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INCIDENCE OF RICE GRAINS MYCOFLORA AT VARYING STORAGE INTERVALS IN DISTRICT SARGODHA, PUNJAB, PAKISTAN

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ABSTRACT

Background Seed quality has a leading role in agricultural productivity, and affected by different pathogenic fungi. These fungi cause abortion, rotting and necrosis in seeds which leads to poor germination and crop stand.

Methodology Identification and incidence of major seed borne mycoflora associated with rice grains in Sargodha district was assessed using blotter paper method.

Results Different pathogenic and saprophytic fungal species were found on rice seeds. After 15 days interval, pathogenic fungi *Bipolaris* spp. and *Fusarium moniliforme* were observed in the range of 5-52%, while, saprophytic species ranged from 5-45%. After 30 days interval, the trend of incidence of different fungi was same as it was after 15 days. However, after 45 days of storage period, incidence of saprophytic fungi was higher compared to pathogenic fungi. *Drechslera carobnum*, *Aspergillus flavus* and *Curvularia lunata* showed higher incidence of 45, 40 and 35%, respectively, while incidence of *Aspergillus candidus*, *Alternaria* spp., *Aspergillus niger* and *Botrytis* spp. remained low. Pathogenic fungi, *F. moniliforme* and *Bipolaris* spp. exhibited very low incidence of 8% and 2%, respectively. After 60 days of storage period, overall incidence of all fungi remained low.

Conclusion The presence of saprophytic fungi with rice grains indicated the risk of mycotoxins in the area while presence of pathogenic fungi showed the prevalence of inoculum of diseases caused by them which may become future threat in Sargodha district.

INTRODUCTION

Seeds have a leading role in agricultural productivity for providing food to humans and animals, as well as used for plants propagation. The cereal crops make about 90% of cultivated crops, and give tow-half of the universal per capita energy intake (Pimentel 2009). According to Gooding et al. (1981), among 19 major crops whose seeds are used for food, 15 are from the grass (Poaceae) and legume (Leguminosae) families. Cereal grains including barley, maize, millet, oats, rice, sorghum and wheat are the cheap source of proteins and carbohydrates.

Mycoflora infected seeds are shriveled, elongated, crumbled and could seem whitened and chalky (Reddy et al. 2010). Contamination may also cause seed decay, damping off and ultimately yield losses (Howell 2007). There are number of different *Fusarium* species which are responsible for mycotoxin production (Fuminosin) (Kritzinger et al. 2003). Grains mycoflora such as *Aspergillus flavus* consume carbohydrates of seeds and produce aflatoxin. Also, decrease the lipid and carbohydrate contents of wheat, soybean and fababean seeds (Aziz and Mahrous 2004).

Seed-borne microbial losses in the grains are not reported yet due to rare typical symptoms on seed surfaces (Nishikawa et al. 2006). It may differ with location and time depending on the storage and weather conditions (FAO 2016). The main factor involved in the spoilage of grains is moisture. In humid conditions, storage fungi attack the grains rapidly and cause serious losses to the grains. While, under storage conditions, the most isolated fungi are

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Aspergillus and *Alternaria* spp. in wheat (Rahman et al. 2018). Seeds are considered as a prime mean for transferring plant pathogens over long range and affect the growth and efficiency of crop plants (Weber et al. 2001).

Rice (*Oryzae sativa* L.) belongs to family Poaceae (true grass) having two domesticated species of genus *Oryzae*. The production of rice is increasing day by day, and it has become the staple food of 114 countries across the world. Rice having a total area of 168.8 million hectares, and only continent Asia consumes 90% of world's rice production (FAO 2016). In Pakistan, it is the most important cereal crop after wheat (GOP 2017).

Diseases constitute the most important factor responsible for the low yield and quality of rice in Pakistan. In rice, most of the diseases are carried through seed and cause a great loss in production. Different pathogens such as *Alternaria alternate, A. padwickii, Aspergillus niger, C. lunata, Fusarium miniliforme, Nigrospora oryzae, Drechslera oryzae, F. semitectum, F. oxysporum, F. solani, Pyricularia oryzae* and the species of *Phoma, Cercospora, Chaetomium, Myrothecium, Sclerotium, Penicillium* and *Colletotrichum* have been found to be associated with seeds of different varieties of rice collected from different parts of Pakistan (Khan et al. 2000; Javaid et al. 2002). Among these, the fungi, *Rhizoctonia solani, P. oryzae, F. moniliforme* and *Bipolaris oryzae* are pathogenic (Khan et al. 1990; Gill et al. 1999; Wahid et al. 2001).

