International Journal of Advanced Research in Biological Sciences
ISSN : 2348-8069
www.ijarbs.com

Research Article

Detection of seed born fungi associated with different rice varieties and chemical control

Irshad Ahmad[1], Khalid Bhutta[2] and Muhammad Ashraf[3], Muhsin Waheed[4]

[1,2,3]Assistant Director Agriculture (Plant Protection) Pest Warning & Quality Control of Pesticides, Layyah, Muzafargarh[2], Multan[3]
[4]Assistant Agronomist, NES-PAK, Gujranwala
*Corresponding author: *mirshad1960@gmail.com*

Abstract

The study was conducted to evaluate the detection and identification of fungi associated with seeds of different varieties. The percentage infection of F1 (Helminthosporium) in course varieties of rice (28.11%, 17.014%) showed significant effect in agar plate and blotter paper method compared to other seed borne fungi. In coarse varieties 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.

Keywords: Seed borne fungi, rice fine and course, chemical control.

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. Rice is an important cereal crop in the world as well as in Pakistan, having a good potential to meet the food requirement of increasing population and adaptability in our soil and climatic conditions. It is being consumed by large population of the world; especially it is leading crop in Asia. Two billion people in China, India and Bangladesh along with millions more in other countries depend on rice for more than half the protein and calories they consume (Khan *et al*., 2000). It accounts for 5.5 % of value added in agriculture and 1.1 percent in GDP of Pakistan. Our country grows enough high quality rice having typical aroma and good taste to meet both domestic demand and for exports. 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 3 % less than the target (Anonymous, 2009). Its production area is mainly concentrated in Punjab (1728.4 thousand ha) and Sindh (598.1 thousand ha) and on small scale in Baluchistan (193.9 thousand ha) and NWFP (60.8 thousand ha) (Anonymous, 2007a). Although Pakistan is not among the top rice producing or consuming countries, but it is the third largest exporter of rice in the world (Anonymous, 1994). Fine (basmati) varieties occupied 62 percent of the total area cultivated contributing only 50 percent in the total production. On the other side coarse varieties occupied 29 percent of the total area contributing 41 percent in total production, 9 percent of the area was sown with other varieties that contributed 9 percent in total production (anonymous, 2007b). The average yield of the crop in our country is lower in comparison with that of the world, though its production area is increasing day by day. 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. As a result of this the productivity and quality of grains and seeds can be reduced considerably. The infected seeds may fail to germinate, transmit disease from seed to seedling and from seedling to growing plants (Fakir *et al*., 2002). Diseases like brown leaf spot stem rot and bacterial leaf blight caused by the pathogen 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) were the main causes of rice yield reduction in our country. Crop loses caused by the disease may reach up to 20% in out break cases, like in Japan 20-50% loss was observed while yield losses of 15% and 3.7% were reported in India and Thailand respectively. On the later stage the losses could be higher than 70% (Rood, 2004 and khokhar, 1990) reported the field incidence ranging from 10-70 percent in individuals field. These seed-borne pathogens effect the germination of seeds and act as a main source of spreading different diseases under field condition. Thus the primary need is to sow disease-free and high quality seed for a high yield. Its yields are poor as compared to other countries. Yield for Australia, Egypt, Swaziland, Greece, Spain and Pakistan. The yield of basmati rice is 8062, 7724, 7500, 7228, 6285 and 1809 Kgs per hectare respectively (FAO, 1993). 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. These chemical treatments ensure the enhancement of germination percentage, resultantly good crop stand with more plant population in a field and ultimately more yield obtained. By keeping in view the above mentioned facts, the present study carried out to isolate and identify the seed borne mycoflora of course and fine rice varieties their effect on seed germination and management through seed dressing fungicides.

Materials and Methods

Rice seed sample collection

The study was conducted to evaluate the detection and identification of fungi associated with seeds of different varieties. Fifteen varieties of rice (8 coarse and 7 fine) collected from Rice Research Institute, Kala Shah Kaku.

Detection of Seed-borne Mycoflora on paddy

These samples assessed for the detection of seed borne fungi through blotter paper and potato dextrose agar method (ISTA, 2003). These seed lots surface sterilized with the 0.5 % $HgCl_2$. While other fifteen Petri plates used for surface sterilized seeds as 12 seeds in each Petri plate (11 seeds all around & 1 at the centre of the plate). The plated plates incubated for 7 days at 25 °C. After incubation fungi developed on each seed were examined under different magnifications of a stereomicroscope and were identified by colony, color, speculation type and available literature and percent incidence was recorded (Booth, 1971; Sutton, 1980; Mathur 2003).

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

Blotter paper methods

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 identified and counted.

