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SEED-BORNE MYCOFLORA OF RICE VARIETIES SINDH BASMATI, IRRI-9 AND DR-83 WITH FIRST REPORT OF SEED TRANSMISSION OF *MACROPHOMINA PHASEOLINA* IN RICE FROM SINDH, PAKISTAN

Muhammad Mujeeb Akram and Saleem Shahzad*

Department of Agriculture and Agribusiness Management, University of Karachi, Karachi-75270, Pakistan
**Corresponding Author's email: sshahzad@uok.edu.pk*

ABSTRACT

Rice (*Oryza sativa* L.) is the third largest crop of Pakistan and the sixth biggest exporter of rice. There are several factors which are responsible for low yield but major cause is the infectious diseases especially these caused by seed-borne pathogens which also cause losses at different stages including storage. In the current study seeds of rice cultivars *viz*., Sindh Basmati, IRRI-9 and DR-83 were collected from various rice mills godowns at Karachi during 2012 and 2013. Five samples of each variety were tested for seed-borne mycoflora using Standard Blotter and Agar plate methods with and without deep-freezing. The highest numbers i.e. 29 species of fungi were isolated from IRRI-9 as compared to 19 from Basmati and 18 from DR-83. *Macrophomina phaseolina* was isolated seeds of all the tested varieties that appeared to be the first report of the fungus from seeds of these varieties in Sindh, Pakistan.

Keywords: Rice, seed-borne mycoflora, *Macrophomina phaseolina*, Sindh, Pakistan

INTRODUCTION

Rice (*Oryza sativa* L.), a member of the family Poaceae is native to tropical and subtropical southern Asia. It is the third largest crop in Pakistan. Globally, Pakistan is the fourteenth significant rice producing nation and the sixth biggest exporter of rice that contributes 6% on the planet's rice send out. Rice is developed on around ten percent of the aggregate arable land in Pakistan that amounts up to around 2.963 million hectares. In 2008–2009, the rice creation was 6.952 million tons that contributed 5.5% of the aggregate farming production in that duration and made a absent 1.6% offer of the nation's GDP (Zafar, 2009).

In Pakistan the Rice crop production in 2017-18 was about 7442 million tonnes which was low as compared to other Asian countries (Anon, 2018). There are several factors which are responsible for low yield but major cause is the infectious diseases specially those caused by seed-borne pathogens such as *Drechslera oryzae, Fusarium moniliforme, F. solani, Pyricularia oryzae, Rhizoctonia solani, Sarocladium oryzae, Sclerotium oryzae, Trichoconiella padwickii* and *Xanthomonas campestris* pv. *oryzae* (Wahid *et al*., 2001; Gill *et al*., 1999). These pathogens cause damage to the rice crop during seed germination, seedling establishment, growth and reproductive phases, storage and transit. The infected seeds either could not germinate or produced diseased seedling and also the infected plants (Fakir *et al*., 2002). The present report describes the seed-borne mycoflora of three varieties of rice i.e. IRRI-9, DR-83 and Sindh Basmati that are cultivated in Sindh. Of these, Sindh Basmati and DR-83 were also evaluated for the seed-borne mycoflora by Khan *et al*. (1988) whereas IRRI-9 is evaluated for the first time.

MATERIALS AND METHODS

Seeds of rice cultivars, Basmati, IRRI-9 and DR-83 were collected from various rice mills godowns at Karachi during 2012 and 2013. The rice mills obtained these varieties from farmers in Thatta, Badin, Shikarpur, Larkana and Jacobabad districts of the Province of Sindh, Pakistan. Five samples of each variety from different locations were tested for seed-borne mycoflora. Isolations were carried out at the Pest and Disease Research Lab (PDRL), Department of Agriculture & Agribusiness Management, University of Karachi using Standard Blotter (Mathur and Neergaard, 1970), Agar plate (Muskett and Malone, 1941) and Deep-freezing (DF) (Limonard, 1968) methods as suggested by ISTA (2003). In each method, the isolations were made from 200 non-surface sterilized (untreated) as well as surface-sterilized (treated) seeds. The seeds were soaked in 1% sodium hypochlorite solution for 5 minutes for surface-sterilization.

