JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2012. Vol. **6**(3), p. 1427-1431

Biological Control of Blast Disease (*Magnaporthe grisea*) of Rice by some Phyllosphere Fungi *In vitro*

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(Received: 06 Decenber 2011; accepted: 12 January 2012)

Rice blast caused by *Magnaporthe grisea* is one of the major diseases affecting the rice production. Application of beneficial antagonistic fungi to protect the disease may be an alternative strategies to chemical control. In present the study, fungi isolated from the phyllosphere of the rice leaves of South Assam were used to screen their antagonistic ability against *M. grisea* under *in vitro* condition. Among, 11 isolates, *Aspergillus niger* gave significantly higher inhibition of mycelial growth of *M. grisea*.

Key words: Blast of rice, *M. grisea,* Antagonistic fungi.

Rice (*Oryza sativa*) plays a major role in the nutrition of the people around the world and after wheat is the most important agricultural product (Yamaguchi *et al*., 2008). There are various factors that reduce rice production, the most important of which are pests, diseases and weeds (Yamaguchi *et al*., 2008). Blast caused by *M. grisea is one of* the major diseases affecting rice cultivation. Rice blast is a widespread and damaging disease of cultivated rice caused by the fungus *M. grisea* (Rossman *et al*.,1990). It is the most destructive pathogen of rice worldwide; around 50% of production may be lost in a field moderately affected by infection. Each year the fungus destroys rice enough to feed an estimated 60 million people (Zeigler *et al*.,1994). Though, these diseases are being managed through fungicides, their adverse effects on environment and beneficial micro-organisms are quite evident. These have caused many scientists to conduct research into the integrated control of fungal diseases, including biological controls using antagonistic microorganisms. Different antagonistic fungi inhibit several plant pathogenic fungi. But, their activity varied among the different fungi (Deweger *et al.*, 1986). In view of this, we have evaluated several fungi isolated from the phyllosphere of rice for their antagonistic ability against *M. grisea* causing Blast disease in rice cultivated in Assam, India.

MATERIALS AND METHODS

Dual plate culture technique (Rabindran and Vidyasekaran, 1996) was followed for examining the microbial interactions. Antagonistic fungi were inoculated at one side of petridishes (1 cm away from the edge) containing Oatmeal agar medium. 5 mm discs of *M. grisea* were plug from 7 days old OMA cultures with cork borer and placed at the opposite side of petridishes 5cm away from the antagonistic fungi towards the edge of petriplates facing towards and opposing each other. The plates were then incubated for 7 days at

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24±2^{O}C. *M. grisea* inoculated alone in triplicates without other fungi were served as control. There were three replications for each isolate against the pathogen and also for the control. The colony grows on both sides i.e. towards and opposing each other from loci was measured. Observations on mycelial growth of test pathogens were recorded and percent inhibition of pathogen growth was calculated. The parameters used for the assessment of colony interaction were width of inhibition or intermingled zone between both colonies. The percent inhibition of pathogen growth was calculated by using Fokkema (1973) formulae:

% Inhibition= 100x $r_1 - r_2/r_1$

r_1= radial growth of *M. grisea* in control.

r2== radial growth of *M. grisea* in dual inoculation.

RESULTS AND DISCUSSION

The pathogen *M. grisea* was kindly supplied by MTCC (1477) IMTECH Chandigarh. Antagonists fungal *spp*. were isolated from the phyllosphere of the rice leaves. Isolation were done in specific for fungi (PDA, OMA and Czapek Dox Agar Medium) by leaf washing technique (Dickinson, 1971). All the cultures were maintained at 27^{0}C and the fungal isolates were identified by morphological and microscopical characteristics according to Subramaniam (1971), Ellis (1971,1976) and Raper and Fennell, (1965) maintained in the laboratory of Microbiology, Department of Life science and Bioinformatics, Assam University. The antagonistic effect of these fungal isolates was evaluated against *M. grisea*.

