Distribution of Postharvest Mycoflora on Some Cereal Grains

Rajurkar S.K[a], Taware A.S[b]
[a]Department of Botany, Deogiri college, Station road, Aurangabad 431005, MS, India
[b]Department of Botany, Deogiri college, Station road, Aurangabad 431005, MS, India

Abstract

In India, though percentage of cereal yield has been increased during few years, infestation of fungi result in reduction of market value of grains. The present study deals with some associated fungi of Jowar, Wheat and Maize. Standard Blotter method and Czepakdox agar method was used for investigation. Seed borne mycoflora of Jowar, Wheat and Maize comprises number of fungal species *Aspergillus nige*r and *Rhizopus nigricans* exhibited higher percentage frequency and relative percentage. *Aspergillus flavus*, *Alternaria tenuis*, *Mucor* sp., *Penicillium* sp. were reported.

KEYWORDS: Cereal seeds, Seed mycoflora, Percentage frequency, Relative frequency

INTRODUCTION

Cereal grains are the excellent sources of most of the nutrients. The quality of grains is determined by various characteristics depend upon type of use and type of product. In India out of many cereals Wheat, Jowar, and maize has prime importance. India is the second most ranker in the production of Wheat and Rice, as well as a remarkable increase in yield of maize was observed in last few years (http://www.iisc.ernet.in/insa/ch21.pdf)

In the last two decades the nature and composition of utilization of sorghum grains has undergone a change from staple food to industrial uses such as livestock and poultry feed, potable alcohol, starch and ethanol production (Kleih *et al*. 2000).

By considering significance of all these grains it is necessary to retain their quality after harvest i.e. during storage. The major factor which affecting quality of grain, is the infestation of fungi on storage seed. The color and test of grain has been affected by attack of fungi which noticeably reduces its market value.

Fungi affect the quality of grain through increase in fatty acid, reduction in germination, mustiness and finally spoilage of grain. The importance of fungi is also due to production of toxins that causes health hazard in human and animals. Fungal development in grains is influenced by temperature humidity and period of storage. Survey of literature shows that a number of fungi viz., *Alternaria alternata*, *Aspergillus* spp., *Bipolaris maydis*, *Fusarium moniliforme*, *Fusarium* spp., *Cephalosporium* spp., *Helminthosporium* spp., *Mucor* sp., and *Penicillium* spp., have been reported from maize seed (Hafiz, 1986; Amin *et al*., 1985; Ahmad *et al.*, 1993; Anne *et al*., 2000; Niaz and Dawar 2009; Dasjardin *et al*., 2006; Mohammed *et al.*, 2001; Tulin & Askun, 2006).

Many times Infestation of fungi has been observed during storage, which proves hazardous for the seed. The associated fungi may be pathogenic, weak parasites or saprophytes. They may be associated internally or externally with the seed. Seed transmitted pathogens cause disease at various stages of crop growth from germination up to crop maturity and heavy losses have been observed caused by seed borne pathogens is

in various crops. By taking account of all these effects it is essential to search out fungi occurred commonly on the some cereal grains.

MEATERALS AND METHODS

Seed samples were collected from Aurangabad district of Maharashtra. The collected seeds were dried under sunlight to reduce the seed moisture and were stored in cloth bags at room temperature. The seeds were subjected for seed health test as per the recommendation of international seed health testing association (ISTS, 1976).

Standard Blotter method (SBM)

Blotter paper disc were dipped in sterilized distilled water and placed in sterilized petriplate. Excess of water removed from the blotter by inverting the plate. Ten seeds were placed using sterilized forceps in a laminar air flow hood. The seeds were incubated 20 ± 2^0C for 7 days in alternating cycles or 12 hours NUV light and 12 hr. darkness .The seed were examined on 7^{th} days for association under binocular microscope.

Czapek Dox Agar Method (CZA)

In this method, known quantity of agar (20 gm) , powder was dissolved in 1 liter of boiling distilled water along with 2 gm sodium nitrate, 30 gm sucrose , 1 gm potassium phosphate , 0.5 gm potassium chloride and 0.5 magnesium sulphate to prepare Czapek Dox Agar media. This media is then autoclaved at about 15Lbs at 40 ^{0}C for 15 min. after few minutes approximately 10- 15 ml of media was poured in to sterilized petriplates in the laminar air flow hood. After solidification seeds were placed in poured petriplate by using sterilized forceps. All seeds were previously surface sterilized with 1 % sodium hypochloride. Incubation procedure is similar to standard blotter method.

