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SURVEY OF POSTHARVEST FUNGI ASSOCIATED WITH SORGHUM GRAINS PRODUCED IN KARNATAKA (INDIA)

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Abstract: Sorghum is an important cereal produced as staple diet in Karnataka state of India and is prone to fungal infection during pre- and postharvest period. This paper reports the frequency and relative percentage of fungi associated with sorghum grain harvested in Karnataka State in 2004 and 2005. A total of 44 sorghum samples were analyzed for postharvest fungi by direct plating method on PDA and MGA 2.5 agar medium. 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%), *F. anthophilum* (65.0%), *A. niger* (59.1%). These data indicate possible health hazards for humans and animals upon consumption of such contaminated food grain by toxigenic moulds.

Key words: *Aspergillus*, *Fusarium*, frequency, fungi, relative percentage, sorghum

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal after rice, wheat, maize and barley. It is the staple food grain for over 750 million people who live in the semi-arid tropics of Africa, Asia, and Latin America (Diener *et al.* 1981; Wilson *et al.* 1995). Global production of sorghum is currently estimated for 57.6 million tonnes, with Asian countries contributing 20% of the total production. Within Asia, India is the largest producer of sorghum grain. In India, major states that produce sorghum are Karnataka, Andhra Pradesh, Uttar Pradesh and Madhya Pradesh. In Karnataka, during rainy season sorghum is grown on the area of 0.30 million ha, producing 0.29 million tonnes of grain while the post-rainy season, sorghum accounts for 0.34 million ha producing 0.35 million tonnes (Anonymous 2003).

The sorghum crop is grown in dry conditions where other cereal crops yield much less. It is also grown with limited water resources and usually without the application of any fertilizers or other inputs. Therefore, it is often referred to as "coarse grain" or "poor people's crops" because it is grain mainly consumed by the poor people (Hulluka and Esele 1992). The sorghum crop is still a principal source of energy, protein, vitamins and minerals for millions of poor people in these regions (Bhat *et al.* 1997). Besides its traditional use as food crop, has other alternative uses such as livestock and poultry feed, potable alcohol, starch, ethanol production and for hundreds of industrial purposes (Kleih *et al.* 2000). However, fungi associated with sorghum are of serious concern due to their toxigenic potential. The risk of contamination by mycotoxins in sorghum is related to the kind of fungi associated.

A number of fungal species associated with sorghum belong to the genera *Fusarium*, *Aspergillus* and *Penicillium*, which have been known to produce mycotoxins that cause mycotoxicosis in animals and humans (Anonymous 2001). In some cereal grain-producing countries, the fungi of freshly harvested grains have been very well documented (Williams and McDonald 1983; El-Maghraby *et al.* 1995; Placinta *et al.* 1999). There is a lack of accurate data on the frequency and relative percentage of fungi in sorghum 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 recongnize the frequency and a relative percentage of particular species within the genera of fungi in sorghum grains produced in different districts of Karnataka, India.

MATERIALS AND METHODS

Collection of samples

A total of 44 sorghum samples (approximately 1 000 g) were collected from markets, local stores, agricultural cooperatives and farm fields from different districts of Karnataka state during the three harvest seasons of 2004 and

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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 a relative percentage of fungi, sorghum grains were plated on potato dextrose agar (PDA) medium by agar plating method (Mathur and Kongsdal 2003). Since PDA medium was unsuitable for the isolation of *Fusarium* species from the natural sources, malachite green agar 2.5 (MGA 2.5) medium was also used as recommended by Bragulat *et al.* (2004). Four hundred sorghum grains 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 plates at the rate of 10 seeds per plate (9 cm in diameter). The plates were incubated under alternating periods of 12 h darkness and 12 h of daylight at 25±2°C for 7 days.