In non-deep-freezing method (NDF), the treated and untreated seeds were transferred to Petri-dishes containing either Potato Dextrose Agar (PDA) or 3 layers of moistened blotter paper. The plates were incubated at 25±1°C under 12 hours alternating cycles of darkness and artificial day light (ADL) supplied by cool white fluorescent tubes for 7-8 days.

In deep-freezing method, treated and untreated seeds were placed in Petri-dishes containing either potato dextrose agar (PDA) or 3 layers of moistened blotter paper and incubated at 25°C for 24 hours followed by incubation at -20°C in a deep-freezer for 24 h. The dishes were then incubated at 25 ± 1°C under 12 h ADL and 12 hours darkens for 7 days. Fungi growing out from the seeds were examined under stereo microscope and identified up to generic level. Identification up to species level was made using a compound microscope after Reference to Ellis (1971, 1976), Domsch *et al*. (1980), Samson *et al*. (1984) and Nelson *et al*. (1983). The incidence percentage for each fungus in every method was calculated using the following formula:

Incidence (%) = (No. of seeds infected by a fungus / Total number of seed assessed) X 100

Mean values were calculated to obtain average percentages of infected grains in each sample. For the average values of all the samples of a variety, the standard errors were also calculated using the following formula (Chandel *et al.*, 2002):

Standard error = standard deviation / square-root of the number of samples

RESULTS

1. Variety DR-83: Fifteen species were isolated using blotter method (Table 1). Untreated NDF seeds yielded the highest numbers of fungi (10); treated NDF seeds gave 9 species. However, the frequency of isolation of fungi in treated seeds was less as compared to their frequency in untreated seeds. Only 4 species were isolated from treated and untreated DF seeds *Fusarium oxysporum* was isolated from the DF seeds but not from NDF seeds. *Fusarium oxysporum* was isolated in high frequency from treated DF seeds whereas *Fusarium moniliforme* and *Drechslera oryzae* were isolated only from treated DF seeds. *Macrophomina phaseolina,* the charcoal rot fungus was isolated treated and untreated NDF seeds but not from DF seeds.

Eighteen species of fungi were isolated from DR-83 seeds using agar plate method where all the fungi showed their presence in all the samples with variable frequencies (Table 2). Untreated NDF seeds placed on PDA gave 13 species of fungi whereas treated NFD seeds gave 12 species. Similarly, untreated DF seeds showed the presence of 13 species but treated DF seeds yielded only three species of fungi *viz*., *Alternaria alternata, Curvularia lunata* and *Fusarium oxysporum.* The frequency of isolation of *F. oxysporum* was significantly higher in treated DF seeds as compared to other treatments. *Macrophomina phaseolina* was isolated from treated NDF and untreated DF seeds whereas *Trichoderma harzianum* was isolated from untreated NDF seeds only.

Table 1. Frequency of fungi in rice variety DR-83 isolated using blotter plate method.

