Pak. J. Phytopathol.,
Vol. 24(2):133-136, 2012.

DETECTION OF SEED BORNE MYCOFLORA OF DIFFERENT COARSE AND FINE RICE VARIETIES AND THEIR MANAGEMENT THROUGH SEED TREATMENTS

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ABSTRACT

During the present research work seed samples of fifteen varieties of rice (8 coarse and 7 fine) collected from Rice Research Institute, Kala Shah Kaku were processed for the detection and identification of fungi associated with seeds of different varieties. The percentage infection of *Helminthosporium oryzae* were recorded maximum in course varieties (28.11%, 17.014%) in case of agar plate and blotter paper method respectively, compared with other seed borne fungi. In the case of the fine varieties the percentage infection of *Helminthosporium oryzae* was significantly higher in agar plate method i.e. 40.47% as compared with all other seed borne fungi. The percentage infection of *Curvularia* spp. was observed to be much higher in blotter paper technique. The level of seed-borne fungal infestation was significantly higher in fine varieties as compared to coarse varieties. Coarse varieties were found to be less contaminated as compared to fine varieties. During management trials through seed treatment, maximum germination percentages were recorded in coarse varieties as compared to fine varieties. In coarse varieties Dithane-M showed the best germination results 86.67% followed by Derosal (85%), Thiophenate methyl (85%), Copper oxychloride (80%), Trimeltox forte (74.17%), Alert plus (67.50%), Acrobat (66.67%), Tazolen (64.17%) and Distilled water (35.83%) which was used as control showed the minimum results respectively. In fine varieties Derosal showed the best germination results (92.50%) as compared to control (32.50%).
Key words: Rice varieties, Seed borne fungi, chemical treatments.

INTRODUCTION

Rice (*Oryzae sativa L*.) belongs to family *Poaceae* ("true grass") with two domesticated species of genus *oryzae. Oryzae sativa* is native to tropical and subtropical southern Asia, while the African rice, *Oryzae glaberrima*, native to West Africa. Rice is the third largest crop after wheat and cotton in Pakistan. Rice is highly valuable cash crop; also major export item of Pakistan. Our country grows enough high quality rice, having typical aroma and good taste, to meet both domestic demand and for export. Area and production of rice for the year 2008-09 were set at 2963 thousand hectares and 6952 thousand tones respectively. Yield of rice crop was 2346 kilogram per hectares, which was low in comparison to other Asian countries, though its production area is increasing day by day. (Anonymous, 2010). There are many factors which are responsible for low yield but a major constraint to production is the infectious diseases e. g rice blast (*Pyricularia oryzae* Cav.) brown leaf spot (*Bipolaris oryzae spp*.) stem rot (S*clerotium oryzae* Catt.) and foot rot disease (*Fusarium moniliforme*). Most of these infectious problems are seed-borne in nature which causes enormous losses both in storage as well as in the field. These pathogens are known to cause damage at different stages like storage, seed germination, seedling establishment, vegetative growth and reproductive phase. The infected seeds may fail to germinate, transmit disease from seed to seedling and from seedling to growing plants (Fakir *et al*. 2002). Most seed borne diseases like brown leaf spot, rice blast, stem rot and bacterial leaf blight are caused by the pathogens like *Drechslera oryzae, Fusarium moniliforme, Pyricularia oryzae, Rhizoctonia solani, Sarocladium oryzae, Sclerotium oryzae, Trichoconiella padwickii* and *Xanthomonas compestris* pv *oryzae* (Bhutta and Hussain 1998, Khan *et al*. 1990, Wahid *et al*. 2001, Gill *et al*. 1999), are the main causes of rice yield reduction in our country. Seed health is a well recognized factor in the modern agricultural science for desired plant population and good harvest. Seed treatment by chemicals is the best, environmentally safe and economical way to keep good seed health condition, because in this management practice mostly a very low dose (1-1.5 g/kg) of chemicals were used as compared to foliar application. By keeping in view the above mentioned facts, the present study was carried out to isolate and identify the seed borne mycoflora of coarse and fine rice varieties and the effect on seed germination and management through seed dressing fungicides was investigated.

MATERIALS AND METHODS

The experiment was conducted at Seed Health Testing Lab. Department of Plant Pathology, University of Agriculture, Faisalabad during the year 2009-10 to investigate the health, germination and seedling vigor of rice seeds. Fifteen varieties of rice (8 coarse and 7 fine) collected from Rice Research Institute, Kala Shah Kaku. These samples

assessed for the detection of seed borne fungi through blotter paper and potato dextrose agar method followed by ISTA (2003). These seed lots were surface sterilized with the 0.5 % $HgCl_2$. Four hundred seeds were taken from each of fifteen seed samples in Petri plates containing blotter paper in such a way (11 seeds were placed all around & 1 in the centre of Plate). The Petri plates containing seeds were kept in the lab for period of seven days at 22°C ± 1and sufficient light 12 hours. After that Petri plates of seed samples were examined under low power stereo microscope. Pathogenic and saprophytic fungi were counted and identified under different magnifications of a stereomicroscope and were identified by colony growth, color, sporulation type and available literature (Booth 1971, Sutton 1980, Mathur, 2003) and percent incidence was recorded.

