The Journal of Animal & Plant Sciences, 21(2): 2011, Page: 193-196
ISSN: 1018-7081

SEED-BORNE MYCOFLORA OF STORED RICE GRAINS AND ITS CHEMICAL CONTROL

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ABSTRACT

Stored grains of five varieties of rice (*Oryza sativa* L.) viz. KS-282, Basmati-385, Basmati-370, Basmati Kernal and Basmati-198 were studied to investigate the occurrence of seed-borne mycoflora using blotter paper method. There was 27%, 19%, 17%, 16% and 14% mycoflora found associated with the seeds of Basmati kernel, Basmati-385, Basmati-370, Basmati-198 and KS-282, respectively. Four fungal species namely *Fusarium moniliforme, Alternaria* sp., *Helminthosporium* sp. and *Curvularia* sp. were isolated from different test rice varieties. Four chemical fungicides namely antracal, topsin, mencozeb and derosal were used to investigate their effect on seed-borne mycoflora of rice. Seed treatment with different fungicides exhibited insignificant effect on the occurrence of *F. moniliforme* and *Alternaria* sp. Antracal completely stopped the growth of *Helminthosporium* sp. and *Curvularia* sp. The other three fungicides markedly suppressed the growth of *Helminthosporium* by 50%. Similarly, topsin and mencozeb suppressed the growth of *Curvularia* sp. by 50%.

Key words: Chemical control, rice, seed-borne mycoflora.

INTRODUCTION

Microorganisms play an important role in affecting the quality of seed, of which fungi are the largest group. These pathogens are disastrous as they reduce seed vigour and weaken the plant at its initial growth stages. Seed-borne diseases caused by fungi are relatively difficult to control as the fungal hyphae get established and become dormant. Many diseases of economically important crops are seed-borne like bakanae disease of rice (*Fusarium moniliforme* Sheld), loose smut [*Ustilago tritici* (Pers.) Rostrup], flag smut (*Urocystis tritici* Koern.), karnal bunt [*Neovossia indica* (Mitra) Mundkar] and ear cockle of wheat (*Anguillulina tritici* Gerv. & Bened.) (Javaid and Anjum 2006). If seed infected or contaminated by a pathogen that is also soil-borne, is sown in non-infested soil, the pathogen may be established in that soil. *Tilletia contraversa,* the cause of dwarf bunt of wheat, being both seed- and soil-borne, may be established in new areas through use of contaminated seeds (Neergaard, 1986). Apart from being seed-borne pathogens, fungi may grow on storage products. Most of the storage fungi are species of *Aspergillus* and *Penicillium.* These fungi may decrease seed germinability, cause seed disclouration, produce toxins that may be injurious to man and domestic animals, and may reduce seed weight also (Neergaard, 1986).

Rice is the world's most important food crop second to wheat, feeding over two billion people in Asia alone. Rice falls next to wheat as a staple food of Pakistan. It is also an important commercial crop and ranks as one of the major foreign exchange earners after cotton crop. It occupies about 10% of the total cropped area and on average 1/3 of its production is exported every year. About 2.2252 m ha are put under this crop annually with production potential of 4.4785 m tons and average yield of 2013 kg ha^{-1} (Anonymous, 2004), which is too low as compared to world's average and many rice growing countries of the world. Among several other factors contributing towards low yield of this crop in Pakistan, the most important are diseases. Most of the diseases of rice are carried though seed and cause enormous losses to the crop. Fungi including *Alternaria alternata, A. padwickii, A. longissima, Aspergillus niger, Curvularia oryzae, C. lunata, Drchslera oryzae, Fusarium miniliforme, F. semitectum, F. oxysporum, F. soalni, Pyricularia oryzae,* and species of *Phoma, Cercospora, Chaetomium, Sclerotium, Pecicillium, Myrothecium* and *Colletotrichum* have been isolated from seeds of different varieties of rice collected from different regions of the country (Wahid *et al.,* 1993, 2001; Khan, 1999, 2000; Javaid *et al.,* 2002). The present study was carried out to identify the fungi associated with seeds of different rice varieties and their chemical control.