S. no.	Fungus	No Deep-Freezing				Deep-Freezing			
		Treated		Untreated		Treated		Untreated	
		Infected samples (%)	Infected seeds (%)	Infected samples (%)	Infected seeds (%)	Infected samples (%)	Infected seeds (%)	Infected samples (%)	Infected seeds (%)
1	*Alternaria alternata*	100	5 ± 0.2	100	3.5 ± 0.7	-	-	-	-
2	*Aspergillus sulphureus*	100	1 ± 2	100	23.5 ± 5.2	-	-	-	-
3	*Aspergillus clavatus*	80	0.5 ± 0.1	100	4 ± 0.8	-	-	-	-
4	*Aspergillus flavus*	-	-	-	-	-	-	100	5.5 ± 0.5
5	*Aspergillus fumigatus*	100	5 ± 0.3	-	-	-	-	100	27.5 ± 1.3
6	*Aspergillus niger*	100	4 ± 0.5	100	1 ± 0.2	-	-	-	-
7	*Aspergillus raphani*	100	1.5 ± 0.1	80	1 ± 0.2	-	-	-	-
8	*Aspergillus terreus*	100	1 ± 0.2	100	1 ± 0.2	-	-	100	3.5 ± 0.4
9	*Drechslera oryzae*	-	-	-	-	100	21.5 ± 2.5	-	-
10	*Fusarium moniliforme*	-	-	-	-	100	2.5 ± 0.1	-	-
11	*Fusarium oxysporum*	-	-	-	-	100	28.5 ± 1.2	100	3.5 ± 1.2
12	*Macrophomina phaseolina*	100	1.5 ± 0.1	100	3 ± 0.6	-	-	-	-
13	*Mucor* sp.	100	4 ± 0.5	100	4.5 ± 1.0	100	2.5 ± 0.4	-	-
14	*Rhizopus stolonifer*	-	-	100	7 ± 1.5	-	-		-
15	*Trichoderma harzianum*	-	-	20	0.5 ± 0.1	-	-		-

Table 2. Frequency of fungi in rice variety DR-83 isolated using PDA plate method.

S. no.	Fungus	No Deep-Freezing				Deep-Freezing			
		Treated		Untreated		Treated		Untreated	
		Infected samples (%)	Infected seeds (%)	Infected samples (%)	Infected seeds (%)	Infected samples (%)	Infected seeds (%)	Infected samples (%)	Infected seeds (%)
1	*Alternaria alternata*	100	5 ± 2.3	100	4.5 ± 1.2	100	8 ± 1.4	100	1 ± 0.2
2	*Aspergillus sulphureus*	100	6.5 ± 2.9	100	2.5 ± 0.8	-	-	100	12 ± 4.3
3	*Aspergillus clavatus*	100	3.5 ± 1.5	100	2 ± 0	-	-	-	-
4	*Aspergillus flavus*	100	1.5 ± 0.6	100	8.5 ± 1.2	-	-	100	3.5 ± 0.3
5	*Aspergillus fumigatus*	100	4.5 ± 2.0	-	-	-	-	100	0.5 ± 0.1
6	*Aspergillus nidulans*	-	-	-	-	-	-	100	8 ± 1.6
7	*Aspergillus niger*	-	-	100	1 ± 0.1	-	-	100	0.2 ± 0.1
8	*Aspergillus raphani*	-	-	80	0.5 ± 0.1	-	-	100	2 ± 0.3
9	*Aspergillus terreus*	100	3.5 ± 1.5	100	4 ± 0.6	-	-	100	1 ± 0.2
10	*Curvularia lunata*	100	5.5 ± 2.2	100	2 ± 0.3	100	4 ± 0.6	100	8.5 ± 2.0
11	*Drechslera oryzae*	100	3 ± 1.3	100	2.5 ± 0.2	-	-	-	-
12	*Fusarium moniliforme*	-	-	100	4 ± 0.8	-	-	-	-
13	*Fusarium oxysporum*	100	1 ± 0.4	100	2 ± 0.5	100	30.5 ± 1.6	100	22 ± 1.6
14	*Macrophomina phaseolina*	100	1.6 ± 0.7	-	-	-	-	100	3.5± 0.6
15	*Mucor* sp.	-	-	100	5 ± 0.1	-	-	-	-
16	*Penicillium oxalicum*	100	1.5 ± 0.6	-	-	-	-	100	2 ± 0.3
17	*Rhizopus stolonifer*	100	3 ± 1.3	-	-	-	-	100	2.5 ± 0.35
18	*Trichoderma harzianum*	-	-	100	2 ± 0.4	-	-	-	-

Table 3. Frequency of fungi in rice variety Basmati isolated using blotter plate method.

