi	Bi
Fusarium fujikuroi	% inhibition	46.67	30.76	13.33	87.15
	IMZ (cm)	-	0.3	-	0.25
	IHZ (cm)	0.2	-	0.1	-
	Grade	Bi	Bi	C	Bi
Microdochium fisheri	% inhibition	50.00	52.00	48.25	65.35
	IMZ (cm)	-	0.2	-	0.6
	IHZ (cm)	0.2	-	0.1	-
	Grade	C	Bi	C	Bi
Nigrospora oryzae	% inhibition	40.00	45.00	35.50	60.64
	IMZ (cm)	0.2	0.1	-	0.2
	IHZ (cm)	-	-	-	-
	Grade	Bi	Bi	C	Bi

IMZ= Intermingling zone, IHZ = Inhibition zone, and '-'= not applicable

Grade 3 (Type Bi) = Intermingling growth where the test fungus grew over the test pathogen either above or below or both resulting in suppression of growth of the test pathogen.

Grade 4 (Type C) = Slight inhibition where both the test pathogen and test fungus approached each other until almost in contact, leaving a narrow demarcation line (1-2 mm) based on Skidmore and Dickinson(1976).

Similar observation was also noticed in the present study reported that *Trichoderma viride*, *Aspergillus flavus*, *A. niger* and *A. terreus* were used against five important rice pathogenic fungi for the purpose of biological control[25]. In dual culture technique maximum growth inhibition was recorded for *Trichoderma* spp. against different pathogenic fungi[26].

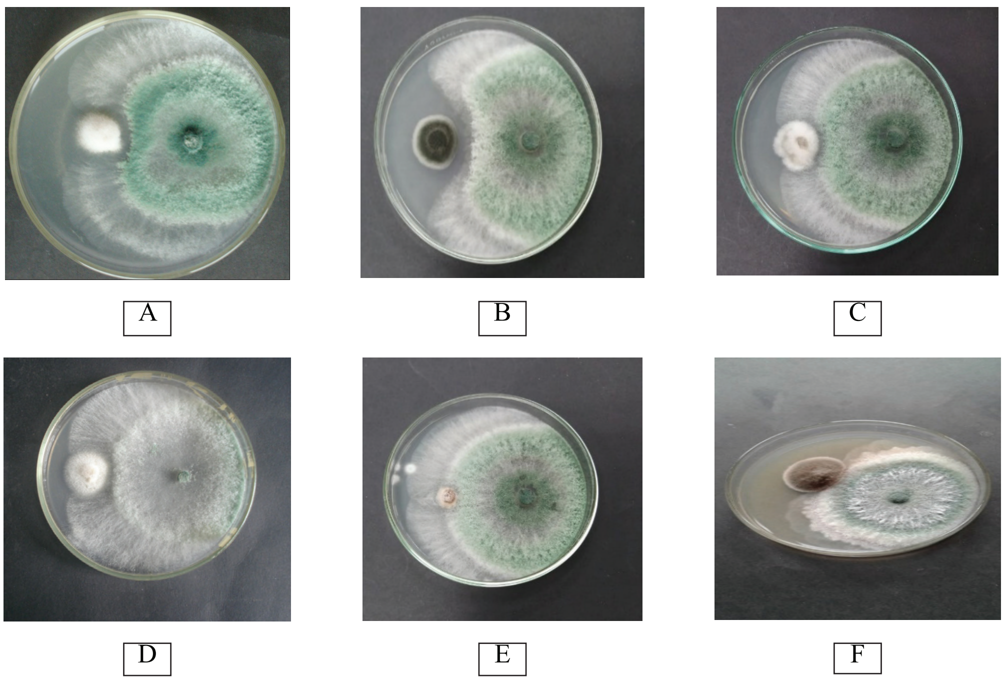

Fig. 1. Photograph showing colony interaction between different test fungi and *Trichoderma viride.*

A. *Bipolaris oryzae* B. *Curvularia lunata* C. *Fusarium equiseti*
D. *Fusarium fujikuroi* E. *Microdochium fisheri* and F. *Nigrospora oryzae*

Table 2 shows the effect of volatile substances emanating from the cultures of the soil fungi on the radial growth of the test pathogens varied from 13.88 to 82.63%. The maximum inhibition (82.63%) was recorded owing to *T. viride* against *N. oryzae* followed by *B. oryzae* (65.35%), *F. equiseti* (63.90%), *M. fisheri* (62.40%), *F. fujikuroi* (57.12%) and *C. lunata* (54.37%) volatile metabolites after 7 days of incubation at 25 ± 2°C.

