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Mycological Evaluation of Maize Grains Produced in Karnataka (India) for the Post Harvest Fungal Contamination

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Abstract: Maize is an important cereal produced and exported from Karnataka, India. The risk of contamination by mycotoxins in maize is related to the high incidence of fungi. This paper reports on the identification of post harvest fungi of maize grains harvested in Karnataka state in 2004 and 2005. A total of 86 maize samples were analyzed for frequency and relative density of internal mycoflora by direct plating method on PDA and MGA 2.5 agar medium. The most prevalent fungal genera occurring on maize grains were species of *Fusarium* and *Aspergillus*. The other genera included *Penicillium, Drechslera, Nigrospora, Curvularia, Alternaria*, *Chaetomium* and *Phoma*. The data revealed the high frequency of *Fusarium* species (96.5%) and the high relative density of *Aspergillus* species (41.7%) among the 17 fungal genera recoded. The predominant fungi recorded *F. verticillioides*, *F. anthophilum*, *F. proliferatum*, *A. flavus*, *A. niger* and *A. ochraceous* respectively. These data is of immense value for assessing the possible health hazards in humans and animals upon consumption of such contaminated food grains by toxigenic moulds.

Key words: *Aspergillus* · *Fusarium* · Frequency · Maize · Relative density

INTRODUCTION

India is predominantly an agricultural based country with nearly three fourths of the people dependent on agriculture. India with its diverse agro climatic conditions produces a variety of food crops throughout the year. Maize (*Zea mays* L.) is the third most important crop after rice and wheat. Most of the maize produced in India, mainly comes from the states of Karnataka andhra Pradesh, Bihar, Punjab, Uttar Pradesh and Madhya Pradesh [1]. Karnataka is the leading producer and exporter of maize as it falls under the maize belt of India and produces 15% of India's total produce [2].

Maize is used in more ways than any other cereals by humans, as a feed grain, as a fodder crop and for hundreds of industrial purposes. Its grain, stalk, leaves, cobs and tassel have commercial value. It is also the main cereal feed ingredient, which constitutes 30-35% of poultry rations [3]. However, maize kernals are prone to pre and post-harvest fungal infection and it is often unavoidable and it is a worldwide problem. The susceptibility of maize grains to various fungi has been very well documented [4].

A number of fungal species associated with maize belongs to the genera *Fusarium*, *Aspergillus* and *Penicillium*, which have been known to produce mycotoxins that cause mycotoxicosis in animals and humans. In some grain-producing countries, the mycoflora of freshly harvested grains has been very well documented [5-9]. There is a lack of accurate data on the frequency and relative density of *mycoflora* in maize grains. Because of these reasons, it has not been possible to develop effective management strategies to prevent fungal infection and bio-deterioration of grains. Hence, this study was undertaken to know the frequency of mycoflora and their relative density in maize grains produced in different districts of Karnataka, India.

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MATERIALS AND METHODS

Collection of Samples: A total of 86 maize samples (approximately 1000g) from 15 districts of Karnataka state were collected from markets, local stores, agricultural cooperatives and farm fields (after the harvest) during the three harvest seasons of 2004 and 2005. Samples were brought to the laboratory in sterile plastic bags and kept at 4°C. All the samples were subjected to mycological analysis.

Mycological Analysis: To know the frequency and relative density of fungi, maize kernels were plated on potato dextrose agar (PDA) medium by agar plating method [10]. Since, PDA medium was unsuitable for the isolation of *Fusarium* species from the natural sources, MGA 2.5 medium as recommended by Bragulat *et al.* [11] was also used. Two hundred (200) maize kernels from each sample were surface sterilized with 2% sodium-hypochlorite solution for 3 min. and rinsed twice with sterile distilled water. Samples were then plated on PDA and MGA 2.5 agar plates at the rate of 10 kernels per plate. The plates were incubated under alternating periods of 12h darkness and 12h of daylight at 25±2°C for 7 days.