S. no.	Fungus	No Deep-Freezing				Deep-Freezing			
		Treated		Untreated		Treated		Untreated	
		Infected samples (%)	Infected seeds (%)	Infected samples (%)	Infected seeds (%)	Infected samples (%)	Infected seeds (%)	Infected samples (%)	Infected seeds (%)
1	*Alternaria alternata*	100	1.2 ± 0.5	100	1 ± 0.4	-	-	100	1 ± 0.1
2	*Aspergillus clavatus*	100	1.5 ± 0.6	100	11.5 ± 5.1	-	-	100	4.5 ± 0.9
3	*Aspergillus flavus*	-	-	100	26 ± 11.6	-	-	100	12.5 ± 2.5
4	*Aspergillus fumigatus*	-	-	100	3.6 ± 1.6	-	-	100	6 ± 1.5
5	*Aspergillus niger*	100	1 ± 0.4	100	4.5 ± 2.0	-	-		-
6	*Aspergillus rhizopodus*	-	-	100	2 ± 0.8	-	-	100	1.5 ± 0.2
7	*Aspergillus terreus*	100	15.5 ± 6.9	100	6 ± 2.6	100	14.5 ± 2.7	100	6.5 ± 0.7
8	*Curvularia clavata*	20	0.2 ± 0.1	60	0.5 ± 0.2	-	-	-	-
9	*Curvularia lunata*	-	-	100	1.5 ± 0.6	-	-	-	-
10	*Drechslera* sp.	100	0.5 ± 0.2	100	3 ± 1.1	100	6.5 ± 0.7	100	4 ± 0.4
11	*Fusarium oxysporum*	20	0.5 ± 0.2	80	2.5 ± 1.1	100	1 ± 0.2	100	1.5 ± 0.1
12	*Fusarium semitectum*	-	-	-	-	100	21 ± 1.6	100	21 ± 2.03
13	*Macrophomina phaseolina*	100	1 ± 0.4	100	2.5 ± 1.1	-	-	-	-
14	*Mucor* sp.	-	-	-	-	-	-	100	2.5 ± 0.4
15	*Nigrospora orzyae*	-	-	100	1 ± 0.4	-	-	-	-

Table 4. Frequency of fungi in rice variety Basmati isolated using PDA plate method.

S. no.	Fungus	No Deep-Freezing				Deep-Freezing			
		Treated		Untreated		Treated		Untreated	
		Infected samples (%)	Infected seeds (%)	Infected samples (%)	Infected seeds (%)	Infected samples (%)	Infected seeds (%)	Infected samples (%)	Infected seeds (%)
1	*Alternaria alternata*	-	-	60	0.5 ± 0.2	40	21 ± 1.6	40	0.22 ± 0.1
2	*Aspergillus clavatus*	100	5.5 ± 2.4	100	5.5 ± 2.4	-	-	100	1 ± 0.2
3	*Aspergillus flavus*	-	-	100	7 ± 3.1	-	-	100	11.5 ± 1.6
4	*Aspergillus fumigatus*	-	-	100	4 ± 1.7	-	-	100	3 ± 0.5
5	*Aspergillus nidulans*	-	-	100	1.5 ± 0.6	-	-	-	-
6	*Aspergillus niger*	100	17 ± 7.6	100	11.5 ± 5.1	-	-	100	11.5 ± 1.5
7	*Aspergillus rhizopodus*	-	-	100	12 ± 5.3	-	-	100	9 ± 0.6
8	*Aspergillus terreus*	100	4.5 ± 2.0	100	1.5 ± 0.6	100	1 ± 0.1	100	2 ± 0.3
9	*Curvularia clavata*	100	1 ± 0.4	80	1 ± 0.4	100	6 ± 0.4	100	1.5 ± 0.4
10	*Curvularia lunata*	-	-	60	0.5 ± 0.2	-	-	-	-
11	*Drechslera* sp.	-	-	60	0.5 ± 0.2	100	9 ± 2.0	-	-
12	*Fusarium moniliforme*	-	-	-	-	100	3.5 ± 0.6	-	-
13	*Fusarium oxysporum*	100	2 ± 0.8	100	1 ± 0.2	100	12 ± 1.4	100	0.5 ± 0.1
14	*Macrophomina phaseolina*	100	3 ± 1.3	80	0.5 ± 0.4	-	-	-	-
15	*Mucor* sp.	-	-	100	1 ± 0.4	-	-	100	1.5 ± 0.2
16	*Nigrospora orzyae*	100	2.5 ± 0.6	100	2 ± 1.870	-	-	-	-
17	*Penicillium* sp.	-	-	60	0.5 ± 0.2	-	-	80	0.5 ± 0.1
18	*Rhizopus stolonifer*	-	-	40	0.5 ± 0.2	-	-	-	-

