International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 4, Issue 4 - 2017

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.04.001

Mycoflora associated with grain discolouration of common rice (*Oryza sativa* L.) cultivars and their management

Azher Mustafa and Muhammmad Mohsan*
Plant Pathology Research Institute Faisalabad
*Corresponding author

Abstract

Six fungal species *Alternaria alternata*, *Dreschelera oryzae*, *Curvularia oryzae*, *Fusarium moniliforme*, *Cercospora oryzae* and *Helminthosporium oryzae* has been found associated with discoloured panicles of different varieties in the field. *Helminthosporium oryzae* and *Alternaria alternata* were the predominant species on all the varities. Similarly among the twelve treatment including control, Nativo and Kumulus outclassed all the chemicals in reducing discoloured panicle by 49.59% and 51.34% respectively as compared to control 67.40%.and hence improveing the paddy yield. Kumulus was the best treatment in reducing grain infection.

Keywords: Mycoflora, Rice, treatment.

Introduction

In Pakistan rice is important cash crop of the country and overall national economy.Rice accounts 2.7% of the value added in agriculture and 0.6% of GDP. Rice ranks second amongst the staple food grain crops in the country and it has a major source of earnings. In recent years rice export from Pakistan is 1.92 million US dollar in 2012-13.Pakistan mainly export rice to UAE,Iran,Afghanistan, Kenya and Saudia Arabia. Rice growing area in 2012-13 is 2.341 million hectares with production 5.541 million tons with average yield 2398 Kg/hectare (Annonymous 2012-13).

The grain yield/unit area is reducing due to various factors among which diseases/biotic stresses are major causes of low yield. Rice crop is attacked by 76 various diseases. Rice crop in the field is attacked by different types of organisms including fungi and bacteria. As a consequence of seed born infection grains are discoloured. Fungi of different taxa especially ascomycetes and mitisporic fungi such as *Alternaria*, *Cladosporium*, *Colletotrichum*, *Diplodia*, *Fusarium* and *Cochliobolous* attack grains and legumes in the field having high moisture percentage in the seed (24-25%) are the main cause of grain discolouration. (Saini *et al* 2012and Nghiem 1993)

Grain discoloration of rice is a complex disease due to infection by certain microorganisms on the glumes, kernels, or both. The fungi that are reported to be associated with discoloration of grains are *B. oryzae, A. padwickii, Pyricularia oryzae, F. moniliforme, F. grminearum, Nigrosporaoryzae, Epicoccumnigrum, Curvularia spp, Phomasorghina, Dichotomophthoropsis nymphacearum* and *Heterosporium echinunulatum etc.* (Ou, 1985). The pathogens found associated with discolored grains has also been reported by Ilyas and Javaid (1995), Khan *et al*., (2000) and Javaid*et al*., (2002).

Symptoms of grain discoloration appears externally on the glumes or internally on the kernels, or both, the dots varied in size, shape and colors, and caused by a large number of fungi and bacteria associated Rice grain discolouration has a complex etiology and cannot be diagnosed prior to harvest. (Ou 1983, Pizzatti and Cortesi 2008).

Phat *et al*., (2005) reported that rice yield loss due to pests and diseases has been noticed more and more seriously. Grain discoloration is considered as one of popular problems. Rice grain discolouration is one of the most important rice disease all over the world. Its intensity varies according to season and locality (Ou 1985).Different factors such as lodging, frequent rains,high relative humidity and cloudy weather prevailing particularly from booting to maturity influence the grain discolouration

The disease has the potential to reduce the yield of rice as much as 75% in severely affected regions due to reduction in grain weight, floret sterility, inhibition of seed germination, reduction of stands, as well as the year-to-year transmission because of the seed borne nature of the pathogen (Trung, et al., 1993). The pathogen causing grain discolouration have direct influence on both quality and quantity of seeds. Infected seeds are not preferred for quality seeds posing a serious problem in seed certification and marketing.(Pham *et al.* 2001)

Different chemicals were used to control seed borne fungi of rice (Habib *et al.* 2012). Arshad *et al* (2005) tested different fungicides against fungi isolated from discoloured seed of different rice verities. Different fungicides for the management of grain discolouration of rice in the field (Adhikari and Bhowmick 2010).Although grain discolouration diseases has been a minor problem in the past. But in recent years it is becoming a serious problem if no attention is given to the disease. Keeping in view all the factors a field experiment was conducted to evaluate some commercially available fungicides for the effective management of the problems.