Screening of seeds for mycoflora

The incubated seeds were screened on seventh day by using stereo binocular microscope. The fungi on the seeds were identified by observing characteristic feature such as the form, length and arrangement of conidiophores, size, septation color, chain formation, conidia and their arrangement on the conidiophores, appearance of spore masses, characteristic of mycelium, density of colony etc.The frequency of fungi and relative percentage of particular species with in a genus of fungi was calculated using the formula of Ghiasian *et al.* (2004).

RESULTS AND DISCUSSION

In order to find out cereal seed mycoflora standard Blotter method and CZ agar method were used. In analysis three species of *Aspergillus, Alternaria alternata*, Sp. of Rhizopus and sp. of *Cladosporium* were observed on cereals seed by standard blotter method.

Standard Blotter Method

The present mycological investigation shows that in the Jowar seeds *Aspergillus niger* has highest percentage frequency (16%) as compared to other observed fungal species. In wheat seed mycoflora *Aspergillus flavus* showed higher percentage frequency (8%). The scenario is different in the maize seed mycoflora where *Alternaria alternate* (10%) and Rhizopus sp. has equal percentage frequency. Data revealed that *Aspergillus niger* had a lower degree infection on the wheat and maize seeds. It was noted from the data the *Cladosporium* sp. exhibited lower frequency percentage on maize seeds (Fig. I)

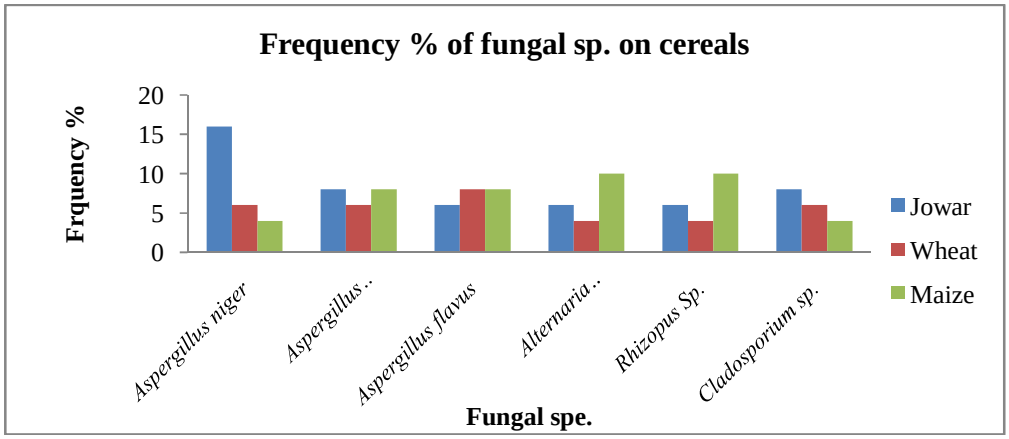

Fig. I: Observed frequency percentage of fungal species on cereals by Standard Blotter Method.

Mycological analysis of some cereal seed further revealed that relative percentage of *Aspergillus niger* (32 %) was high among observed fungi. When compared all relative percentage, it was observed that relative percentage of *Aspergillus fumigatus* was nearby same in Jowar, Wheat, Maize seeds. *Alternaria alternate*, *Rhizopus* sp., *Cladosorium* sp. showed nearly equal in all three cereal seeds (Fig. II)

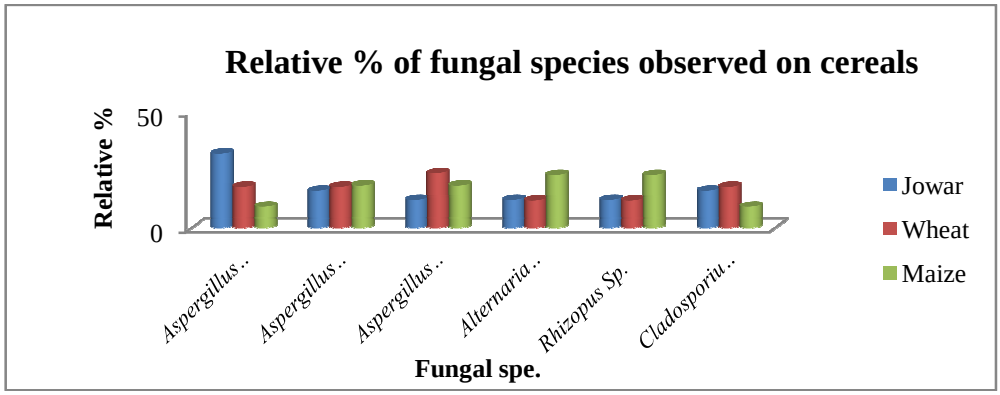

Fig. II: Observed Relative percentage of fungal species on cereals by Standard Blotter Method