$$\text{Incidence} = \frac{\text{Number of infested seeds}}{\text{Number of plated seeds}} \times 100$$

During the management of seed borne pathogens plastic trays (18 x 9) were used for both treated and non treated trials in sand medium. Forty seeds were sown in four lines in each plastic tray 10 seeds/line. The effect of different fungicides was recorded at 4, 7 and 14 days after sowing. The sensitivity of different fungi to different fungicide @ 2g /kg (Derosal, Dithane M-45, Trimeltoxe forte, Acrobat, Alert plus, Copper oxychloride, Thiophenate methyl, Tazolen) was studied using modified Borum and Sinclair's technique (1968). A weighed quantity of each fungicide was added to the plastic trays having rice seeds. All treatments were arranged in completely randomized design (CRD) and observed the mean comparison by using Duncan multiple range test (DMR).

RESULTS AND DISCUSSION

The variation in number, type of fungi and their frequency of occurrence on different varieties of rice were recorded. Detection of seed borne fungi through blotter paper method in coarse varieties revealed that *Helminthosporium spp* was the most predominant fungus in all the samples tested with a range from 13.883-20.85% and *Alternaria alternata* (12.5-19.45%), *Aspergillus niger* (12.5-16.667%), *Fusarium moniliforme* (9.73-16.66%), *Rhizopus spp* (8.333-18.350%), *Aspergillus flavus* (4.15-12.50%), *Curvularia spp* (0.00-8.35%) was observed respectively. Similarly in agar plate method *Helminthosporium spp* was again predominant fungus in all the samples tested with a range from 22.23-31.95% and *Alternaria alternata* (16.66-20.83%), *Aspergillus niger* (16.65-20.85%), *Rhizopus spp (*13.83-16.65%), *Fusarium moniliforme* (12.5-18.05%), *Aspergillus flavus* (9.73-19.43%), *Curvularia spp* (6.95-11.11%) was observed, respectively. The prevalence of fungi varied with respect to variety and detection method of seed samples collected. Higher infestation was recorded in agar plate method as compared to blotter paper method in coarse varieties. Seed borne mycoflora of rice showed variation in their composition depending on variety and detection of seed samples collected. Detection of seed borne fungi through Blotter paper method in fine varieties *Curvularia spp* was the most predominant fungus observed in all the samples tested with a range from 23.6-45.83% followed by *Aspergillus flavus* (18.05-34.73%), *Rhizopus spp* (19.45-27.78%), *Alternaria alternata* (15.26-26.38%), *Fusarium moniliforme* (13.88-29.16%), *Aspergillus niger* (9.735-33.333%), *Helminthosporium spp* (6.93-26.38%) was observed respectively. Similarly in agar plate method *Helminthosporium spp* was the most predominant fungus observed in all the samples tested with a range from 34.716-45.83% followed by *Alternaria alternata* (25.00-36.11%), *Aspergillus niger* (19.45-36.11%), *Fusarium moniliforme* (20.83-26.38%), *Rhizopus spp* (18.05-29.15%), *Aspergillus flavus* (13.88-25.00%), *Curvularia spp* (4.16-26.7%) was observed respectively (Table –1).

The prevalence of fungi varied with respect to variety and detection of seed samples collected. Higher infestation was recorded in agar plate method as compared to blotter paper method in fine varieties. Our findings follow the conclusions of Riaz *et al. (*1995) who worked on seeds of 225 accessions of rice, collected from the provinces of Sindh, Baluchistan and North West Frontier Province in 1984 and 1987, maintained in the Plant Genetic Resources Institute's gene bank were evaluated for seed borne fungi through blotter paper method and agar plate method. Most of the accessions were contaminated with species of16 fungal genera *Alternaria* and *Helminthosporium spp*. occurred most frequently, followed by *Curvularia*, *Fusarium* and *Aspergilus spp*.

Accessions collected from southern Pakistan showed a higher degree of contamination than those from the north. Our results conflicted with the results of Bhutta (1997) who tested cotton seeds by using Agar plate, Blotter paper methods and newspaper method and conducted that blotter paper method was best for seed health testing. The results also resembled to work done by Tasleem *et al.* (2000) who detected seed borne fungi of rice from central Punjab and their control from 20 rice seed samples yielded *Fusarium moniliforme, Fusarium semitectum, Alternaria padwikii, Alternaria alternata, Curvularia oryzae, Drechslera oryzae and Aspergillus niger*. Average external and internal infestation was 46.79 and 16.77% respectively. Our findings also follow the conclusion of Franco *et al.* (2001) who conducted a survey in Brazil during the cropping season to determine fungi associated with irrigated rice. The fungi identified were *Pyricularia*

Table 1: Comparative study of percentage infection of seed borne fungi through blotter paper method and agar plate method in (8 course and 7 fine) varieties of rice