Identification of Fungi: The fungal colonies on maize kernels were visualized using stereo-binocular microscope (Magnus MS24). Representative isolates of fungal species were transferred onto Potato Dextrose Agar (PDA) and Spezieller Nahrstoffarmer Agar (SNA) medium to study the macro and micro morphological characteristics. All the isolates of *Fusarium* species were identified up to the species level by using fungal keys and manuals [12, 13, 14]. The isolation frequency and relative density of each fungal species was calculated as per the procedure of Ghiasian *et al.* [15].

Statistical Analysis: The Statistical Presentation System Software (SPSS) was used to calculate the means, standard errors and standard deviations. One-way ANOVA was applied to the data to determine differences.

RESULTS AND DISCUSSION

In the semi-arid tropics of Asia and Africa people depend on cereals such as sorghum, maize, wheat etc. as their staple diet. The quality of these grains is often lowered, following colonization by fungi with subsequent mycotoxin contamination [16, 17]. Due to non-scientific agricultural practices, intermittent rain at the time of harvest and poor storage facilities, grains are pre-disposed to fungal infection and grain bio-deterioration [18]. The grain bio-deterioration is a dynamic process, which leads to the loss of physical and nutritional qualities, which eventually render grains unsuitable for human consumption. Throughout the world, much attention has been given to know the frequency and relative density of mycoflora occurring on maize grains. However, data on frequency and relative density of fungal species on maize grains is very limited in Karnataka, India.

Mycological examination of 86 maize samples collected from different districts of Karnataka by agar plating method revealed the occurrence of 17 mycoflora such as *Fusarium, Aspergillus, Curvularia, Alternaria, Penicillium, Drechslera, Chaetomium, Phoma, Cladosporium, Nigrospora, Epicoccum, Trichothecium, Rhizopus, Trichoderma, Mucor, Paecilomyces* and *Geotrichium* species. The most dominant fungal genera were *Fusarium* and *Aspergillus* with the frequency 96.5% and 94.2 % respectively. The fungal genera such as *Penicillium* (77.9%), *Nigrospora* (48.8%), *Chaetomium* (37.2%), *Drechslera* (25.6%) and others were also isolated. The species of *Aspergillus* was the predominant mycoflora with 41.7% relative density. The species of *Fusarium* (27.5%) and *Penicillium* (13.7%) were the other most important fungal genera recorded with high relative density (Fig. 1). The relative density of these fungi indicated the extent of pre-harvest contamination and relative damage to physiological and biochemical quality parameters of maize kernels. These fungi when grow on kernels, can reduce the germination along with loss of carbohydrate, protein and oil content, increases the moisture content, free fatty acid and thus reduce the dry matter content [16]. The fungal growth also causes discolouration of grain, heating, mustiness, dry matter loss and production of several secondary metabolites such as mycotoxins, which are potentially dangerous to humans and animals [5, 18].

The incidence of *Fusarium* species on agar (MGA 2.5) revealed the occurrence of nine different *Fusarium* species with high frequency and relative density. The study showed that, high frequency of *F. verticillioides* (86.0%), *F. anthophilum* (60.5%), *F. proliferatum* (42.2%) and *F. pallidoroseum* (39.5%) respectively. The low frequency o f *F. graminearum* and *F. solani* (4.7% and 3.5%) were also recorded.

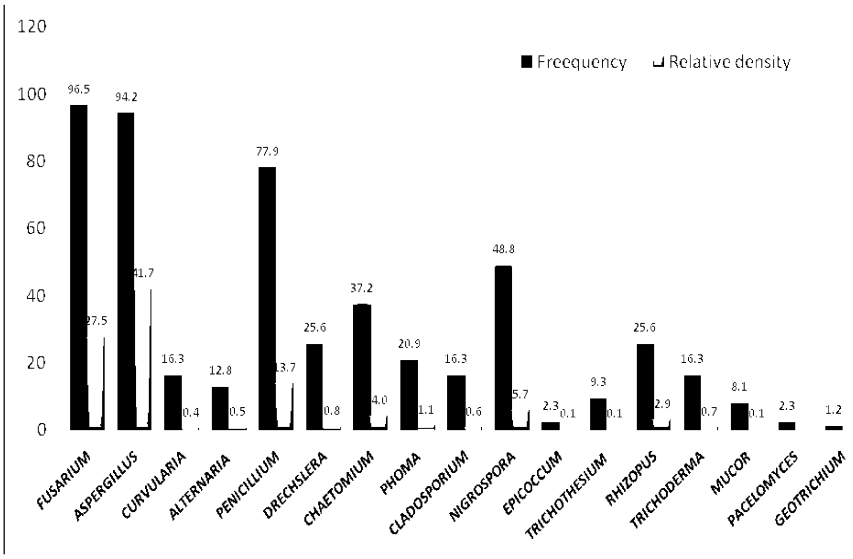

Fig. 1: Fungal genera isolated from maize samples collected from Karnataka, India

