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A SEED BORNE MYCOFLORA ASSOCIATED WITH LOCAL AND IMPORTED PADDY SEED LOTS IN PAKISTAN

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A B S T R A C T

In the present study, 110 local and 125 imported rice (*Oryzae sativa* L.) seed samples comprising of 28 different varieties were analyzed for seed health using standard blotter paper method. Eighteen fungal species belonging to 8 fungal genera viz., *Alternaria, Bipolaris. Curvularia, Fusarium, Nigrospora, Pyricularia, Phom*a and *Tellitia* were found associated with local and imported paddy seed lots. Local paddy seed lots were found more infected with seed-borne fungi as compared to imported paddy seed lots. On local paddy seed lots, *Curvularia lunata* (10.2%) was most observed as predominant fungal species followed by *Fusarium moniliforme* (6.5%). On the other hand, in imported paddy seed lots *Tellitia barclyana* was found maximum in infection percentage (90%) followed by *Fusarium moniliforme* (7.6%).

Keywords: Mycoflora, paddy, rice, seed-borne.

INTRODUCTION

Rice (*Oryzae sativa* L.) belongs to the family Poaceae and is cultivated throughout the tropical and subtropical region of the world (Ezuka and Kaku, 2000). According to the statistics of the year 2013-14 Rice was grown over an area of 2789.2 thousand hectares with the total production of 6798.1 thousand tons (GOP, 2014). Rice is considered as a major crop of the world especially in Asian countries such as Pakistan, India, Bangladesh, China, Vietnam, and Korea. Rice is placed on second position in cereal cultivation around the world and contributes significantly in the national economy as an export item as well as staple food (Zahid *et al.,* 2005). Rice seed is affected by wide range of bacterial and fungal pathogens. So far more than 100 different fungi have been detected on rice seeds (Neergard, 1977). A total of 31 seed bone diseases are associated with rice seed in which 30 are caused by fungi (Ou, 1985). Disease infected seed cause establishment of inoculum in the disease free soil and also contribute towards the lesser seed germination, higher seed discoloration, reduces seed weight and produces toxins that may be injurious to humans and domestic animals. Several seed borne fungi including *Fusarium, Aspergillus* and *Penicillium* are associated with the production of mycotoxins such as Aflatoxin (AFB1), Ochratoxin, Fuminosin (B1, B2) and Zearalenone (Reddy *et al.,* 2010).International Agency for Research on Cancer has reported that Aflatoxin (AFB1) and Fuminosin B1 are categorized as carcinogenic in humans. Consumption of toxin contaminated food in human and animals has reported severe mycotoxicoses (Peraica and Domijan, 2001; Reddy *et al*., 2007). Considering the importance of rice crop in Pakistan, little work has done on seed borne mycoflora as compared to other foreign countries.

Fungi including *Alternaria padwickii, Curvularia lunata, Curvularia oryzae, Fusarium moniliforme, Fusarium oxysporum, Fusarium semitectum, Pyricularia oryzae* and species of *Phoma* have also been isolated from seeds of different regions of the country. (Wahid *et al.,* 1993 & 2001; Khan *et al*., 2000; Javaid *et al*., 2002.). Some species like *Fusarium moniliforme, F. semitectum, Alternaria padwickii, A. alternate, Curvularia Oryzae, Drechlera Oryzae* were found pathogenic in nature with 46.79% to 16.77% external and internal infestation

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(Khan *et al*., 2000). In another study, seeds of five different varieties i.e., Basmati kernel, Basmati-385, B-370, B-190 and KS-282 were found infected with 27% 19%, 17%, 16%, and 14% seed borne mycoflora respectively using Blotter paper method (Butt *et al.,* 2007). Brief literature cited above shows that information regarding seed borne mycoflora of rice in Pakistan is not sufficient. Therefore following study was carried out to update existing literature on seed borne fungi associated with rice.

MATERIALS AND METHODS

Experiment was conducted in Federal Seed Certification and Registration Department, Islamabad in 2012. A total of 235 seed sample consisting of 28 different local and imported rice varieties (Table: 1) were collected and analyzed using standard Blotter paper method. Four hundred seeds of each sample were placed in a sterile Petri plates containing three layers of (Whatman No.1) moist filter paper. In each Petri plate 25 seeds were plated. The plates were incubated at 25±1°C under alternate cycles of 12 hour of light and 12 hours of darkness for 7 days. The seeds were examined on eighth day under the stereo binocular microscope and fungi were identified using reference manual Dematiaceous Hyphomycetes (Ellis, 1971). The fungi associated with rice seed samples were recorded and expressed individually.

Table 1: Inventory of rice seed samples.