Causal Organisms	Coarse Varieties				Fine Varieties			
	Agar Plate Method		Blotter Paper Method		Agar Plate Method		Blotter Paper Method	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Helminthosporium spp	28.11	22.233-31.95	17.014	13.883-20.85	40.47	34.716-45.835	13.886	6.935-26.383
Alternaria alternate	19.44	16.667-20.835	15.623	12.5-19.45	31.75	25.00-36.115	21.228	15.265-26.383
Aspergillus niger	19.27	16.65-20.85	14.75	12.5-16.667	31.15	19.45-36.115	17.066	9.735-33.333
Fusarium moniliforme	14.75	12.5-18.05	14.404	9.733-16.667	23.61	20.835-26.386	20.438	13.885-29.167
Rhizsopus spp.	15.09	13.833-16.65	11.15	8.333-18.350	22.19	18.05-29.15	22.821	19.45-27.785
Aspergillus flavus	14.06	9.733-19.435	9.2	4.15-12.50	18.05	13.885-25.00	23.614	18.05-34.733
Curvularia spp.	8.68	6.95-11.115	3.98	0.00-8.35	12.700	4.167-26.4	39.478	23.6-45.835

Table No. 2: Effect of different seed dressing fungicides on seed germination and recovery of fungi

Chemicals (C)	Variety (V)		Means
	Coarse	Fine	
Distilled water (control)	35.83 K	32.50 K	34.17 E
Dithane-M45	86.67 AB	82.50 BC	84.58 AB
Trimeltox forte	74.17 DEF	70.00 FGH	72.08 C
Acrobat	66.67 GHI	60.00 IJ	63.33 D
Alert plus	67.50 FGH	60.00 IJ	63.75 D
Derosal	85.00 B	92.50 A	88.75 A
Tazolen	64.17 HI	55.00 J	59.58 D
Thiophenate methyl	85.00 B	77.50 CDE	81.25 B
Copper oxy chloride	80.00 BCD	72.50 EFG	76.25 C
Means	71.67 SA	66.94 B	

LSD value (C) = 4.663, LSD value (V) = 2.198, LSD value (V x C) = 6.59

oryzae (0.04%), *Helminthosporium oryzae (*Ito*) Ishiyama, cochliobolus miyabeanus* (2.6%), *Curvularia lunta (Cochliobolus lunatus)* (4.9%), *Alternaria sp.* (6.3%), *Fusarium sp.* (1.8%), *Phoma sp.* (11.1%), *Cladosporium sp.* (11.6%), *Epicoccum sp.* (1.5%), *Aspergillus sp.* (7.6%), *Penicillium sp.* (34.4%), *Gerlachia oryzae, monographella albescens* (18.8%). *Trichoconiella padwickii, Alternaria padwickii* (8.4%) and others (5.1%).The results resembled to the work done by Naeem-Khalid *et al.* (2001) and Javed *et al.* (2002) who investigated the incidence of mycoflora, their frequency and impact on seed germination of rice cultivars. The incidence of different fungal populations was observed including six field fungi viz. *Alternaria padwickii, Curvularia spp, Bipolaris oryzae, Cochliobolus miyabeanus, Fusarium moniliforme, Gibberella fjuikuroi,* and five storage fungi *viz. Aspergillus niger, Aspergillus flavus, Penicillium spp, Rhizopus stolonifer, Chaeomium.* During chemical treatment trials for management of seed borne fungi coarse varieties are resistant varieties in comparison with fine varieties. Maximum germination percentages were recorded in coarse varieties as compared to fine varieties. In coarse varities, Dithane-M showed the best results 86.67% followed by Derosal (85%), Thiophenate methyl (85%), Copper oxychloride (80%), Trimeltox forte (74.17%), Alert plus (67.50%), Acrobat (66.67%), Tazolen (64.17%) and distilled water (35.83%) which was used as control showed the minimum results respectively. In fine varieties Derosal showed the best results 92.50% followed by Dithane-M (82.50%), Thiophenate methyl (77.50), Copperoxy chloride (72.50%), Trimeltox forte (70%), Acrobat (60%), Alert plus (60%), Tazolen (55%) and Distilled water (32.50%) used as control showed the minimum results respectively. Our findings also follow the conclusion of Bhalli *et al.* (2001) who evaluated eight fungicides viz. Apron, Benlate, Derosal, Copperoxy chloride, Ridomil, Seore, Topaz and Topsin-M to control the mycelial

growth of *Fusarium moniliforme*. Derosal was best to inhibiting the mycelial growth *in-vitro*. The results resembled to the work done by Farid *et al.* (2002) who tested the twelve seed samples of rice and all were found infected by *Bipolaris oryzae* the cause of brown spot disease. Highest (5.5%) and lowest (1.5%) incidence was found in sample of Bhabokhali and Mahozompur, respectively. Four fungicides viz. Bavistin, Thiophenate methyl, Tilt 250 EC and Dithane M-45 were evaluated against *Bipolaris oryzae*. Dithane M-45 was best with 100% reduction of the prevalence of the pathogen and inhibited the mycelial growth at 0.3% of the seed weight as seed treatments and 500 ppm as mycelial growth inhibition test followed Tilt 250 EC; Thiophenate methyl and Bavistin. All test fungicides were effective against *Bipolaris oryzae* at higher concentration and showed the maximum germination of seedlings in comparison to control.

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