Table 1: *Fusarium* species isolated from maize samples collected from Karnataka, India

Sl. No.	Name of the Fungus	Total No. of Isolates	Frequency	Relative density
1	*F. verticillioides*	2308	86.0	44.5
2	*F. proliferatum*	1475	44.2	28.5
3	*F. oxysporum*	12	08.1	00.2
4	*F. anthophilum*	1001	60.5	19.3
5	*F. pallidoroseum*	223	39.5	04.3
6	*F. sporotrichioides*	74	23.3	01.4
7	*F. solani*	4	03.5	0.10
8	*F. decemcellulare*	75	08.1	01.4
9	*F. graminearum*	6	04.7	00.1

Table 2: A *spergillus* species isolated from maize samples collected from Karnataka, India

Sl.No.	Name of the Fungus	Total No. of Isolates	Frequency	Relative density
1	*A. flavus*	3632	84.9	46.2
2	*A. niger*	3744	83.7	47.6
3	*A. flavus columnaris*	90	19.8	01.1
4	*A. versicolor*	75	18.6	01.0
5	*A. candidus*	111	09.3	01.4
6	*A. fumigatus*	11	04.7	00.1
7	*A. ochraceous*	177	31.4	02.3
8	*A. nidulans*	5	02.3	00.1
9	*A. oryzae*	20	03.5	00.3
10	*A. tamari*	1	01.2	0.01

Similarly *F. verticillioides* was the predominant *Fusarium* species recorded with 44.5% relative density. *F. proliferatum* (28.5%) and *F. anthophilum* (19.5%) were the other *Fusarium* species recorded with high relative density (Table 1). It should be noted that, *F. verticillioides* was isolated from almost all the samples with high frequency (86.0%). Similar data has been obtained in the State of Parana, Brazil where they detected 98.7% incidence *Fusarium* species on corn [19]. In the same region, *F. moniliforme* (=*F. verticillioides*) was the most frequently isolated fungal species, followed by *F. proliferatum, F. subglutinans* and *F. graminearum* respectively. In Italy, Logrieco *et al*. [20] reported the occurrence of *F. moniliforme* in pre-harvest maize ears (yellow hybrids) and it was the predominant species in infected ear kernels. *F. moniliforme* is considered to be

Table 3: Other fungal species isolated from maize samples collected from Karnataka, India

Sl. No.	Name of the Fungus	Total No. of Isolates	Frequency	Relative density
1	*Curvularia lunata*	40	08.1	51.9
2	*Curvularia* spp.	37	08.1	48.1
3	*Drechslera oryzae*	137	19.8	86.2
4	*Drechslera* spp.	22	05.8	13.8
5	*Chaetomium indicum*	256	20.9	33.5
6	*Chaetomium globosum*	189	12.8	24.7
7	*Chaetomium* spp.	319	32.6	41.8
8	*Phoma lingam*	15	04.7	07.1
9	*Phoma* spp.	196	17.4	92.9
10	*Cladosporium cladosporioides*	26	02.3	21.5
11	*Cladosporium herbarum*	3	01.2	02.5
12	*Cladosporium* spp.	92	15.1	76.0
13	*Nigrospora oryzae*	313	26.7	29.1
14	*Nigrospora* spp.	763	38.4	70.9
15	*Rhizopus stolonifera*	139	07.0	25.0
16	*Rhizopus nigricans*	5	01.2	00.9
17	*Rhizopus* spp.	411	20.9	74.1
18	*Trichoderma harzianum*	3	02.3	02.4
19	*Trichoderma viride*	37	07.0	29.1
20	*Trichoderma* spp.	87	12.8	68.5

the most common *Fusarium* species occurring in tropical and subtropical climates and the most prevalent fungus associated with corn in USA [4]. The survey conducted worldwide also showed that, *F. verticillioides*, *F. proliferatum* and *F. anthophilum* are most frequently isolated species in maize and are able to produce fumonisins [7-9].