Table 5. Frequency of fungi in rice variety IRRI-9 isolated using blotter plate method.

S. no.	Fungus	No Deep-Freezing				Deep-Freezing			
		Treated		Untreated		Treated		Untreated	
		Infected samples (%)	Infected seeds (%)	Infected samples (%)	Infected seeds (%)	Infected samples (%)	Infected seeds (%)	Infected samples (%)	Infected seeds (%)
1	*Alternaria alternata*	-	-	100	3 ± 0.4	-	-	-	-
2	*Aspergillus sulphureus*	-	-	100	17 ± 3.9	-	-	-	-
3	*Aspergillus candidus*	100	10.6 ± 1.4	-	-	-	-	-	-
4	*Aspergillus clavatus*	100	6.9 ± 3.0	-	-	-	-	-	-
5	*Aspergillus flavus*	-	-	-	-	-	-	100	5.7 ± 0.5
6	*Aspergillus fumigatus*	-	-	-	-	-	-	100	6.2 ± 1.1
7	*Aspergillus heteromorphus*	-	-	100	11.5 ± 1.1	-	-	80	1 ± 0.4
8	*Aspergillus niger*	-	-	100	1 ± 0.2	-	-	60	0.5 ± 0.2
9	*Aspergillus parasiticus*	100	4.1 ± 1.8	-	-	-	-	-	-
10	*Aspergillus raphani*	-	-	-	-	20	0.2 ± 0.2	-	-
11	*Aspergillus rhizopodus*	100	6.9 ± 3.0	-	-	-	-	-	-
12	*Aspergillus* sp.	100	5.5 ± 2.4	-	-	-	-	-	-
13	*Aspergillus terreus*	-	-	100	1 ± 0.1	-	-	100	5 ± 1.6
14	*Chaetomium globossum*	100	1.1 ± 0.4	-	-	-	-	-	-
15	*Colletotrichum* sp.	100	5.5 ± 2.4	-	-	-	-	-	-
16	*Curvularia clavata*	-	-	100	6 ± 0.8	-	-	-	-
17	*Curvularia lunata*	-	-	100	2 ± 0.5	100	8.5 ± 1.4	100	0.4 ± 0
18	*Drechslera oryzae*	-	-	-	-	100	21 ± 3.6	-	-
19	*Fusarium oxysporum*	80	0.7 ± 0.3	100	2 ± 0.1	100	21 ± 2.8	100	2 ± 0.1
20	*Fusarium semitectum*	100	0.8 ± 0.3	-	-	-	-	-	-
21	*Fusarium solani*	60	5.5 ± 2.4	-	-	-	-	-	-
22	*Macrophomina phaseolina*	-	-	100	1 ± 0	100	3.5 ± 0.6	100	2.8 ± 0.6
23	*Mucor* sp.	-	-	100	3 ± 0.5	-	-	-	-
24	*Nigrospora orzyae*	-	-	-	-	100	3.5 ± 0.4	-	-
25	*Rhizopus stolonifer*	100	2.8 ± 1.2	60	27.5 ± 3.4	-	-	-	-
26	*Trichoderma harzianum*	100	6.6 ± 2.9	100	3 ± 0.3	-	-	-	-