Observations by CZ Agar Method

The genus Rhizopus was the most dominant fungi among nine different fungi reported in this study. The second most infestation was exhibited by A. flavus among all three cereals. Percentage frequency of Fusarium was higher in Maize and lower in Jowar and Wheat (Fig.III)

Mycological examinations showed that relative percentage of Rhizopus (30%) was highest in Jowar and wheat but the relative percentage of Fusarium (22.22%) was more in Maize. A. *wentii* observed only in maize and Alternaria alternate recorded only in wheat. A. *niger*, A. *fumigatus* and A. *wentii* have nearby same relative frequency in maize (Fig.IV).

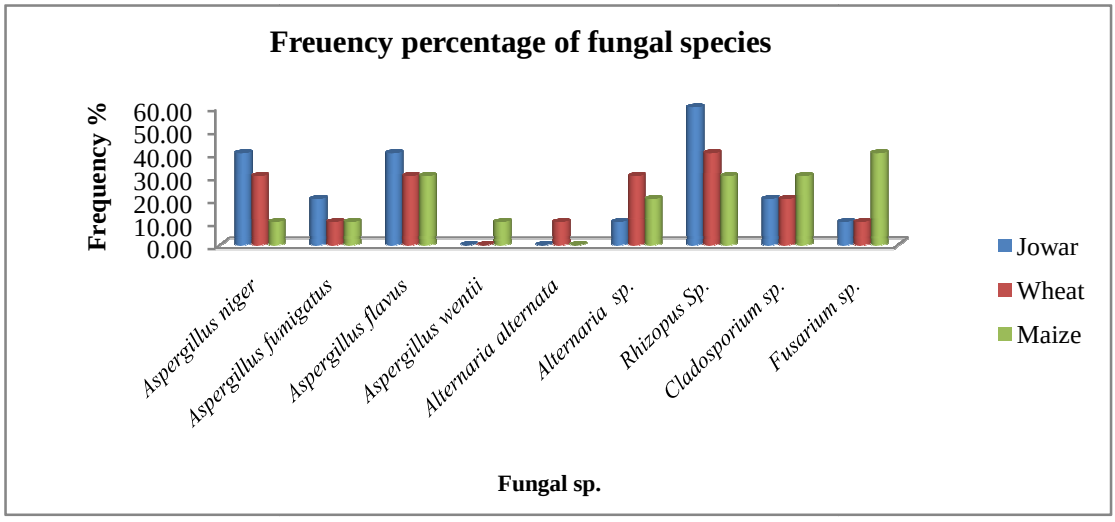

Fig III: Observed frequency percentage of fungal species on cereals by CZ Agar Method.

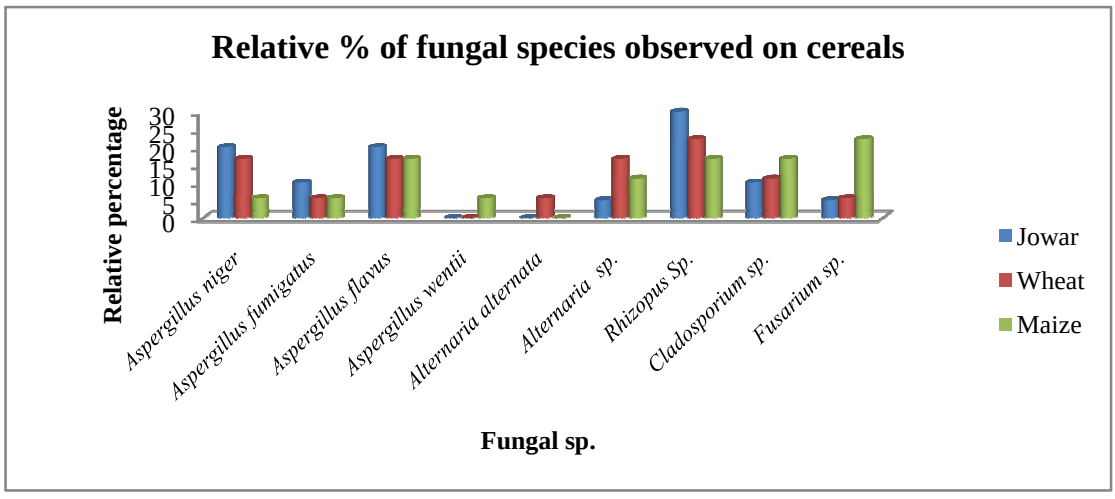

Fig IV: Observed frequency percentage of fungal species on cereals by CZ Agar Method