Identification of fungi

Fungal colonies grown on sorghum grains were observed under stereo-binocular microscope (Magnus MS24) and 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 fungal species were identified up to the species using keys and manuals (Barnett and Hunter 1972; Booth 1977; Keith 1996; Mathur and Kongsdal 2003; Singh *et al.* 1999; Summerell *et al.* 2003; Leslie and Summerell 2006). 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).

$$\text{Frequency [\%]} = \frac{\text{number of samples infected with fungi}}{\text{total number of samples analyzed}} \times 100$$

$$\text{Relative percentage [\%]} = \frac{\text{number of fungal species isolated}}{\text{total number of fungi isolated}} \times 100$$

RESULTS AND DISCUSSION

India with its varied agro-climatic conditions produces a variety of food crops throughout the year. Non-scientific method of agricultural practices, poor storage facilities and unfavourable environmental conditions during pre- and postharvest handling of these crops were responsible for the contamination, infection and colonization by mycotoxin producing fungi (Janardhana *et al.* 1999). 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 (Diener *et al.* 1981; Wilson *et al.* 1995). 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 (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 and their epidemiological significance in cereal crops grown in Karnataka and elsewhere in India.

In the present investigation, mycological examination of 44 sorghum samples revealed the occurrence of 19 fungal genera such as *Fusarium, Aspergillus, Curvularia, Alternaria, Penicillium, Drechslera, Chaetomium, Phoma, Cladosporium, Nigrospora, Epicoccum, Trichothecium, Gonatobotrys, Verticillium, Rhizopus, Trichoderma, Fusidium, Micrococcum* and *Mucor* species. The most dominant fungal genera were *Fusarium* and *Aspergillus* with a high frequency of 93.2 and 88.6% and with a relative percentage of 23.3 and 19.6%, respectively. The other fungal genera such as *Alternaria* (84.1%), *Phoma* (68.2%), *Curvularia* (65.9%), *Penicillium* (61.4%) and others were also isolated with the different level of frequency and relative percentage (Fig. 1).

The genus *Fusarium* was the most dominant fungi among 19 fungi reported in this study. It was report as a natural contaminant in cereals and also in many other

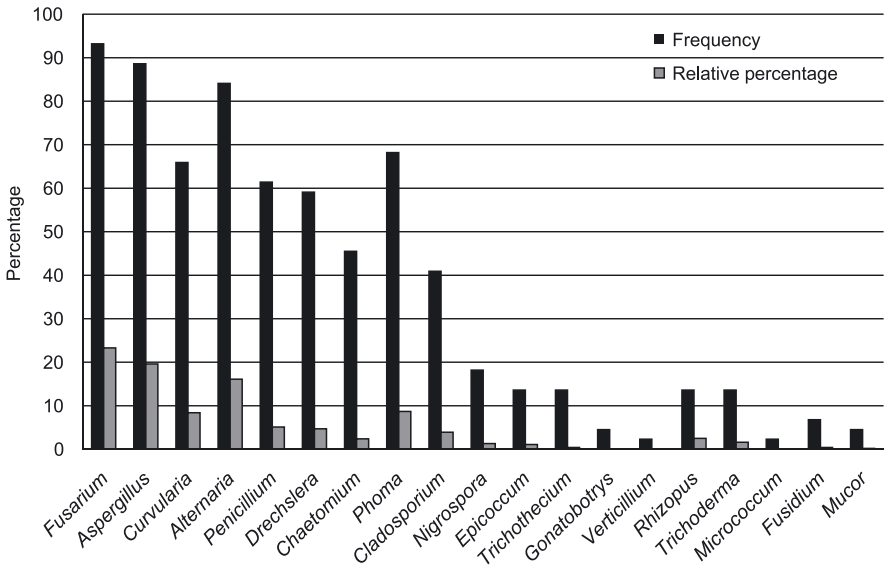

Fig. 1. Fungal genera isolated from sorghum seed samples collected from Karnataka, India

Table 1. *Fusarium* species isolated from sorghum seed samples

No.	Name of the fungus	Total No. of isolates	Frequency [%]	Relative percentage
1	*F. verticillioides*	580	88.6	54.9
2	*F. proliferatum*	69	40.9	06.5
3	*F. oxysporum*	09	13.6	00.9
4	*F. anthophilum*	241	65.9	22.8
5	*F. pallidoroseum*	94	47.7	08.9
6	*F. sporotrichioides*	49	31.8	04.6
7	*F. solani*	14	04.5	01.3
8	*F. graminearum*	1	02.3	00.1