Sr.	Variety	Type	No. of Samples
1	B.2000	local	3
2	B.SUPER	Local	31
3	CRIS-134	local	1
4	CRIS-342	Local	1
5	DR-92	Local	2
6	FH-1000	Local	1
7	FH-901	Local	1
8	IRRI-6	Local	18
9	KHUSBOO-95	Local	2
10	KS-282	Local	21
11	KSK-133	Local	14
12	MEMK	Local	1
13	N-78	Local	1
14	SHARSHAR	Local	4
15	SHADA	local	3
16	SHUA-92	local	6
17	AAS-501	Imported	5
18	ARIZE-403	Imported	41
19	ARIZE-TEJ	Imported	5
20	GNY-50	Imported	18
21	GNY-53	Imported	6
22	HEERA	Imported	1
23	HJ-19	Imported	2
24	HR-40	imported	31
25	HS-777	Imported	1
26	KOMAL	Imported	1
27	MR-40	Imported	13
28	RA-204	Imported	1
	TOTAL		235

RESULTS

A total of 235 seed samples consisting of 28 different local and imported rice varieties were studied for the presence of seed borne mycoflora using standard blotter paper method and results are presented in Figure1 & 2. A total of eight genera of fungi *viz., Alternaria, Bipolaris, Curvularia, Fusarium, Nigrospora, Pyricularia, Phoma* and *Tellitia* consisting of 18 species of fungi were found to be associated with local and imported paddy seed lots.

The results showed that the seed borne fungal infection varied from 1.0% to 10.2% in local varieties. However, in imported varieties infection varied from 1.0 to 9.0 %. In local paddy seed lots the most dominant fungi was *Curvularialunata* that showed mean infection of 10.2% followed by *Fusarium moniliforme* (6.5%), *Fusarium oxysporum* (5.7%), *Fusarium subglutinans*(5.0), *Bipolaris tetramera* (4.0%) and *Fusarium semitectum* (3.1%). The following fungi *viz, Curvulari aoryzae, Fusarium solani, Phoma, Bipolaris oryzae, Alternaria padwickii, Alternaria alternata, Bipolaris sorolianans, Nigrospora oryzae* and *Tellitia barclyana* were observed to an extent of 3.0, 3.0, 2.8, 1.5, 1.3, 1.0, 1.0, 1.0, 1.0 and 1.0%, respectively. The highest percentage of *Curvularia lunata* was observed in variety IRRI-6 i.e. 32%, followed by KS-282 (26%) and KSK-133 (20%). IRRI-6 also had maximum infection of *Fusarium moniliforme* (17%) followed by KS-282 (14.5%). Likewise seeds of FH-901 showed 11% infection of *Fusarium moniliforme* followed by CRIS-134 (6%). Maximum mean infection percentage of 3.87 was found in Variety IRRI-6 to be followed by 3.34 % in KS-282 and 2.43% in KSK-133.Seeds of variety KHUSBOO-95 carried least mean infection percentage of 0.21 followed by 0.25% in MEMK and 0.28% SHADA.

In imported paddy seed samples, *Tellitia barclyana* was observed in high frequency (9.0%) followed by *Fusarium moniliforme* (7.6%), *Curvularia oryzae* (5.0%), *Curvularia lunata* (3.5%), *Alternaria padwickii* (2.75%), *Fusarium* *semitectum* (2.25%), *Bipolaris oryzae* (1.25%), *Phoma, Alternaria tenuis* (1.0%) and *Pyricularia oryzae* (1.0%).. Seeds of HS-777 carried the maximum infection percentage of *Tellitia barclyana* i.e. 33% , similarly in variety HR-40 *Fusarium moniliforme* was maximum in infection percentage i.e. 22% followed by 14.5% in AAS-501. Seeds of four varieties namely B-385, DAGHA-1, GNY-53 and KOMAL have found to be free from the mycoflora infection.