2. Variety Sindh Basmati: Fifteen species of fungi were isolated (Table 3). Untreated NDF Basmati seeds placed on blotter showed the presence of the highest number of fungi (13) whereas treated NDF seeds showed eight species However, the frequency of isolation of fungi isolated from treated seeds was generally lower as compared to their frequency in untreated seeds. Similarly, 10 species were isolated from untreated DF seeds as compared to only four from treated DF seeds. Frequency of *Aspergillus* species was generally reduced in untreated DF seeds as compared to untreated NDF seeds. *Fusarium semitectum* was isolated only from DF seeds where its frequency was more in treated DF seeds as compared to untreated DF seeds. *Macrophomina phaseolina* was isolated from NDF seeds but not from DF seeds. In NDF seeds, the recovery of *F. oxysporum* was only 1.5% from untreated and 2% from treated seeds. *Mucor* sp. was only isolated from untreated DF seeds.

Basmati seeds tested using agar plate method yielded 18 species of fungi (Table 4). Seventeen species were isolated from untreated NDF seeds whereas *Aspergillus rhizopodus* was most frequent. Treated NDF seeds showed the presence of 7 species that showed association with 100% samples. Untreated DF gave 11 species whereas treated DF seeds yielded on five species. *Fusarium moniliforme* was isolated only from treated DF seeds, whereas, *Fusarium oxysporum* was most frequent in treated DF seed. *Macrophomina phaseolina* was isolated only from treated and untreated NDF seed but not found DF seed.

Table 6. Frequency of fungi in rice variety IRRI-9 isolated using PDA plate method.

S. no.	Fungus	No Deep-Freezing				Deep-Freezing			
		Treated		Untreated		Treated		Untreated	
		Infected samples (%)	Infected seeds (%)	Infected samples (%)	Infected seeds (%)	Infected samples (%)	Infected seeds (%)	Infected samples (%)	Infected seeds (%)
1	*Alternaria alternata*	100	3 ± 0.5	100	2.5 ± 1.4	100	1.5 ± 1.1	100	1.5 ± 1.1
2	*Aspergillus sulphureus*	100	4 ± 1	100	1.5 ± 1.1	100	12 ± 4.4	100	13.5 ± 2.2
3	*Aspergillus flavus*	100	1 ± 0.2	100	4 ± 2.9	100	3.5 ± 1.1	100	4 ± 2.9
4	*Aspergillus fumigatus*	100	3 ± 0.6	-	-	100	3 ± 0.6	100	13.5 ± 3.3
5	*Aspergillus*	100	5.5 ± 3.7	100	16 ± 12.2	-	-	-	-
6	*Aspergillus nidulans*	-	-	-	-	100	1 ± 0.2	60	0.5 ± 0.2
7	*Aspergillus niger*	-	-	60	0.5 ± 0.5	-	-	-	-
8	*Aspergillus terreus*	100	3 ± 0.3	100	2.5 ± 1.8	100	2 ± 0.3	100	3 ± 0.4
9	*Curvularia clavata*	100	4 ± 0.7	100	7 ± 5.2	-	-	-	-
10	*Curvularia lunata*	100	4.5 ± 0.4	100	3 ± 2.6	100	1 ± 0.7	100	3 ± 1.6
11	*Drechslera oryzae*	100	2 ± 0.3	100	1.5 ± 1.1	-	-	100	21 ± 2.1
12	*Fusarium moniliforme*	-	-	60	0.5 ± 0.4	-	-	60	0.5 ± 0.2
13	*Fusarium oxysporum*	100	1.5 ± 0.6	100	1 ± 0.7	100	8.5 ± 2.4	100	2 ± 1.5
14	*Macrophomina phaseolina*	100	4 ± 0.4	100	22.5 ± 1.8	-	-	-	-
15	*Mucor* sp.	-	-	100	2.5 ± 1.8	-	-	-	-
16	*Penicillium oxalicum*	100	1 ± 0.1	-	-	100	2 ± 0.3	100	2 ± 1.4
17	*Rhizopus stolonifer*	-	-	100	0.5 ± 0.2	100	2.5 ± 0.6	100	1.5 ± 1.1
18	*Trichoderma harzianum*	100	5.5 ± 0.6	100	1 ± 0.7	100	2.5 ± 0.5	100	0.8 ± 0.6