Materials and Methods

Rice panicles with discolored grains of different rice varieties were collected from field research areas of Rice Research Institute, Kala Shah Kaku. Discolored grains separated from their panicles and kept in Petri plates. Seeds were tested according to (ISTA 1996) (400 seeds per sample per treatment) method. Seeds were placed on blotter paper containing Petri plates (ten seeds per plate at equal distance) and incubated at 28°C for 4-5 days. The seeds were tested for infestation of pathogens under steriobinoucular microscope and under trinocular microscope under 100X magnification and identified with the help of literature Booth, 1971 ; Barnet and Hunter 1972.; Agarwal, 1989 ; Ellis, 1980).The frequency distribution of different pathogens was calculated as under;

$$\% \text{ Distribution Frequency} = \frac{\text{No of seed Infested with the pathogen}}{\text{Total no of seed plated}} \times 100$$

Field Trial

A field trial was conducted at the Rice Research Institute, Kala Shah Kaku during 2013-14.Commercially grown cultivar super nursery was sown at June 25, 2013 in the field area and transplanted on July 31, 2013 at a spacing of 22.5 cm x 22.5 cm using two seedlings/ hill. Standard agronomic practices were followed to raise the crop. Ten fungicides at their recommended doses were tested against untreated check in a randomized complete block design (RCBD) with three replicates keeping a plot size of 5 m x 2 m. Chemicals were sprayed thrice at booting, late dough stage. Data were recorded at 10 days after the last spray by taking random samples on % panicles affected meter square and grain infection percentage were recorded. One square meter area was selected and panicles were selected randomly. At harvesting grain yields were recorded randomly selected Plot of one square meter area. Data recorded was subjected to statistical analysis

Results

Sixty samples of different rice varieties were tested through blotter method. Total of six fungal species including *Alternaria alternata, Curvularia oryzae, Fusarium moniliforme, Dreschlera oryzae, Cercospora oryzae* and *Helminthosporium oryzae* with different detection frequency. *Alternaria alternata* and *Helminthosporium oryzae* were the predominant fungal species detected among the all fungi on almost all the samples tested (Table 1).

MYCOFLORA ASSOCIATED WITH DIFFERENT RICE CULTIVARS

Sr No	Name of variety/Line	Infected seed					
		Cercospora Oryzae	*Alternaria alternata*	*Helminthosporium oryzae*	*Dreschelera oryzae*	*Fusairium moniliforme*	*Curvularia oryzae*
1	Basmati 515	6.75	35.25	16.50	12.50	2.25	7.35
2	Super Basmati	4.50	14.50	7.75	6.25	5.25	11.25
3	Bas 198	5.50	9.75	8.75	9.75	-	4.25
4	Bas 385	3.75	16.25	11.75	15.25	2.75	6.25
5	Pk 386	2.85	12.75	13.25	17.50	.75	6.50
7	Bas 370	2.75	14.25	19.25	4.50	1.25	7.50
8	Basmati -2000	5.50	17.25	64.25	11.75	3.50	14.25
9	PK 8677-18-1-7-18	5.25	20.25	37.25	13.25	6.25	13.25
10	PK 8647-11-1-1	3.25	11.25	36.5	5.75	5.50	9.50
11	PK 8431-1-2-1-2-3	4.35	15.75	16.50	9.25	4.50	10.50
12	PK-8892-4-1-4-1	5.25	16.25	24.25	14.25	3.75	11.25
13	PK 8685-5-1-1-1	6.75	18.75	22.5	6.75	2.25	35.35
14	KSk 434	8.25	23.50	31.25	9.75	4.25	24.50
15	KSK 282	3.25	14.25	24.25	7.50	3.50	23.25