Effective management of seed borne diseases of rice can be accomplished through the use of disease free seeds (Akter and Hossain 2015). Present research was designed to study the incidence of mycoflora on rice seeds of Sargodha market at different storage intervals.

MATERIALS AND METHODS

The experiment was conducted in the laboratory of Plant Pathology, College of Agriculture, University of Sargodha. The fungal contamination of different types were determined in accordance with their development on seeds using blotter paper method.

Collection and identification of seed samples

Seed samples of rice were collected from local grain market of Sargodha, Punjab, Pakistan. Seeds were disinfected with 1% sodium hypochlorite followed by three washings with distilled sterilized water. For disease incidence of rice grain mycoflora, 400 seeds were taken in total. Seventeen disinfected seeds of rice were placed on three layers of moist blotter paper in each plastic petri-plate and incubated at 24±2°C (ISTA 2005). For fungal growth, samples were observed after 15, 30, 45 and 60 days interval under stereomicroscope (Signaboubo et al. 2016). Fungi which were not identified directly from colony growth on seeds were sub-cultured on PDA plates and purified by hyphal tip culture technique. Identification was done on the basis of their colony growth, colony color, and morphological characters of fruiting bodies and spores/ conidia by using a compound microscope. Completely randomized design (CRD) was used for these experiments and each treatment contained three replications. Percent disease incidence (PDI) of different pathogens was recorded as under;

$$\text{PDI} = \frac{\text{Number of diseased seed}}{\text{Total number of seed examined}} \times 100$$

Error bar graphs were plotted using Microsoft excel 2013 to show the incidence of different mycoflora at varying storage intervals.

RESULTS AND DISCUSSION

After 15 days of storage period, variation in percent incidence of various mycoflora, both pathogenic and saprophytic, was observed. Pathogenic fungi *Bipolaris* spp. and *F. moniliforme* were observed in the range of 5% and 52%, respectively, while saprophytic species were observed in the range of 5-45% (Figure 1). Saprophytic fungus *A. candidus* showed maximum incidence of 45%, while, lowest incidence was observed in case of saprophytic fungus *Penicillium capsulatum* i.e. 5%. saprophytic fungi, *Alternaria* spp., *A. niger*, *A. flavus*, *C. lunata*, *D. carobnum* and *Mucor* spp. showed higher incidence compared to *Abisidia* spp., *Botrytis* spp., *Fusarium cladosporium* and *Rhizopus* spp. (Figure 1). After 30 days of storage period same trend was observed (Figure 2). However, after 45 days of storage period, incidence of saprophytic fungi was higher compared to pathogenic fungi (Figure 3). Fungi including *D. carobnum*, *A. flavus* and *C. lunata* showed higher incidence of 45, 40 and 35%, respectively, while, incidence of *A. candidus*, *Alternaria* spp., *A. niger* and *Botrytis* spp. remained low i.e. 18, 16, 12 and 15%, respectively. Pathogenic fungi, *F. moniliforme* and *Bipolaris* spp. exhibited very low incidence of 8 and 2%, respectively (Figure 3). After 60 days of storage period, overall incidence of all fungi remained low (Figure 4). Fungi *Alternaria* spp., *D. carobnum* and *Mucor* spp. showed maximum incidence of 24, 20 and 22%, respectively. While, second maximum incidence was observed in fungi, *A. candidus* (16%) *C. lunata* (14%) and *Cladosporium* spp. (12%). While, fungi *F. moniliforme*