MATERIALS AND METHODS

Isolation of seed-borne mycoflora of rice: Experiment was conducted in 2007. Seed samples of five rice varieties namely KS-282, Basmati-385, Basmati-370, Basmati Kernal and Basmati-198 were collected from Rice Research Institute, Kala Shah Kahoo, Pakistan. Two

hundred seeds of each sample were randomly selected. Seeds were surface sterilized with 0.1% mercuric chloride for 4 minutes followed by four washings with sterilized water. The surface sterilized seeds were transferred on three layered filter paper beds in 9 cm diameter sterilized Petri plates. There were 25 seeds in each Petri plates. Each treatment was replicated 8 times. Filter papers were kept moist with sterilized distilled water. The fungi appeared on rice seeds were isolated and identified. The percentage frequency of occurrence of various fungal species was calculated as follows:

$$\text{Frequency of occurrence (\%)} = \frac{\text{No. of seeds on which a fungal species occurs}}{\text{Total No. of seeds}} \times 100$$

Chemical control of seed-borne mycoflora of rice: Four chemical fungicides namely antracal, topsin, mencozeb and derosal were used to investigate their effect on seed-borne mycoflora of rice var. Basmati 385. Five hundred seeds of Basmati 385 were surface sterilized with 0.1% mercuric chloride. One hundred seeds were soaked in 0.2% solutions of each of the four chemical fungicides for 24 hours. Seeds for control treatment were similarly soaked in sterilized distilled water. Seeds of each treatment were transferred on potato dextrose agar medium in 9 cm diameter plates. There were four replicates of each treatment with 25 seeds in each replicate. Fungal colonies appeared on seeds in different treatments were isolated, purified and identified.

Statistical analysis: All the data were analyzed by one way analysis of variance followed by Duncan's Multiple Range Test to separate the treatment means at $P \leq 0.05$ (Steel *et al.,* 1997).

RESULTS AND DISCUSSION

Highest percentage of mycoflora (27%) was found associated with seeds of Basmati kernel that was significantly higher than mycoflora associated with all other rice varieties. There was 19%, 17%, 16% and 14% mycoflora found associated with the seeds of Basmati-385, Basmati-370, Basmati-198 and KS 282, respectively (Fig. 1).

Four fungal species namely *Fusarium moniliforme, Alternaria* sp., *Helminthosporium* sp. and *Curvularia* sp. were found associated with the seeds of different varieties of rice. There was a variation in percentage occurrence of different fungal species in seeds of different varieties of rice (Fig. 2). Earlier workers have reported *Pyricularia oryzae, Alternaria alternata, A. padwickii, A. longissima, Curvularia oryzae, C. lunata, Drchslera oryzae, Aspergillus niger, Fusarium miniliforme, F. semitectum, F. oxysporum, F. soalni,* and species of *Phoma, Cercospora, Chaetomium, Sclerotium, Pecicillium, Myrothecium* and *Colletotrichum* from seeds of different varieties of rice (Wahid *et al.,* 2001; Khan, 2000; Javaid *et al.,* 2002; Nguefack *et al.,* 2007). Highest percentage of *F. moniliforme* (11%) was recorded in seeds of Basmati kernel that was significantly higher than its occurrence in seeds of all other varieties. There was 6%, 4%, 5% and 3% occurrence of this fungal species in seeds of Basmati-385, Basmati-198, Basmati-370, and KS 282, respectively (Fig. 1A). Highest percentage of *Alternaria* sp. was also found in seeds of Basmati kernel (7.5%) followed by Basmati-385 (6%), Basmati-198 (6%), KS 282 (4.5%) and Basmati-370 (4%). Difference in occurrence of *Alternaria* sp. among the various rice varieties was insignificant (Fig. 2B). Likewise, highest percentage of *Helminthosporium* sp. was recorded in seeds of Basmati kernel (4%) followed by 3.5% in seeds of each of KS 282 and Basmati-370, and 2.5% in seeds of Basmati-385, Basmati-198. The difference in percentage occurrence of *Helminthosporium* sp. in seeds of different rice varieties was, however, insignificant (Fig. 2C). Highest percentage of occurrence of *Curvularia* sp. (4%) was recorded in Basmati kernel and Basmati-198 followed by Basmati-385 (3.5%), Basmati-370 (3%) and KS-282 (2.5%). Difference among the various rice varieties was insignificant (Fig.2D).