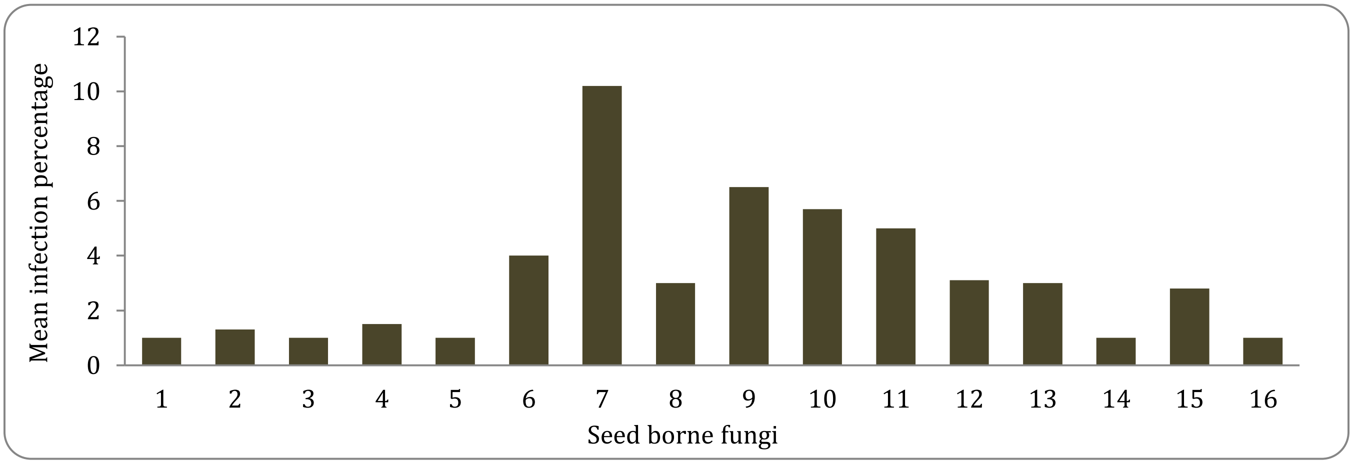

Figure 1. Mean infection percentage of fungi observed in local paddy seed lots.

1. *Alternaria alternata*, 2. *Alternaria padwickii*, 3. *Alternaria radicina*, 4. *Bipolaris oryzae*, 5. *Bipolaris sorolianans*, 6. *Bipolaris tetramera*7. *Curvularia lunata,*8.*Curvularia oryzae*, 9. *Fusarium moniliforme*, 10. *Fusarium oxysporum*, 11. *Fusarium subglutinans*, 12. *Fusarium semitectum*, 13. *Fusarium solani*, 14. *Nigrospora oryzae*, 15. *Phoma*, 16. *Tellitia barclyana*.

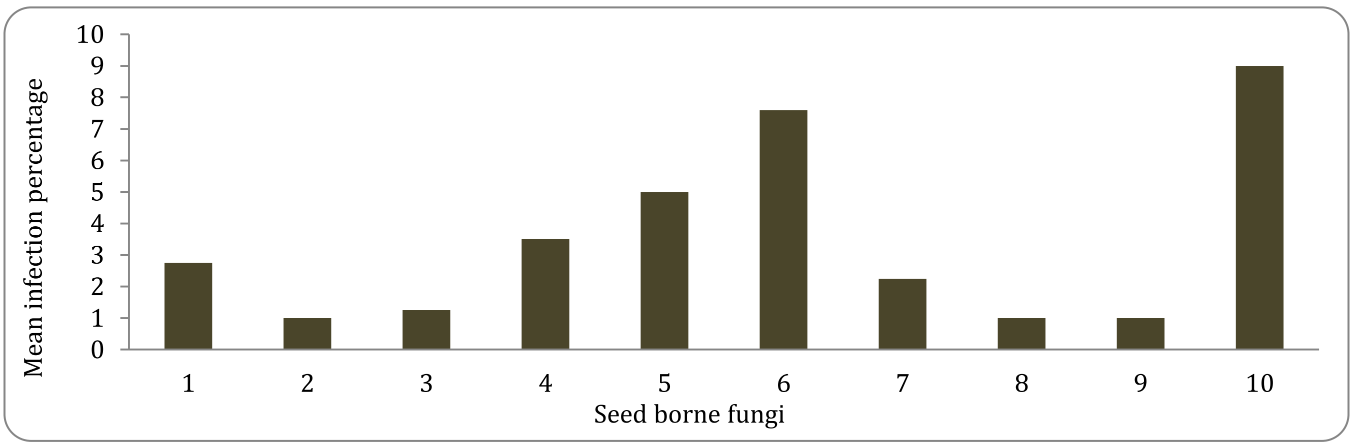

Figure 2. Mean Infection percentage of imported paddy seed lots.

1. *Alternaria alternata*, 2. *Alternaria tenuis*, 3.*Bipolaris oryzae*, 4.*Curvularia lunata,* 5. *Curvularia oryzae,* 6. *Fusarium moniliforme*,7. *Fusarium semitectum*, 8. *Phoma,*9. *Pyricularia oryzae*, 10. *Tellitia barclya*

DISCUSSION

The present study was conducted to check the presence of diverse mycoflora both in local and imported paddy seed lots. All fungal pathogens found in this study are known to be seed borne in nature (Richardson, 1990). Rice crop is affected by a wide range of fungal pathogen and most of them are seed borne, which have been identified from rice seeds in Pakistan from time to time. Earlier work carried out by Khan *et al.* (2000) reported the presence of *Fusarium moniliforme, F. semitectum, Alternaria alternata, A. padwickii, Curvularia oryzae, C. lunata, Pyricularia oryzae* and species of *Phoma* and *Nigrospora* on different varieties of rice in Pakistan. These pathogens are also known to cause several important diseases of rice, for example *Bipolaris oryzae* brown spot of rice, *Fusarium moniliforme*are associated with bakanae foot rot, rice blast caused by *Phyricularia oryzae* and Stackburn disease caused by *Alternaria padwickii. Aspergillus* species are common contaminants of rice seeds also *Alternaria* and *Penicillium* species have been reported (Park *et al.,* 2005; Sales and Yoshizawa, 2005).