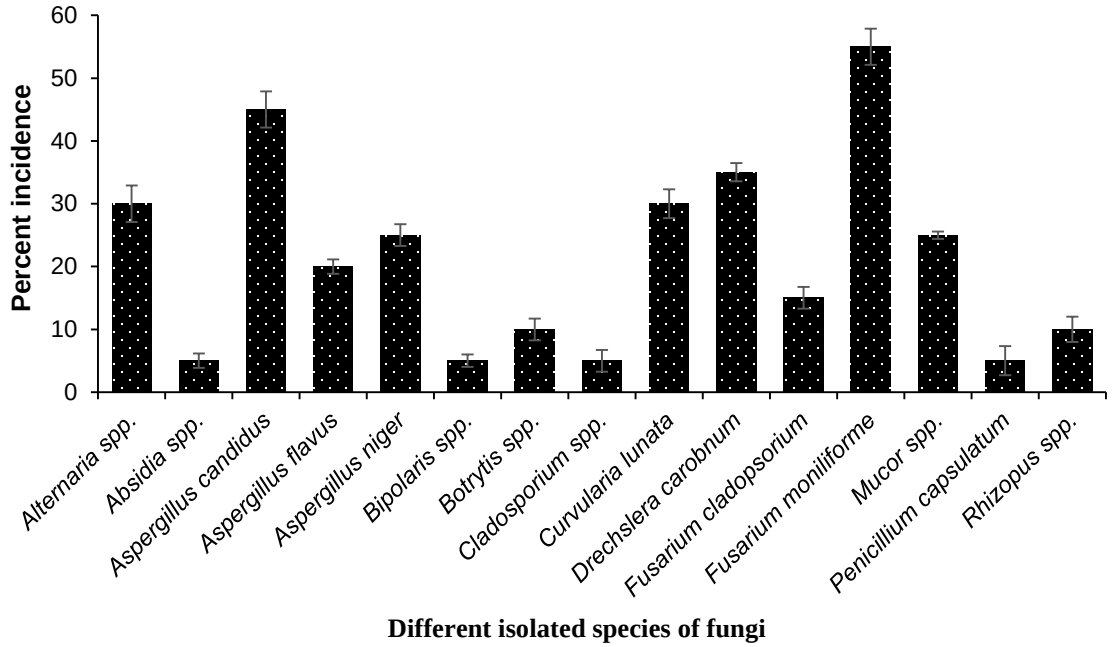

Figure 1 Percentage incidence of different fungi on rice seeds after 15 days storage period

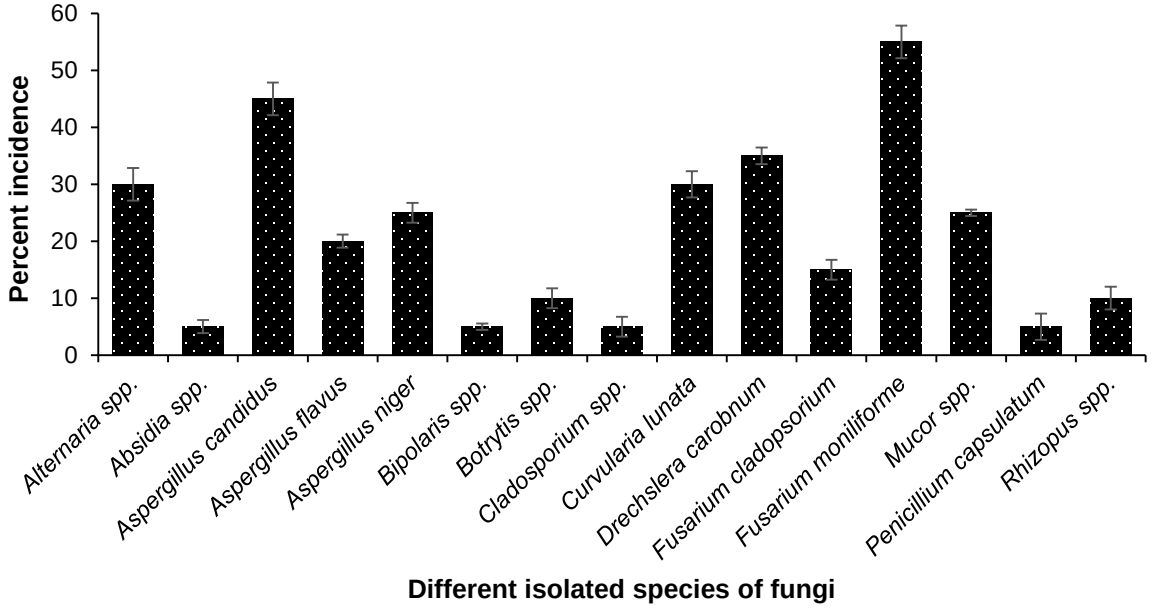

Figure 2 Percentage incidence of different fungi on rice seeds after 30 days storage period