Data regarding the effect of various fungicides on the occurrence of various seed-borne fungal species is presented in Fig. 3. Seed treatment with different fungicides exhibited insignificant effect on the occurrence of *F. moniliforme* and *Alternaria* sp. In contrast, antracal completely stopped the growth of *Helminthosporium* sp. and *Curvularia* sp. The other three fungicides markedly suppressed the growth of *Helminthosporium* by 50%. Similarly, Topsin and mencozeb suppressed the growth of *Curvularia* sp. by 50%. Earlier studies also showed that different chemical fungicides exhibit variable effects on seed-borne mycoflora of rice (Ekefan *et al.,* 2006; Thobunluepop *et al.,* 2008).

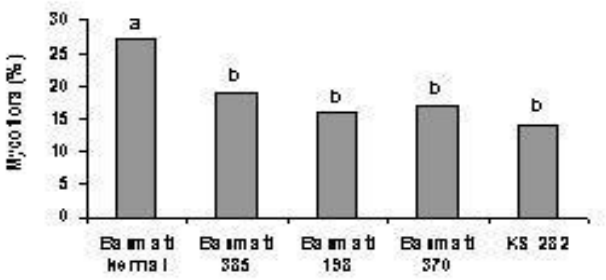

Fig. 1: Percentage mycoflora associated with different rice varieties. Values with different letters show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range test.