M. grisea

Conidia of *M. grisea*

Test pathogen

M. grisea was confirmed as fungal pathogens that caused a blast disease on rice. The information in Table 1 illustrates the growth of *M. grisea* on three different media – Potato dextrose agar, Czapeck dox agar and Oatmeal agar.

The result have shown better growth of *M. grisea* on Oatmeal agar compared to Czapeck dox agar and Potato dextrose agar.

M. grisea on OMA

M. grisea on CDA

M. grisea on PDA

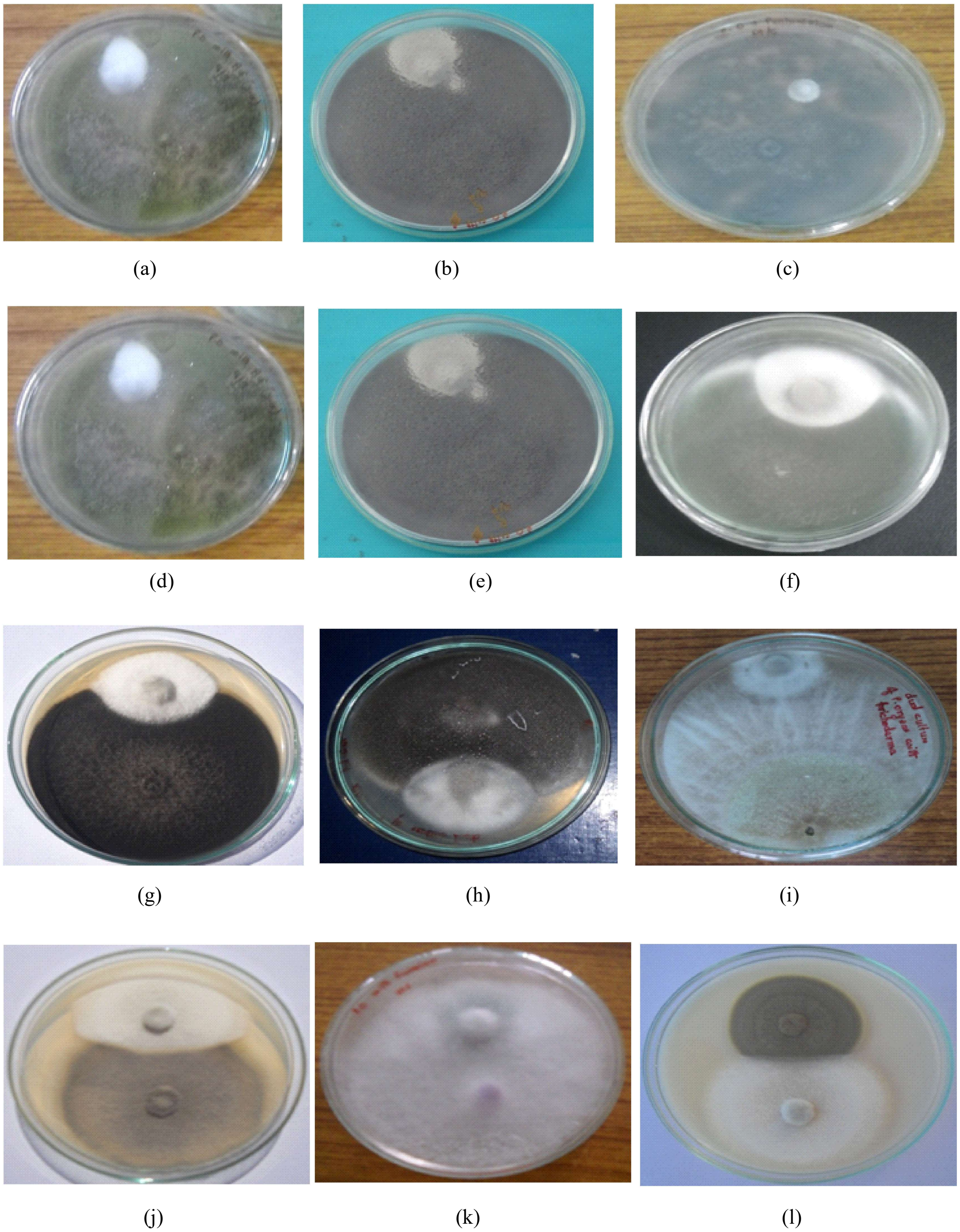

Fig. 1. Photograph of dual culture of *M. grisea* with various antagonists
a) Control; b) *Aspergillus niger;* c) *Penicillium* spp.; d) *A. flavus;* e) *Curvularia oryzae;* f) *A. fumigatus;* g) *C. lunata;* h) *Drechlera* spp.; i) *Trichoderma viride;* j) *Alternaria alternata;* k) *Fusarium* spp.; l) *Cladosporium* spp.

Table 1. Selection of Medium for cultures.

Organisms		Czapeck (cm)	PDA (cm)	Oatmeal agar(cm)
M. grisea	Replicate1	3.4	4.7	5.9
	Replicate 2	3.85	4.45	5.85
	Replicate 3	3.9	4.5	6.05
Mean value±S.E		3.72 ± 0.129	4.55 ± 0.062	5.93 ± 0.049

Table 2: *In vitro* antagonism of fungal *spp*. against *M. grisea.*

S. No.	Test Mycoflora	Control (mm) (r_1)	Interaction (mm) (r_2)	%Growth inhibition of *M. grisea*	Growth of antagonistic (mm)
1.	*Aspergillus niger*	32±0.595	3±0.59	90.62	47
2.	*Penicillium spp.*	32±0.595	4.3±0.34	87.5	45.7
3.	*A. flavus*	32±0.595	5.0±0.00	84.38	45
4.	*Curvularia oryzae*	32±0.595	6±0.59	81.25	44
5.	*A. fumigatus*	32±0.595	6.3±0.68	80.31	43.7
6.	*C. lunata*	32±0.595	7.7±1.23	75.94	42.3
7.	*Fusarium spp.*	32±0.595	8.0±0.59	75	42
8.	*Drechlera spp.*	32±0.595	9±0.59	71.88	41
9.	*Trichoderma viride*	32±0.595	15±0.59	53.12	35
10.	*Alternaria alternata*	32±0.595	15±0.59	53.12	35
11.	*Cladosporium spp.*	32±0.595	21±0.59	34.37	29