Our present results are in agreement with that recorded by several investigators (Beuchat 1981; Pitt and Hocking

1985; El-Zawahry *et al.*, 1991) who revealed 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. Results of previous studies conducted by (Elangovan *et al.,* 1999) that showed all rice grains have significant differences in contaminating speciesand in their infection counts within seeds. The most frequent contaminant in 15 rice samples was the genus's *Aspergillus*. Only species, namely *A. flavus, A. niger, A. fumigatus, A. terreus and A. candidus* dominated as contaminants in different parts of the rice grains. A high incidence of *A. flavus* was found in the seed mycoflora of rice bran, while Hafez *et al.* (2004) reported that 120 species belonging to 38 genera in 64 paddy samples from Egypt. They found *A. flavus, A. sydowi, A. terreus, A. fumigatus, A. ochraceus, P. chrysogenum, P. corylophilum, F. oxysporum, Alternaria tenuis, Cladosporium cladosporioides, Trichoderma viride* and *Mucor racemosus* more frequently on glucose agar.

Several studies have reported detectable levels of aflatoxins and ochratoxin A in rice from different countries, including Cuba (Escobar and Regueiro, 2002), Korea (Park *et al.,* 2005), Malaysia (Abdullah *et al.,* 1998), Sri Lanka (Bandara *et al.,* 1991), Philippines (Sales and Yoshizawa, 2005), United Arab Emirates (Osman *et al.,* 1999), and Côte d'Ivoire (Sangare-Tigori *et al.,* 2006). Aflatoxigenic fungi are the main risk among the contamination of rice products, because they produce several mycotoxins that can cause problems in humans and animals that consume distinctive fractions of rice grain (Osborne, 2002 Ueno, 2004; Sales and Yoshizawa 2005). The four major aflatoxins are known as aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2). Aflatoxins have been shown to be potent mutagens, teratogens and carcinogens (Kotsonis *et al.,* 2001). Contamination of cereal commodities by moulds and mycotoxins results in dry matter, quality, and nutritional losses and represents a significant hazard to the food chain. Moulds and mycotoxin contamination is highly risky. It can seriously affect consumer health. The most well-known among the mycotoxins are aflatoxins (AF), which are usually predominant in foods and feeds (Binder *et al.,* 2007). The chronic effects at low dose exposure of AF consist of a number of adverse effects such as reduces growth rate and feed efficiency, decreased liver and kidney function and suppression of the immune system, and it can result in severe illness (Shivachandra *et al.,* 2003; Reddy *et al.,* 2010).

The effects of these seed borne pathogens on rice crop are also devastating. Previous literature reveals that seed borne pathogens resulted in huge quantitative and qualitative losses (Dors *et al.,* 2011). Rice blast epidemic in Korea during the year of 1970 caused major food crises and yield losses were estimated upto 10-50 percent. Similarly great Bengal famine was recorded in Bengal, India in 1942-43 caused by Brown spot of rice (*Helminthosporium oryzae*) that ruined the whole rice crop and about 4-5 million people were affected (Agrios, 2005). Another most important affect that results from seed borne diseases or seed transmission is that it increases the chances of introduction and establishment of seed borne pathogen in new areas. Neergard (1979) reported the introduction of Asian races of Blast fungus caused by *Pyricularia oryzae* to Burkina Faso with the seeds of new rice varieties from the International Rice Research Institute (IRRI) that devastated the whole rice crop in that country during the year 1971. These seed borne pathogens may permanently contaminate the soil and contribute towards inoculum build up that will eventually be a serious threat for a new crop.

CONCLUSION

The outcome of the present study reveals that seed-borne pathogens are present on most of the local and imported rice varieties. However, it was noticed that imported varieties were less infected as compared to local. It was also noticed that some particular varieties were infected with specific seed borne fungi. Pathogen free seed is the fundamental contribution in agriculture. Although the imported varieties are treated with chemicals for maintaining quarantine regulations, but from the present study it was revealed that seed borne pathogens were associated with those seeds. So the seed health status of imported and local rice seed needs to be improved to establish pathogen free propagation material.

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