Based on these findings grain discolouration is complex in which different pathogens are involved. Similar association of various fungal species were found with samples from different location Arshad *et al* (2009). Also the association of different fungal species with discoloured paddy was reoprtedd by (Habib *et al* 2012.) Rice panicles infested with grain discoloration disease were collected from different rice areas and eight pathogens namely *Bipolaris oryzae, Alternaria alternata, Alternaria padwickii, Drechslera oryzae, Fusarium moniliforme, Curvularia oryzae, Nigrospora oryzae* and *Aspergillus niger* were isolated from diseased samples. (Arshad *et al* 2009.Siani 2012)The association of these pathogens with discolored grains has also been reported by different researchers Ilyas and Javaid (1995), Khan *et al*., (2000) and Javaid *et al*., (2002). The association of these fungi with seeds/grains suggests the transmission of pathogens through seeds, which cause rotting of seeds in the soil, seedling and inflorescence abnormality at later stage (Ou, 1985; Neergaard, 1970).

Results Field Trial

All the fungicides tested significantly reduced the incidence of grain discolouration and increased the grain yield over untreated check plot statistically at P>0.05% (Table 1). Among these, Nativo performed better in with lowest panicle infection (49.597%) and improved paddy yield (3.92 t/ha), as compared to untreated control (67.047 %) and 2.49 t/ha respectively. Similar behavior was observed in case of treatment Kumuls. The lowest decrease in incidence of panicle infection was observed in case of treatment Mancozeb. Remaining other fungicides showed intermediate response and statistically at par in reducing panicle infection as compared to untreated control. Grain infection also reduced and minimum grain infection percentage was found in case of treatment Kumulus.

Different fungicides Copperoxycholoride, Ditane M45, Thiophonate Methyl, Alert Plus and Trimaltox forte were tested to control seed borne fungi of different rice varieties was reorted by Habib*et al* (2010). Adhikari and Bhowmic 2010) reported that Tebuconazole, Propineb, Mancozeb were found effective against grain discolouration in the field experiment and improved paddy yield and combination of fungicides (Carbendazim+ mancozeb) which reduced panicle infection and improve paddy yield.

Similar type of findings was reported by different workers in an experiment on use of Dithane M 45 and Ceresan controlled some seed borne fungi of rice. Anvil with1-1.5 liter per hectare applied at 5-10 days after flowering was recommended to control grain discolouration (Hai 1996). Severity of grain discolouration was reduced by using Bonanaza 100 FL with 400 cc per hectare (Cuong 1998).

Table 1: Efficacy of different spray fungicides for the control of paddy grain discolouration

Sr. No	Treatments	Chemical Name	Dose/acer	Infected panicle	Infected grain	Yield (t/ha)
1	**Surpunch 12% WP**	Difenconazole+Validamycin	250gm	52.697CDE	30.667DE	3.687AB
2	**Nativo 75 WDG**	Tebuconazole	65gm	49.597E	31.667CDE	3.920A
3	**Castle 50% WP**	Kasugamycin+Copperoxycholoride	250gm	54.857BCDE	35.333BCDE	3.446BC
4	**Topsin-M**	Thiophonate methyl	250gm	51.340DE	30.333DE	3.3683BCD
5	**Kumulus 80WG**	Sulfer	800gm	49.597E	28.333E	3.5667AB
6	**Proway 45% EW**	Procholoraz	200ml	55.157BCDE	40.667BCDE	3.0657DE
7	**Mancozeb**	Etylenbisdithiocarbamate	250gm	61.477AB	45.667BC	3.1663CDE
8	**Score 250EC**	Difenconazole	125ml	52.083CDE	43.667BCD	3.5187BC
9	**Sulfex Gold 80% WDG**	Sulfer	800gm	58.623BC	45.667BC	2.9320E
10	**Gemstar Super 325SC**	Azoxystrobin+Difenconazole	120ml	56.843BCD	47.000B	3.0737DE
11	**Cordate 4WP**	Kasugamycin	300gm	56.487BCD	48.667B	3.1697CDE
12	**Control**	Water(H_2O)	100ltr	67.407A	67.667A	2.4900F
			LSD Value	6.656	14.713	0.3689

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How to cite this article:
Azher Mustafa and Muhammmad Mohsan. (2017). Mycoflora associated with grain discolouration of common rice (*Oryza sativa* L.) cultivars and their management. Int. J. Adv. Res. Biol. Sci. 4(4): 1-5.
DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.04.001