Potato dextrose agar plate method

In this method the culture media was sterilized at 121ºC at pressure of 15 pounds per square inch for 15 minutes. Before pouring into Petri-plates streptomycin sulephate @ 30 µ gm/ml was added to warm media to check the bacterial growth. After cooling the media in Petri-plates seeds of different rice varieties plated immediately without washing the treated seeds. The Petri plates containing the rice seeds were kept in incubator at 25 ±1 °C and sufficient light 12 hours and dark in 12 hours. After 3-7 days, seed samples were studies under stereo microscope. Pathogenic as well as saprophytic fungi were identified and counted.

Effect of different seed dressing fungicides on seed germination and recovery of fungi

Different seed samples were collected in a tray having medium i. e. sand medium. Plastic trays (18 x 9) were used for this purpose both in treated and non treated trials. Forty seeds were sown in four lines in each plastic tray 10 seeds ⁄ line. 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 complete randomize design (CRD) and observe 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 are recorded. Detection of seed borne fungi through blotter paper method in course varieties *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* (9.733-16.667%), *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 fugus in all the samples tested with a range from 22.233-31.95% and *Alternaria alternata* (16.667-20.835%), *Aspergillus niger* (16.65-20.85%), *Rhizopus spp (*13.833-16.65%), *Fusarium moniliforme* (12.5-18.05%), *Aspergillus flavus* (9.733-19.435%), *Curvularia spp* (6.95-11.115%) was observed respectively. The prevalence of fungi varied with respect to variety and detection of seed samples collected. Higher infestation was recorded in agar plate method as compare to blotter paper method in course 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 23.6-45.835% followed by *Aspergillus flavus* (18.05-34.733%), *Rhizopus spp* (19.45-27.785%), *Alternaria alternata* (15.265-26.383%), *Fusarium moniliforme* (13.885-29.167%), *Aspergillus niger* (9.735-33.333%), *Helminthosporium spp* (6.935-26.383%) 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 34.716-45.835% followed by *Alternaria alternata* (25.00-36.115%), *Aspergillus niger* (19.45-36.115%), *Fusarium moniliforme* (20.835-26.386%), *Rhizopus spp* (18.05-29.15%), *Aspergillus flavus* (13.885-25.00%), *Curvularia spp* (4.167-26.7%) was observed respectively. The prevalence of fungi varied with respect to variety and detection of seed samples collected. Higher infestation was recorded in agar plate method as compare 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

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	Course Varieties				Fine Varieties			
	Agar Plate Method		Blotter Paper Method		Agar Plate Method		Blotter Paper Method	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
F1=Helminthosporium	28.11	22.233-31.95	17.014	13.883-20.85	40.47	34.716-45.835	13.886	6.935-26.383
F2=*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
F3=*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
F4=*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
F5=*Rhizopus 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
F6=*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
F7=*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 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) =6.594

methods and news paper method and conducted that blotter paper method was best for seed health testing. He also found that blotter paper method yielded higher incidence of pathogenic fungi. But in our results PDA method proved effective for the detection of pathogenic fungi. The results also resembled to work done by Tasleem et al *et al.* (2000) who detect 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 46.79and16.77%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 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 other (5.1%)*. The results resembled to the work done by Naeem- Khalid *et al.*, (2001) who investigated the incidence of mycoflora, their frequency and impact on seed germination of rice cultivars Dilrosh-97, Basmati 370, Basmati 385, Pakhal and IRRI-6 were investigated .the highest fungal incidence was observed in Dilrosh-97 while the lowest was observed in Pakhal. The remaining cultivars were recorded moderate incidence. Six field fungi viz*. Alternaria padwickii, Curvularia spp, Bipolaris oryzae, Cochliobolus miyabeanus, Fusarium moniliforme, Gibberella fjuikuroi, five storage fungi viz. Aspergillus niger, Aspergillus flavus, Penicillium spp, Rhizopus stolonifer, Chaeomium. This study strongly recommends the use of chemical treated seed in raising healthy nursery seedlings of rice.* Javed *et al.*, (2002) analyzed 873 paddy seed samples during 1994-2001 for routine health test. Standard blotter paper method was used and a working sample of 400 seeds was tested in replicates of 25 seeds per Petri dish. From these samples four known pathogenic fungi viz. *Alternaria, Cercospora, Drechslera oryzae and Fusarium* and 14 other genera viz. *Alternaria, Aspergillus, Chaetomium, Colletotrichum, Curvularia, Drechslera, Epicocum, Fusarium, Myrothecium, Penicillium, Phoma, Pyricularia, Rhizopus and Sclerotium* were recorded.

Course varieties are resistance varieties as compare to fine varieties. So maximam germination percentages were recorded in course varieties as compare to fine varieties. In course 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), Copperoxychloride (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) were 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 show the maximum germination of seedlings as caompare to control.