3. Variety IRRI-9: Twenty six species were isolated (Table 5). Untreated NDF seeds placed on blotter showed the presence of the highest number of fungi (13) whereas treated NDF seeds showed eight species. However, the frequency of isolation of fungi isolated from treated seeds was generally lower as compared to their frequency in untreated seeds. Similarly, 10 species were isolated from untreated DF seeds as compared to only six from treated DF seeds. Frequency of *Aspergillus* species was generally reduced in untreated DF seeds as compared to untreated NDF seeds. *Fusarium semitectum* and *Fusarium oxysporum were* isolated only from treated NDF seeds. *Macrophomina phaseolina* was isolated from NDF seeds but not from DF seeds. In seeds not exposed to deep-freezing, whereas the recovery of *F. oxysporum* was 2% or less from untreated NDF and not *Drechslera oryzae* and *Alternaria alternata* as compared to 21% from treated DF seeds. *Aspergillus raphani*, *Drechslera oryzae* and *Nigrospora orzyae* were isolated only from treated DF seeds.

Eighteen species of fungi were isolated from IRRI-9 seeds in agar plate method (Table 6). The highest numbers of fungi (15) were isolated from untreated NDF seeds whereas treated NDF seeds showed presence of 13 fungi. Similarly, 14 species were isolated from untreated DF seeds as compared to 11 from treated DF seeds. *Fusarium moniliforme* was isolated from untreated NDF and DF seed by not from treated seeds. *Fusarium oxysporum* was isolated from treated as well as untreated NDF and DF seed with highest frequency in treated DF seed. *Macrophomina phaseolina* was isolated in from NDF seed where it frequency was higher in untreated seeds as compared to treated seeds.

DISCUSSION

Rice crop is infected by variety of fungal pathogens and most of them are seed-borne (Richardson, 1990). Seed-borne pathogens produce huge quantitative and qualitative losses in rice (Dors *et al*., 2011). In the year 1970, rice blast disease produced an epidemic and caused up to 10-50% losses in yield in Korea (Mew *et al*., 2004). A large number of seed-borne fungi also produce toxic metabolites that often kill the embryo (Vidyasekaran *et al*., 1970) and can make the seeds toxic to man and animal. These fungi can also synthesize proteolytic, pectinolytic and cellulolytic enzymes (Mathur and Gupta., 1981).

Several report have been made on the association of fungi with the rice seeds (Mendoza and Molina, 1980; Mia and Mathur, 1983; Agarwal *et al*. 1989; Sisterna *et al*. 1994; Purushattam *et al*. 1996; Sharma *et al*., 1997; Bicca *et al*., 1998; Fakir, 2000; Naeem *et al*., 2001; Fakir *et al*., 2002, 2003; Rahman *et al*., 2002; Nahar, 2003, Mathur *et al*., 2004; Portapuglia, 2004; Islam *et al*., 2007). Similarly, Neninger *et al*. (2003) reported 99 species belonging to 59 genera of fungi including both the field as well as storage fungi. It has been reported that most grain samples were contaminated by soil fungi such as *Alternaria*, *Fusarium* and *Cladosporium* and by storage fungi such as *Aspergillus* and *Penicillium* spp. (Beuchat, 1981; Pitt and Hocking, 1985; El-Zawahry *et al.*, 1991). During the present studies, 33 species of fungi belonging to 14 genera were isolated from rice seeds that included storage as well as field fungi and corroborate well with the results of the previous studies.

Macrophomina phaseolina was isolated from all the varieties of rice tested during the present studies. It appears to be the first report of *M. phaseolina* from seeds of Sindh Basmati, IRRI-9 and DR-83 varieties of rice from Sindh, Pakistan. Rehman *et al.* (2018) reported *M. phaseolina* from seeds of rice variety Super Sindh collected from Punjab, Pakistan. Parate and Lajewar (1987) also reported *M. phaseolina* in rice seeds from Central India.