Table 2. *Aspergillus* species isolated from sorghum seed samples

No.	Name of the fungus	Total No. of isolates	Frequency [%]	Relative percentage
1	*A. flavus*	455	72.7	51.1
2	*A. niger*	297	59.1	33.3
3	*A. flavus columnaris*	33	13.6	03.7
4	*A. versicolor*	37	20.5	04.2
5	*A. candidus*	32	20.5	03.6
6	*A. fumigatus*	04	04.5	00.4
7	*A. ochraceous*	24	20.5	02.7
8	*A. sydowii*	01	02.3	00.1
9	*VA. nidulans*	08	04.5	00.9

Table 3. Other fungal species isolated from sorghum seed samples

No.	Name of the fungus	Total No. of isolates	Frequency [%]	Relative percentage
1	*C. lunata*	233	43.2	60.8
2	*Curvularia* spp.	150	34.1	39.2
3	*Alternaria alternata*	675	84.1	93.7
4	*Alternaria* spp.	46	09.1	06.3
5	*D. oryzae*	161	38.6	75.6
6	*D. tritici-repentis*	03	04.5	01.4
7	*Drechslera* spp.	49	22.7	23.0
8	*C. indicum*	42	13.6	38.5
9	*C. globosum*	37	15.9	33.9
10	*Chaetomium* spp.	30	20.5	27.5
11	*P. lingam*	48	09.1	12.2
12	*Phoma* spp.	347	56.8	87.8
13	*C. cladosporoides*	66	22.7	37.1
14	*C. herbarum*	32	09.1	18.0
15	*Cladosporium* spp.	80	22.7	44.9
16	*N. oryzae*	03	02.3	04.9
17	*Nigrospora* spp.	58	18.2	95.1
18	*R. stolonifera*	14	02.3	12.2
19	*Rhizopus* spp.	101	11.4	87.8
20	*T. harzianum*	57	06.8	79.2
21	*T. viride*	13	04.5	18.1
22	*Trichoderma* spp.	02	02.3	02.8

agricultural commodities (Leslie *et al.* 1990). *Fusarium* is one of the most economically important genera of fungi causing diseases on a wide variety of plants and plant derived products (Rheeder and Marasas 1998; Summerell *et al.* 2003). A widespread distribution of *Fusarium* species may be attributed to the ability of these fungi to grow on a wide range of substrates and their efficient mechanism of spore dispersal (Nelson *et al.* 1994). Considering the importance of the genus *Fusarium*, all the isolates of *Fusarium* species were identified up to the species level. The study showed the presence of nine *Fusarium* species. A high frequency of *F. verticillioides* (86.6%), *F. anthophilum* (65.9%), *F. pallidoroseum* (47.7%) and *F. proliferatum* (40.9%) recorded on sorghum samples. A low frequency of *F. solani* and *F. graminearum* (4.5 and 2.3%) recorded in sorghum samples, respectively. Further, the study on relative percentage showed that the *F. verticillioides* (54.9%) and *F. anthophilum* (22.8%) were the dominant *Fusarium* species with a high relative percentage followed by *F. pallidoroseum* and *F. proliferatum* with 8.9 and 6.5% relative percentage respectively (Table 1). An important observation made in the present investigation is that *F. verticillioides* was isolated from almost all the samples with high frequency and relative density. The results of this investigation are similar to those obtained by earlier workers and show that *Fusarium* is the most dominant species isolated from maize and sorghum samples (Gonzalez *et al.* 1997). Gamanya and Sibanda (2001) evaluated the distribution of *Fusarium* species in cereals and oilseeds in Zimbabwe. They found that maize and sorghum are the most contaminated cereals when compared to oilseeds. Similar data were obtained in the State of Parana, Brazil where they detected 98.7% incidence *Fusarium* species on corn (Ono *et al.* 1999). 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. The survey conducted worldwide also showed that, *F. verticillioides*, *F. proliferatum* and *F. anthophilum* were the most frequently isolated species in sorghum and were able to produce fumonisins (Da Silva *et al.* 2004). The National Institute of Nutrition (NIN), Hyderabad (India) conducted an epidemiological survey of fumonisins – a class of fusarial toxins in 27 villages belonging to six districts of Andhra Pradesh and alarmingly high levels of fumonisins were detected in sorghum samples (Bhat *et al.* 1997). Another study which correlates with the data obtained in the present work is that a high incidence of *F. verticillioides* was reported from maize and sorghum based animal feedstuffs and poultry feed mixtures produced in Karnataka state (Dass *et al.* 2007).