***In vitro* comparative studies on antagonistic activity of some fungal isolates against *M. grisea* by dual plate culture technique**

In dual culture, the growth of *M. grisea* was variably inhibited by different phyllosphere fungi. Among them *A. niger* showed maximum inhibitory ability against *M. grisea* and this fungi (90.62%) showed the most effective antagonism as compared to others (Table. 1). *A. flavus, A. fumigatus* and *Penicillium sps.* were also able to inhibit the fungal pathogen as compared with other remaining fungi but little less ability in comparing to *A. niger*. According to the experimental results, *A. niger* and *Penicillium spp*. have the most antagonistic efficiency against *M. grisea* .

DISCUSSION

The study has suggested that, few saprophytic phyllosphere fungi may act as biocontrol agents that were evaluated for their efficacy against *M. grisea* . Results have shown that *A. niger* showed the best antagonistic activity. This biocontrol agent demonstrates a powerful antagonistic behaviour in the control of rice diseases blast. It can therefore be concluded that *A. niger* is an effective biological control agent Moreover, *A. niger* is characteristically fast growing, easy to culture, could be manipulated genetically in the laboratory. So, using this fungi as biocontrol agent against blast disease of rice could be made easily available for the management of the disease i,e technology for the fungus can be developed as new biocontrol agents for blast disease of rice plant. The results obtained here pointed out the possible use of fungal *spp.* commercially in rice fields for blast disease suppression. However, further research is needed to elucidate in details the mechanism of action of these fungi.

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