and *Bipolaris* spp. showed very low incidence of 8% and 2%, respectively (Figure 4) . Rice is known as one of the major cereal crops in the world. It has been reported that rice suffers from 60 different diseases in the world. Among these, 27 are found to be seed borne (Fakir et al. 2002). In current study, microscopic characteristics were used for the identification of mycoflora associated with rice, while blotter paper technique was used for isolation (Mathur and Kongsdal 2003). The reason behind using this technique was its high efficiency and economically more reliability (Ahmad and Bhutta 1993; Fakhrunnisa et al. 2006). Our results confirmed that rice grains are highly attacked by different pathogenic

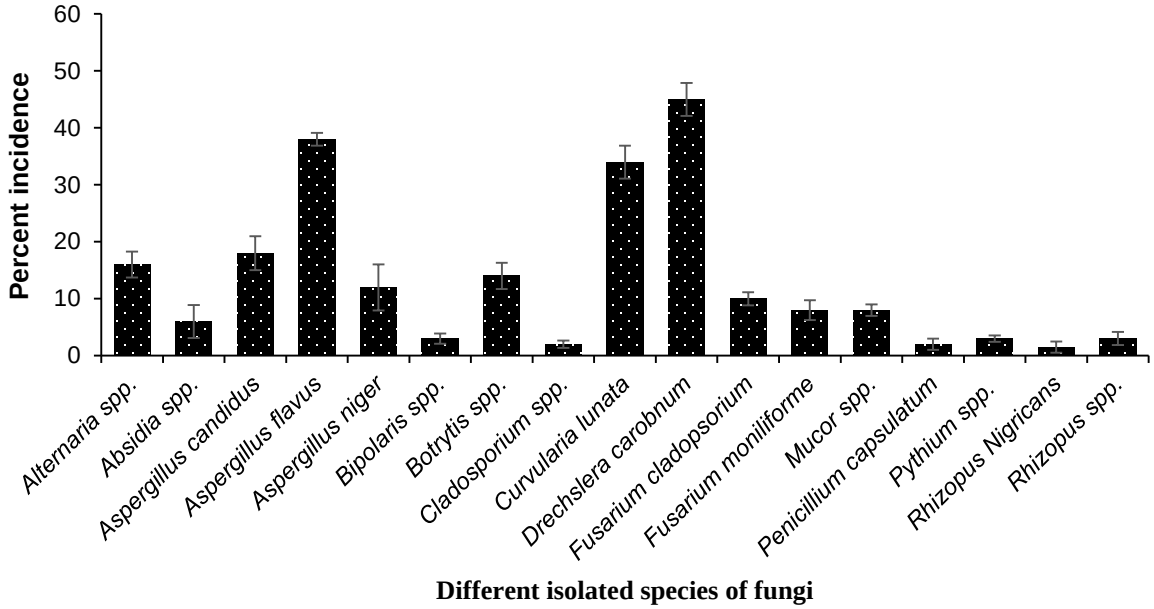

Figure 3 Percentage incidence of different fungi on rice seeds after 45 days storage period