In the present investigation, the predominant *Aspergillus* species isolated as internal mycoflora were *A. flavus* (84.9%), *A. niger* (83.7%), *A. ochraceous* (31.4%), *A. flavus columnaris* (19.8%) and *A. versicolor* (18.6%) respectively. The low frequency *A. nidulans* (2.3%) and *A. tamari* (1.7%) was also recorded. The data on relative density showed that *A. niger* was the predominant *Aspergillus* species with 47.6% followed by *A. flavus* (46.2%) (Table 2). Further, mycological analysis of maize samples for the other internal mycoflora revealed that, *Nigrospora* species (38.4%) was recorded followed by *Chaetomium* species (32.6%). other fungal species such as *Rhizopus* sp. (20.9%), *Drechslera oryzae* (19.8%), *Phoma* sp. (17.4%), *Cladosporium* sp. (15.1%) and *Trichoderma* sp. (12.8%) were also recorded with lower frequency (Table 3). The fungal species frequencies and relative density are given in the Table 1, 2, 3. Similar reports were also available on incidences of fungal species on various cereals [7, 9, 21-25]. Almeida *et al.* [24] reported that *Fusarium*, *Penicillium*, *Aspergillus* and two other filamentous fungal genera were found associated with corn kernels. Ghiasian *et al.* [15] reported that, predominated fungal species such as *Fusarium* (38.5%), *Aspergillus* (8.7%), *Rhizopus* (4.8%), *Penicillium* (4.5%), *Mucor* (1.1%) and four other fungal genera on cereals. Although *Aspergillus* species considered as storage fungi, these can also occur in field conditions. The presence of *A. flavus* in freshly harvested maize was previously observed by Almeida *et al.* [24]. The data on the frequency and relative density of fungi would be of great significance for predicting post-harvest deterioration and extent of mycotoxins contamination in maize grains produced in Karnataka, India. Fungi associated with maize in Karnataka are of serious concern due to their toxigenic potential. The data on the frequency and relative density presented in the present study would be of great importance in this region for predicting the extent of post-harvest infection, colonization, deterioration and extent of mycotoxins in maize grains. This data is of immense value for assessing the possible health hazards in humans and animals upon consumption of such contaminated maize grains by toxigenic moulds. The results of this study are highly useful for further studies on toxin producing fungi and their epidemiological significance in maize crops grown in Karnataka and elsewhere in India.