Mycological studies conducted on sorghum revealed that sorghum was contaminated by nine species of *Aspergillus* (Table 2). The predominant *Aspergillus* species isolated were *A. flavus* (72.7%) and *A. niger* (59.1%) with the relative percentage of 51.1 and 33.3%, respectively. The three *Aspergillus* species such as *A. ochraceus, A. versicolor* and *A. candidus* were recorded with a similar frequency of 20.5%. A low frequency of *A. sydowii* (2.3%) was recorded in table 2. Surveys conducted worldwide also revealed that, *A. flavus* and *A. niger* were known to frequently con-

taminate peanuts and were able to produce mycotoxins such as aflatoxins (Pitt *et al.* 1994; Wilson *et al.* 1995; Gassen 1999). *A. flavus* contamination and aflatoxin production in sorghum is a serious problem in most of sorghum-producing countries where the crop is grown under rain fed conditions (Klich 2007).

Further, mycological analysis of sorghum samples for the other field fungi revealed the occurrence of *Alternaria* species (84.1%), *Phoma* (56.8%) and *Curvularia* species (43.2%), respectively. Some of the other less frequently isolated fungal species such as *Drechslera oryzae* (38.6%), *Cladosporium* species (22.7%), *Chaetomium* species (20.5%) and *Nigrospora* sp. (15.1%) were recorded in table 3. Similar reports are also available on the incidence of fungal species on various cereals (Marasas *et al.* 1984; Castro *et al.* 1995). Ghiasian *et al.* (2004) reported the occurrence of predominant fungal species such as *Fusarium* (38.5%), *Aspergillus* (8.7%), *Rhizopus* (4.8%), *Penicillium* (4.5%) and *Mucor* (1.1%), respectively.

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 sorghum 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 *Fusarium* 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.

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POLISH SUMMARY

PRZEGLĄD GRZYBÓW WYSTĘPUJĄCYCH POZBIOROWO NA NASIONACH SARGA W KARNATAKA (INDIE)

Sorgo jest składnikiem diety w indyjskim stanie Karnataka. W okresie przed- i pozbiorowym sorgo jest podatne na zakażenia wywoływane przez grzyby. W pracy badano częstotliwość oraz względny procent występowania grzybów atakujących ziarno sorgo, zbierane w stanie Karnataka, w latach 2004 i 2005. W celu stwierdzenia występowania grzybów po zbiorze, analizowano próby sorgo wykorzystując metodę bezpośredniego wykładania ziarna na pożywkę zawierającą 2,5% agaru. Najczęściej z ziarna sorgo izolowano grzyby rodzajów *Fusarium* i *Aspergillus*. Innymi występującymi rodzajami były: *Alternaria*, *Phoma*, *Culvularia*, *Penicillium* i *Drechslera*. Uzyskane dane ujawniły wysoką częstotliwość występowania gatunków *Fusarium* (93,2%) i *Aspergillus* (88,6%) stanowiących, odpowiednio, względny procent 23,3 i 19,6 wśród 19 rodzajów. Przeważającymi gatunkami, które bardzo często wykrywano były: *F. verticillioides* (88,6%), *A. flavus* (72,7%), *F. antrophilum* (65,0%), *A. niger* (59,1%). Dane te wskazują, że ziarno sorgo zarażone grzybami wytwarzającymi toksyny, może szkodzić ludziom i zwierzętom.