Results of previous studies conducted by Elangovan *et al*. (1999) showed that all rice grains have significant differences in contaminating species and in their infection counts within seeds. During storage the mycobiota related to the grain can be varied by the cultivar (Gutierrez *et al*., 2001) and the milling fraction (Lima *et al*., 2000). Present work also indicated a different in seed-borne mycoflora of different varieties of rice where the highest number of species were isolated from IRRI-9 followed by Basmati and then by DR-83.

During the present study, *Fusarium oxysporum* was isolated in the highest frequencies from the treated DF seeds that corroborates well with the results of Mathur *et al*. (1975). Similarly, *F. moniliforme* was isolated only from treated DF seeds of DR-83 and Basmati varieties, whereas, *F. semitectum* was isolated only from treated DF seeds in DR-83 and Sindh Basmati, and treated NDF seeds in IRRI-9. *Fusarium solani* was isolated only from treated NDF seeds of IRRI-9 using PDA plate method. It appears that *F. solani* was internally seed-borne but not deep enough to survive deep-freezing. Fakhrunnisa and Hashmi (1992) also found that deep-freezing method increased the mean infection range of *Fusarium* species in rice. It was attributed partly to the ease of observation and partly to the fact that the dead embryo provides nourishment to the developing mycoflora.

Khan *et al*. (2000) mentioned the association of *F. moniliforme, F. semitectum, A. alternata, A. padwickii, C. oryzae, C. lunata, P. oryzae* and species of *Phoma* and *Nigrospora* with seeds of different varieties of rice in Pakistan. The pathogens isolated during the present studies are known to cause important diseases in rice, for example *Bipolaris oryza*e causes brown spot of rice, *F. moniliforme* is associated with bakanae disease, rice blast caused by *Pyricularia oryzae* and Stackburn disease caused by *Alternaria* (Mew and Gonzales, 2002).

Aspergillus, Penicillium and *Alternaria* species are common contaminants of rice seeds (Park *et al*., 2005; Sales and Yoshizawa, 2005). The same was true during the present studies. Frequency of *Aspergillus* species was generally reduced in untreated DF seeds as compared to untreated NDF seeds. No *Aspergillus* species was isolated from treated DF seeds in any treatment except where treated DF IRRI-9 seeds were placed on PDA that showed the presence of *A. sulphureus, A. flavus, A. fumigatus* and *A. nidulans.*

Grain discoloration of rice is an emerging threat to rice crop in Pakistan. Seven pathogens *viz*., *A. alternata*, *A. padwickii*, *D. oryzae*, *F. moniliforme*, *C. oryzae*, *N. oryzae* and *A. niger* have been isolated from seed samples showing symptoms of grain discoloration disease collected from rice areas of NIAB, Faisalabad, Sheikhupura, Samundri, PirMahal and Vehari (Arshad *et al*., 2009). During the present studies, the rice seed samples showed

presence of these pathogens that indicates a potential threat of the outbreak of rice discoloration disease under disease favoring conditions. *Drechslera oryzae* was isolated only from treated DF seeds of DR-83 and IRRI-9 varieties placed on blotter papers, and from treated or untreated seeds NDF seeds of the same varieties placed on PDA. (Fakhrunnisa & Hashmi, 1992) also found the DF seeds showed high percentage of infection by *Drechslera* spp.

Trichoderma harzianum was isolated from NDF seeds of DR-83 and IRRI-9 but not from DF seeds. *Nigrospora oryzae* was isolated only from treated DF seeds of IRRI-9 and treated and untreated NDF seeds of Basmati. Although *T. harzianum* is a well known biocontrol agent but it may cause damages to grains if the storage conditions are improper.

Khan *et al*. (1988) reported that the number of fungal species reduced significantly when seeds were subjected to deep-freezing. The results of the present study also showed that in general, the frequency of isolation of fungi was higher in untreated NDF seeds as compared to treated NDF seeds. Deep-freezing showed reduction in number of fungi isolated from both treated and untreated seeds as compared to NDF seeds. The minimum numbers of species were isolated from treated DF seeds.

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