Table 2. Per cent inhibition of radial growth of test pathogens by volatile metabolites of antagonistic fungi

Antagonists	% inhibition of radial growth of test pathogens					
	Bipolaris oryzae	*Curvularia lunata*	*Fusarium equiseti*	*Fusarium fujikuroi*	*Microdochium fisheri*	*Nigrospora oryzae*
Aspergillus flavus	23.81[d]	33.87[c]	52.32[a]	32.96[c]	30.42[cd]	42.87[b]
A. fumigatus	20.18[d]	13.88[d]	58.17[a]	47.63[b]	46.65[b]	35.60[c]
A. niger	23.72[e]	44.08[c]	62.64[b]	40.75[cd]	36.29[d]	75.00[a]
Trichoderma viride	65.35[b]	54.37[d]	63.90[b]	57. 12[cd]	62.40[bc]	82.63[a]

Volatile metabolites of *A. flavus*, *A. fumigatus*, *A. niger* and *T. viride* showed significant growth inhibition against *Curvularia lunata* and *Fusarium* spp. similar observation was also noticed in the present study(27-28).

Table 3 shows the effects of non-volatile metabolites of antagonistic fungi against test pathogens varied from 65.11 to 93.24%. The maximum inhibition of radial growth was observed with the culture filtrates of *T. viride* against *N. oryzae* (93.24%) followed by *F. fujikuroi* (90.81%), *C. lunata* (88.36%), *F. equiseti* (83.54%), *M. fisheri* (68.40%) and *B. oryzae* (65.11%) at 20% concentration respectively (Table 3).

Table 3. Per cent inhibition of radial growth of test pathogens owing to non-volatile metabolites of antagonistic fungi

Test pathogen	Concentation (%)	% inhibition of radial growth of test pathogens			
		A. flavus	*A. fumigatus*	*A. niger*	*T. viride*
Bipolaris oryzae	5	24.35[c]	50.64[b]	54.43[a]	17.47[d]
	10	32.30[d]	54.32[b]	60.22[a]	36.02[c]
	15	42.48[d]	72.30[b]	78.34[a]	60.22[c]
	20	55.35[d]	77.78[b]	86.55[a]	65.11[c]
Curvularia lunata	5	54.05[c]	42.24[b]	24.42[c]	22.50[c]
	10	62.16[a]	55.47[b]	32.26[d]	42.61[c]
	15	64.01[b]	62.86[b]	47.10[c]	78.38[a]
	20	78.30[b]	72.32[c]	58.02[d]	88.36[a]
Fusarium equiseti	5	15.27[c]	21.31[b]	50.05[a]	22.83[b]
	10	31.78[c]	32.50[c]	64.37[a]	42.07[b]
	15	36.65[c]	36.24[c]	72.09[a]	54.55[b]
	20	42.75[b]	71.14[a]	86.80[a]	83.54[a]

Table 3. Contd.

Test pathogen	Concentation (%)	% inhibition of radial growth of test pathogens			
		A. flavus	*A. fumigatus*	*A. niger*	*T. viride*
Fusarium fujikouri	5	54.50[b]	30.82[c]	31.72[c]	62.97[a]
	10	62.11[ab]	57.05[b]	31.88[c]	75.03[a]
	15	64.04[b]	63.05[b]	64.35[b]	82.73[a]
	20	73.87[b]	64.83[c]	72.93[b]	90.81[a]
Microdochium fish-eri	5	23.95[b]	14.62[c]	23.54[b]	44.57[a]
	10	32.50[b]	4.10[c]	36.09[b]	55.98[a]
	15	36.94[d]	42.70[c]	55.26[b]	65.43[a]
	20	44.59[d]	55.33[c]	62.27[b]	68.40[a]
Nigrospora oryzae	5	63.93[ab]	48.04[c]	60.36[b]	66.64[a]
	10	73.12[a]	58.81[b]	70.75[a]	75.13[a]
	15	82.82[a]	66.96[b]	72.15[b]	84.62[a]
	20	94.10[a]	80.49[b]	82.81[b]	93.24[a]

These results are in agreement with the findings of Bashar and Chakma(29) and Al-ameen *et al.*(30) reported that culture filtrates of *T. viride* and *A. niger* were responsible for maximum inhibition of *Fusarium* spp. at 20% concentration. The present investigation suggests that the isolates of *Trichoderma* may be further exploited as potential biocontrol agents against the fungal pathogens of rice in field trial.

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References

1. Bhuiyan NI, DNR Paul and MA Jabber 2002. Feeding the extra millions by 2025- challenges for rice research and extension in Bangladesh. A key note paper presented in the National Workshop on Rice Research and Extension-2002 held at BRRI, Gazipur, Bangladesh.
2. Ahmed M, M Hossain, K Hassan and CK Dash 2013. Efficacy of different plant extract on reducing seed borne infection and increasing germination of collected rice seed sample. Universal Journal of Plant Science **1**(3):66-73.
3. BBS 2016. Statistical Pocket Book of Bangladesh. Bangladesh Bureau of Statistics, Statistics Division, Ministry of Planning, Government of People's Republic of Bangladesh. Pp. 207.
4. FAO 2016. Food and Agriculture Organization of the United Nations. www. fao.org/ world food situation/csdb/en.

5. Selim M, M Akram, Akhtar MH and M Ashraf 2003. Rice Production Handbook. Pak. Agric. Res. Council, Islamabad.pp.70.

6. Bhutta AR and SA Hussain 1998. Seed borne fungi associated with rice seed lots in Pakistan. Int. Rice Res. Notes **23**(3):26-27.