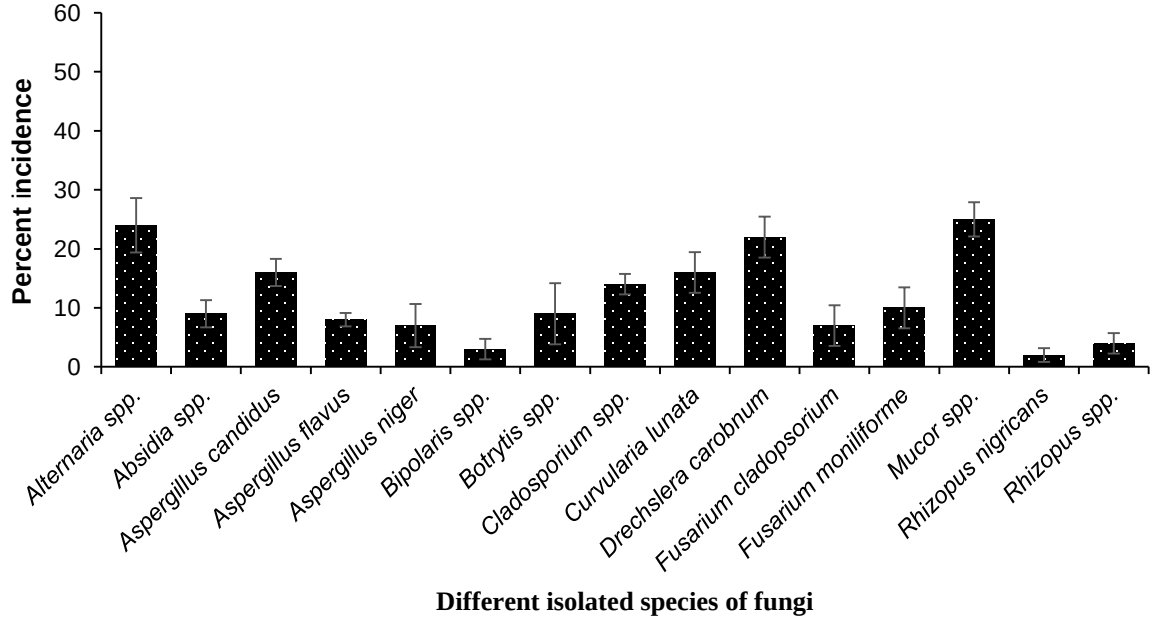

Figure 4 Percentage incidence of different fungi on rice seeds after 60 days storage period

and saprophytic fungi at different storage intervals. Similar results were reported by Ora et al. (2011). They reported that many seed borne fungi attacked cereal grains. They used blotter paper and agar plate methods for the identification of different seed borne pathogens from several rice hybrids and found that *Aspergillus* spp., *B. oryzae, C. lunata, Curvularia globosumm, F. moniliforme, Rhizopus stolonifer, Phoma* spp., *Penicillium* spp., *Alternaria tenuissima, N. oryzae,* and *Xanthomonas oryzae* were found to be the highest in all the rice hybrids. When compared the storage periods, it was observed that incidence of different fungi was higher during early storage periods compared to later. These results were in line with

previous results as reported by Barney et al. (1995), Malaker et al. (2008). Higher incidence of fungi at early storage periods might be due to high moisture level of seeds. Moisture level of seeds is always higher at the early stage of storage and decreased with the passage of time. Barney et al. (1995) also reported that incidence of fungi was more at initial storage period than the later due to higher moisture content of seed. Incidence of *F. moniliforme* was higher during early storage periods. This pathogen caused bakanae disease in rice (Sunder et al. 2014). Higher incidence of this pathogen on rice seeds may explicate partially the presence of this pathogen in rice belt of Sargodha. This fungus is reported to cause discoloration of rice seeds which may lead towards poor germination and seedling establishment, and consequently deteriorate the rice yield and quality (Roy and Baruah 1972). However, the fungus *P. oryzae*, the cause of rice blast, was not isolated at any storage interval. This showed that this fungus is not very common in rice growing areas of Sargodha. Saprophytes like *Aspergillus* spp. were also found frequent during isolation. Although, *A. flavus* produced aflatoxin, however, different species of this genus are also involved in deterioration of seed grains (Vidhysekaran et al. 1970). Another fungus *C. lunata* was also commonly isolated during present study. This fungus is reported to involve in kernel and glume discoloration in rice (Imolehin 1987). Hence, the isolated mycoflora, both pathogenic and saprophytic, have economic importance for seed grains of Sargodha. Similar results were reported by Ashraf et al. (2017).

CONCLUSION

The current study revealed that rice grains collected from Sargodha district were infested with pathogenic and saprophytic fungi which may deteriorate the quality of rice seeds. Further, pathogenic fungi, *F. moniliforme* and *Bipolaris* spp., found associated with rice grains, may become a potential threat if infested seeds with these fungi are used for rice plantation.

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