According to study of Sreenivasa *et al. (2010)* the genera *Fusarium* and *Aspergillus* were the most frequently isolated on sorghum grain. The other genera included *Alternaria, Phoma, Curvularia, Penicillium* and *Drechslera*. The data revealed a high frequency of *Fusarium* species (93.2%) and *Aspergillus* species (88.6%) with a relative percentage of 23.3 and 19.6% among the 19 fungal genera recorded, respectively. The predominant fungal species recorded at high frequency were F. *verticillioides* (88.6%), *A. flavus* (72.7%), *and F. anthophilum* (65.0%), *A. niger* (59.1%). In the present research *A. niger* exhibited dominance on the Jowar seeds by Standard blotter method, but in the CZ agar plate method *Rhizopus* exhibited dominance.

Some other studies also revealed that *Fusarium* and *Aspergillus* species are common fungal contaminants of maize and also produce mycotoxins (Bakan *et al.*, 2002; Verga *et al*., 2005; Anne *et al*., 2000; Curtui *et al.*, 1998 and Susan *et al.*, 2005). Isolated several *Fusarium* species from maize seed viz., *Fusarium moniliforme, F. graminearum, F. proliferatum, F. acuminatum, F. avenaceum, F. clamydosporium, F. equiseti, F. oxysporum, F. semitectum and F. torulosum* produce mycotoxins viz., Toxins deoxyninalenol (DON), 3-acetyl DON,15-acetyl DON, Fusarenon X(FX),T-2 Toxin (T2), Diacetoxyscir phenol (DAS), Zearalenon (ZEA), Fumonisin, Aflatoxin B1, Ochratoxin A(OA) and Citrinum.(CT) respectively.

When different fungi grow on cereals, they can reduce the germination along with the loss of carbohydrate, protein and oil content, the increase of moisture content, free fatty acids and thus reduce the dry matter content (Wilson *et al*. 1995). The fungal growth also causes discoloration of grain, heating, mustiness, dry matter loss, and production of several secondary metabolites such as mycotoxins, which are potentially dangerous to humans and animals (Christensen and Kaufmann 1969; Williams and McDonald 1983). Therefore studies on frequency and their relative percentage are highly useful and required for further studies on toxin producing fungi.

In accordance to the study of Niaz and Dawar S. (2009) about 70% of the maize seed samples were infested with *Aspergillus flavus, A. niger, A. wentii* and *Penicillium* spp. of the three methods used, agar plate method yielded the highest number of fungi as compared to blotter and deep freezing methods.

One of the studies was conducted to determine the fungi associated with wheat *(Triticum vulgare)*, rice *(Oryza sativa)*, maize *(Zea mays)*, soybean *(Glycine max)* and moong *(Vigna radiate)* in storage. Eight different fungi were isolated namely, *Aspergillus niger, Aspergillus flavus, Aspergillus nidulans, Penicillium, Rhizopus, Fusarium* and *Helminthosporium.* The results of the study revealed that among the fungi isolated, three strains have been reported to produce toxic compounds that may pose a serious threat to human health (Baskey *et al*. 2010).

It was understandable from the present study that cereal grains are subject to manifestation of various fungi and CZ agar method was more suitable as compare to blotter method as more number of fungi can be detected.

Data on the frequency and relative percentage of fungi would be of a great significance for predicting the extent of postharvest infection, colonization and subsequent deterioration of cereal grains. Further, it also helps to know the dry matter loss, nutritional changes and the extent of mycotoxin levels during storage. The high frequency and relative percentage of *Rhizopus* and *Aspergillus* species should be of primary concern for policy makers and food experts in this region to reduce the economic

losses caused by these fungi and also to minimize the exposure of human and animal life to the potential risks of mycotoxins.

Acknowledgement

All the authors are very much thankful to the secretary, M.S.P. Mandal and Principal, Deogiri College, Aurangabad for providing laboratory facilities for the present work.