7. Khan SA, SA Anwar and AB Bhutta 1990. Studies on seed borne fungi, bacteria and nematodes of rice in the Punjab. Pak. J. Sci. Ind. Res. **33**(11):489-492.

8. Gill MA, A Wahid, Javed MS and TZ Khan 1999. Major diseases of rice crop in the Punjab and their management strategies. *In*: Proc. 2nd Nat. Conf. Plant Pathol., Sept. 27-29, 1999, Univ. Agri. Faisalabad.

9. Haque AHMM, H Akon, MA Islam, KM Khalequzzaman and MA Ali 2007: Study of seed health, germination and seedling vigor of farmers produced rice seeds. International Journal of Sustainable Crop Production **2**(5):34-39.

10. Gopalakrishnan CA, Kamalakannan and V Valluvaparidasan 2010. Effect of seed-borne *Sarocladium oryzae*, the incitant of rice sheath rot on rice seed quality. J. Plant Prot. Res. **50**(1):98-110.

11. Chowdhury P and S Shamsi 2016. *In vitro* evaluation of fungicides and some plant extracts against rice sheath rot pathogen *Sarocladium oryzae.* Bangladesh J. Sci. Res. **29**(1):47-54.

12. Ramanathan A, Shanmugam V, Raghuchander T and Samiyappan R 2002. Induction of systemic resistance in ragi against blast disease by pseudomonas fluorescence. Ann. P.C. prot. Soc.**10**:313-318.

13. CAB (Commonwealth Agricultural Bureau) 1968. Plant Pathologist's Pocket Book. 1st edn. The Commonwealth Mycological Institute, England. pp. 267.

14. ISTA 1996. International rules for seed testing rules. Seed Science and Technology **4**(1):3-177.

15. Sultana T, MA Bashar and S. Shamsi 2020. Pathogenic potentiality of fungi isolated from seeds of twenty BRRI released rice varieties (*Oryza sativa* L.) *BRC*. **6**(1):810-813.

16. Barnett HL and BB Hunter 1972. Illustrated Genera of Imperfect Fungi. Burgess Pub. Co. U.S.A. pp. 241.

17. Benoit MA and SB Mathur 1970. Identification of species *Curvularia* on rice seed. Proc. Inst. Seed Test. Ass. **35**(1):1-23.

18. Booth C 1971. The Genus *Fusarium.* The Commonwealth Mycological Institute, England. pp. 267.

19. Ellis MB 1976. *More Dematiaceous Hyphomycetes*. The Commonwealth Mycological Institute, England. pp. 507.

20. Sutton BC 1980. *The Coelomycetes*. Fungi Imperfect with Pycnidia, Acervuli and Stroma. The Common Wealth Mycological Institute, England. pp. 696.

21. Amer OE, MA Mahmoud, AMA El-Samawaty and SRM SRM 2011. Non liquid nitrogen-based-method for isolation of DNA from filamentous fungi. Afr. J. Biotechnol. **10**(65):14337-14341.

22. Skidmore A.M. and Dickinson C.H. 1976. Colony interaction and hyphal interference between Septoria nodorum and phylloplane fungi. Trans. Brit. Mycol. Soc. **66**:57-64.

23. Fokkema NJ 1976. Antagonism between fungal saprophytes and pathogens on aerial plant surfaces. *In*: Microbiology of Aerial Plant Surfaces, Dickinson CH and PreeceTF(eds.) Academic Press, London, pp. 486-506.

24. Bashar MA and Rai B 1994. Antagonistic potential of root-region mycoflora of chickpea against *Fusarium oxysporum* f. sp. *ciceri*. Bangladesh J. Bot. **23**(1):13-19.

25. Khair A and CD Subash 2018. Control of seed borne fungi by *Aspergillus* and *Trichoderma.* SSRG International Journal of Agriculture & Environment Science (SSRG-IJAES) **5**(I):34-39.

26. Bhale UN, PM Wagh and JN Rajkonda 2013. Antagonistic confrontations of *Trichoderma* spp. against fruit rot pathogens on Sapodilla (*Manilkara zapota* L.). Journal of Yeast and Fungal Research **4**(1):5-11.

27. Aktar MT, KS Hossain and MA Bashar 2014. Antagonistic potential of rhizosphere fungi against leaf spot and fruit rot pathogens of brinjal. Bangladesh J. Bot. **43**(2):213-217.

28. Helal RB and S Shamsi 2019. Antagonistic potentials of some soil fungi against three postharvest pathogenic fungi of Carica papaya L. Dhaka Univ. J. Biol. Sci. **28**(1):1-7.

29. Bashar MA and M Chakma 2014. *In vitro* control of *Fusarium solani* and *F. oxysporum* the causative agent of brinjal wilt. *Dhaka Univ. J. Biol. Sci*. **23**(1):53-60.

30. AL-Ameen MD, S Hosen, S Shamsi and MA Bashar 2017. Antagonistic potential of soil fungi against post-harvest pathogenic fungi of *Musa sapientum* L. Bangladesh J. Bot. **46**(2):733-738.

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