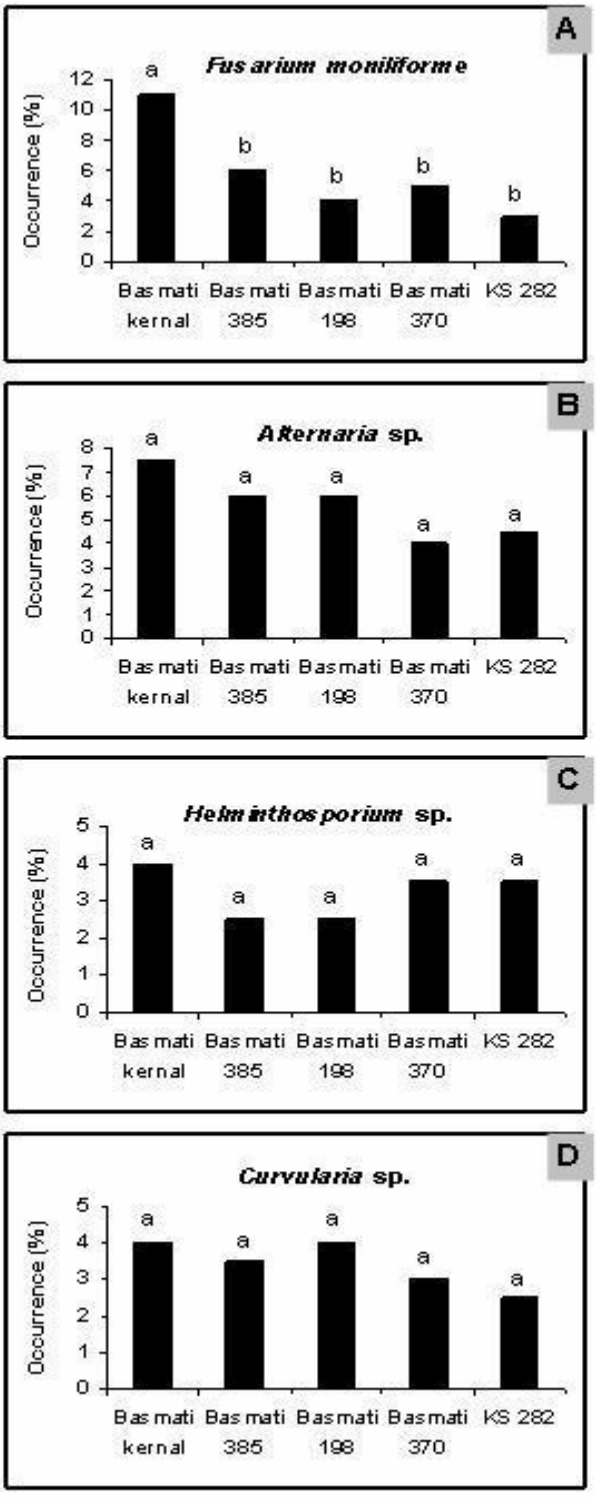

Fig. 2: Occurrence of different seed-borne fungi in five commonly cultivated varieties of rice. Values with different letters show significant difference (P≤0.05) as determined by Duncan's Multiple Range test.

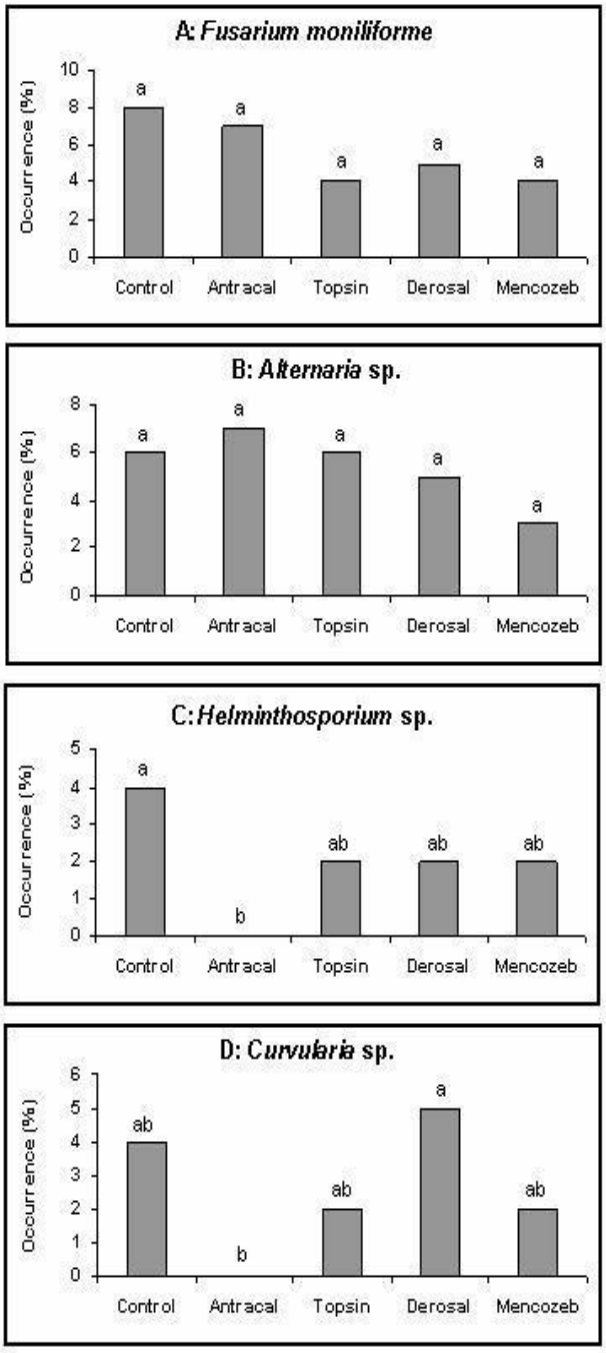

Fig. 3: Effect of four fungicides on seed-borne fungi in rice variety Basmati 385. Values with different letters show significant difference (P≤0.05) as determined by Duncan's Multiple Range test.

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