REFERENCE

Ahmad. I., S. Iftikhar and A.R. Bhutta. (1993). *Seed-borne microorganism in Pakistan: Checklist* 1991. PARC, Islamabad. 32 pp.

Amin, M.H., A.N. Masud and F.S. Farhat. (1985). Fungal spoilage of wheat rice, maize, sorghum and their control. Nuclear Istitute for Agriculture and Biology, Faisalabad. Pakistan. 202 pp.

Anne, E., D. Gyanu, M. Ronald, D. Plattner, C.M. Maragos, K. Shrestha and S.P. MeCormick. (2000). Occurrence of *Fusarium* species and mycotoxins in Nepalese maize and wheat and the effect of traditional processing methods on mycotoxin levels. *J. Aagric. Food Chem*., 48(4): 1377- 1388.

Bakan, B., D. Richard, D. Molard and B. Cahagnier. (2002). Fungal growth and *Fusarium* mycotoxin content in isogenic traditional maize and genetically modified maize grown in France and Spain. *J. Agric. Food Chem*., 50(4): 278-731.

Baskey M., Kumari N., Bedi S. (2010) Mycoflora associated with stored grains and legumes and its public health Importance Industrial Microbiology Explore, 2010, 74–76 Vol. II

Christensen C.M., Kaufmann H.H. (1969). Grain Storage: The Role of Fungi in Quality Loss. University of Minnesota Press, Minneapolis, USA: 36–108.

Curtui V., Usleber E., Dietrich R., Lepschy J. and Martlbauer E. (1998). A survey on the occurrence of mycotoxins in wheat and maize from western Romania. *Journal of Mycopathologia, 143*(2): 97-103

Desjardins A.E., Busman M., Proctor R. and R.J. Stessman. (2006). Wheat kernel black point and fumonisin contamination by *Fusarium proliferatum* [abstract]. National *Fusarium* Head BlightForum Proceedings. 115 pp.

Mohammed, S.S., Abdul-Malik A.Y., Abdul-Sattar M.A. and Al-Abssy A.A.M.. (2001). Mycobiota and Mycotoxin of stored imported wheat Grains in *Egypt*. Botany Department, Faculty of Science, Assiut University, Assiut, Egypt, Biology Department, Faculty of Science, Taiz University, Republic of Yemen.

Ghiasian S.A., Kord-Bacheh P., Rezayat S.M., Maghsood A.H., Taherkhani H. (2004) .Mycoflora of Iranian maize harvested in the main production areas in 2000. *Mycopathology* 158 (1): 113–121

Hafiz, A. (1986). *Plant diseases*. Directorate of publication, Pakistan Agricultural Research Council, Islamabad. 552 pp.

ISTA (1976) International rules for seed testing .Annexer seed Sci, Technol., 4: 5(10; 39)

Kleih Ulrich, Bala Ravi S and Dayakar Rao B. (2000.) Industrial utilization of sorghum in India. Working Paper Series no. 4, Socioeconomics and Policy Program.Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 38 pp.

Niaz I. and Dawar S. (2009) detection of seed borne mycofloraIn maize (*Zea mays* l.) *Pak. J. Bot*., 41(1): 443-451.

Prasada R and Prabhu A.S., (1962.) Leaf blight of wheat caused by a new species of Alternaria. Indian Phytopathology 15, 292-293.

Sreenivasa M. Y., Dass R. S., Janardhana G. R.(2010) Survey of postharvest fungi associated with sorghum grains produced in Karnataka (India). *Journal of Plant Protection Research* vol. 50, no. 3 (2010) DOI: 10.2478/v10045-010-0057-6

Susan, J.M., S. Anderson and P. Brereton. (2005). Determination of Zearalenone in Barely, Maize and Wheat. *Journal of AOAC International*, 88(6): 1733-1740.

Tulin, J.M. and D.I. Askun. (2006). Investigation of Fungal Species Diversity of Maize Kernels. *Journal of Biological Sciences*, 6(2): 275-281.

Verga, B. Toth and J. Teren. (2005). Mycotoxin producing fungi and mycotoxins in foods in Hungary. *Journal of Acta Alimentaria/Akademiai*, 267-275.

Wilson J.P., Cooper H.H., Wilson D.M. (1995). Effect of delayed harvest on contamination of pearl millet grain with mycotoxin producing fungi and mycotoxins. Mycopathology 132 (1): 27–30.

Williams R.J., McDonald D. (1983). Grain moulds in the tropics: problems and importance. Ann. Rev. Phytopathology. 21: 153–178.