REFERENCES

1. Joshi, P.K., N.P. Singh, N.N. Singh, R.V. Gerpacio and P.L. Pingali, 2005. Maize in India: Production systems, constraints and research priorities. pp: 1-50. D.F. CIMMYT. Mexico.
2. Anonymous, 2003. Directorate of Economics and Statistics, Government of Karnataka, Bangalore, Karnataka, India.
3. Kleih, U., S. Bala Ravi, B. Dayakar Rao and B. Yoganand, 2000. Industrial utilization of sorghum in India, 4, International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, AP, India.
4. Munkvold, G.P. and A.E. Desjardins, 1997. Fumonisins in Maize: Can we reduce their occurrence? Plant Disease, 81(6): 556-565.
5. Williams, R.J. and D. Mcdonald, 1983. Grain moulds in the tropics: problems and importance. Annual Reviews in Phytopathol., 21: 153-178.
6. El-Maghraby, O.M.O., I.A. El-Kady and S. Soliman, 1995. Mycoflora and *Fusarium* toxins of three types of corn grains in Egypt with special reference to production of trichothecine toxins. Microbiological Res., 150: 225-232.
7. Gonzalez, H.H.L., S.L. Resnik, R.T. Boca and W.F.O. Marasas, 1995. Mycoflora of Argentinian corn harvested in the main production area in 1990. Mycopathologia, 130: 29-36.
8. Placinta, C.F., J.P. D'Mello and A.M. Macdonald, 1999. A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. Animal Feed Science and Technol., 78: 221-237.
9. Pacin, A.M., H.H.L. Gonzalez, M. Etcheverry, S.L. Resnik, L. Vivas and S. Espin, 2002. Fungi associated with food and feed commodities from Ecuador. Mycopathologia, 156: 87-92.
10. Mathur, S.B. and O. Kongsdal, 2003. Common laboratory seed health-testing methods for detecting fungi. International Seed Testing Association, pp: 234-255 Switzerland.
11. Bragulat, M.R., E. Martinez, G. Castella and F.J. Cabanes, 2004. Selective efficacy of culture media recommended for isolation and enumeration of *Fusarium* species. J. Food Protection, 67: 207-211.
12. Barnett, H.L. and B.B. Hunter, 1972. Illustrated genera of imperfect fungi. III edn. pp: 1-208. Burgess publishing company, Minneapolis, Minneasota, USA.
13. Singh, K., J.C. Frisrad, U. Thrane and S.B. Mathur, 1999. An illustrated manual on identification of some see-borne *Aspergilli, Fusaria, Penicillia* and their mycotoxins. Danish Govt. Institute of seed Pathology for Developing Countries, Denmark.
14. Leslie, J.F. and B.A. Summerell, 2006. The *Fusarium* Laboratory Manual. 1st edn. pp: 1-264. Blackwell Publishing Professional, USA.
15. Ghiasian, S.A., P. Kord-Bacheh, S.M. Rezayat, A.H. Maghsood and H. Taherkhani, 2004. Mycoflora of Iranian maize harvested in the main production areas in 2000. Mycopathologia, 158: 113-121.
16. Diener, U.L., G. Morgan-jones, R.E. Wagener and N.D. Davis, 1981. Toxigenicity of fungi from grain sorghum. Mycopathologia, 75: 23-26.
17. Janardhana, G.R., K.A. Raveesha and H.S. Shetty, 1999. Mycotoxins contamination of maize grains grown in India (Karnataka). Food and Chemical Toxicol., 37: 863-868.
18. Bhattacharya, K. and S. Raha, 2002. Deteriorative changes of maize, groundnut and soybean seeds by fungi in Storage. Mycopathologia, 155: 135-141.
19. Ono, E.Y.S., Y. Sugiura, M. Homechin, M. Kamogae, E. Vizzoni, Y. Ueno and E.Y. Hirooka, 1999. Effect of climatic conditions on natural mycoflora and fumonisins in freshly harvested corn of the State of Parana, Brazil. Mycopathologia, 147: 139-148.
20. Logrieco, A., A. Moretti, A. Ritieni, A. Bottalico and P. Corda, 1995. Occurrence and toxigenicity of *Fusarium proliferatum* from preharvest maize ear rot and associated mycotoxins in Italy. Plant Disease, 79: 727-731.
21. Marasas, W.F.O., P.E. Nelson and T.A. Toussoun, 1984. Toxigenic *Fusarium* species: Identity and mycotoxicology. Pennsylvania State University Press, University Park, USA.
22. Castro, M.F.P.M., L.M.V. Soares and R.R.Z. Furlani, 1995. Mycoflora, aflatoxigenic species and mycotoxins in freshly harvested corn (Zea mays L.): a preliminary study. Reviews in Microbiol., 26: 289-95.
23. Pozzi, C.R., B. Correa, W. Gambale, C.R. Paula, N.O. Chacon-Reche and M.C.A. Meirelles, 1995. Post-harvest and stored corn in Brazil: mycoflora interaction, abiotic factors and mycotoxins occurrence. Food Additives and Contamination, 12: 313-318.
24. Almeida, A.P., B. Correa, M.A.B. Mallozzi, E. Sawazaki and L.M.V. Soares, 2000. Mycoflora and aflatoxin/fumonisin production by fungal isolates from freshly harvested corn hybrids. Brazilian J. Microbiol., 31: 321-326.
25. Dass, R.S., M.Y. Sreenivasa and G.R. Janardhana, 2007. High incidence of *Fusarium verticillioides* in animal and poultry feed mixtures produced in Karnataka, India. Plant Pathol. J., 6(2): 174-178.