References

Anonymous, 2009. Economic Survey of Pakistan Govt. of Pakistan, Ministry of Food & Agriculture, Federal bureau of statistics. Islamabad**.**

Anonymous, 1994. IRRI rice facts. Int. Rice Res. Inst. Manila, Philippines. January, 1994.

Anonymous, 2007 a. Agricultural Statistics of Pakistan Government of Pakistan, Ministry of

Food, Agriculture and Livestock, Economics Wing, Islamabad.

Anonymous, 2007 b. Agricultural Indicators Federal Bureau of Statistics, Statistic Division, Ministry of Economic Affairs and Statistics, Government of Pakistan.

Bhutta, A. R. and Hussain, S. A. 1998. Seed-borne fungi associated with rice seed lots in Pakistan IRRN 23(3):26-27.

Bhutta, A. R. 1997. Comparison of cotton seed health testing method and their economics Pakistan Cotton. 32(3):146-153.

Bhalli, J. A., Aurangzeb, M. and Ilyas, M. B. 2001. Chemical control of bakanae disease of rice caused by Fusarium moniliforme. J. Biol. Sci., (1):483-484.

Booth, C. 1971. The genus Fusarium. Common Wealth Mycological Institute, Kew, Surry, England.

F. A. O., Production Year Book, 1993. (47):68-71.

Farid, K. M., Khalequzzaman, M. D. N. Islam, M. K. Anam and M. T. Islam, 2002. Effect of Fungicides Against Bipolaris oryzae of Rice Under In vitro Condition. Bio. Sci. (1): 4-7.

Fakir, G. A., I. Hossain, M. U. Ahmed, M. A. U. Doullah and Alam, M. 2002. Quality of farmers Boro and T. Aman rice seeds collected before sowing from Bogra, Rajshahi and Rangpur districts of Bangladesh. A paper presented in the review and planning meeting of the Rice Seed Health Improvement (SHIP), PETRRA project held on 17-18 April at BRRI, Gazipur, Bangladesh.

Franco, D. F., Ribeiro, A. S. Nunes, C. D. and Ferreira, F. 2001. Fungi associated with seed of irrigated rice in Rio Grande do Sul. Revista-brsileira-de-Agrociencia. 7(3):235-236.

Gill, M. A., Wahid, A., Javed, M. S. and Khan, T. Z. 1999. Major diseases of rice crop in the Punjab and their management strategies. In: Proc. 2nd Natl. Conf. Plant Patho. Sept. 27-29, Univ. Agri. Faisalabad

ISTA. 2003. International rules for seed testing, rules 2003 (Draper, S.R., Eds.) Zurich, Switzerland, ISTA, pp:1-521.

Javed, M. S., Wahid, A., Idrees, M., Gill, M. A. and Saleem, A. 2002. Seed myco-flora studies in rice. Pak. J. Phy. 14(2):132-134.

Khan, S. A., Anwar, S. A. and Bhutta, A. B. S1990. Studies on seed borne fungi, bacteria, and nematodes of rice in the Punjab. Pak. J. Sci. Ind. Res. 33(11): 489-492.

Khokhar, L. K. 1990. Bakanae and foot rot of rice in Punjab Pakistan IRRN, 15: 3(30).

Khan, J. A., Jamil, F. F. and Gill, M. A. 2000. Screening of rice varieties ⁄lines against bakanae and bacterial leaf blight (BLB). Pak . J. phyto-path. 12(1): 6-11.

Mathur, S. B. and Kongsdal, O. 2003. Common laboratory seed health testing methods for detection fungi, Published by the ISTA.

Naeem, K., Anwar, S. A., Haque, M. I., Riaz, A. and Khan, M. S. A. 2001. Seed-borne fungi and bacteria of rice and their impact on seed germination. Pak. J. Phy.13(1):75-81.

Rood, M. A. 2004. Bakanae in-field yield loss. Rice J. (15):8-10

Riaz, Z., Mitsueda, T. and Ahmad, Z. 1995. Seed-borne fungi of rice collected from Pakista. Plant Genetic-Resources-Newsletter- (103): 39-40

Sutton**,** B. C. 1980. The *Coelomycetes*, P. 696. Common Wealth Mycological Institute, Kew Surrey, England.

Tasleem, U. Z. K., Gill, M. A., Bajwa, K. M. and Khan, M. G. 2000. Screening of rice varieties/lines for resistant against Bakanae disease. Pak. J. Phytopathol. 11(2):166-168.

Wahid, A., Javed, M. S., Idrees, M. and Gill, M. A. 2001. Association of F. solani to rice seeds in the Punjab, Pakistan, Abstracts Published in 3rd Nat. Conf. Pl. Path. 1-3. October at NARC, Islamabad. 41pp.