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Detection of Seed Borne Mycoflora of Sorghum in Turkey

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ABSTRACT

Seed borne mycoflora of 23 sorghum seed samples collected from different localities of Turkey was investigated using blotter, agar plate and deep freezing methods as recommended by ISTA. 19 species (*Absidia* sp, *Acremoniella* sp., *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Aspergillus* sp., *Cladosporium* sp., *Curvularia lunata, Drechslera tetramera, Epicoccum* sp., *Fusarium avenaceum, Fusarium nygamai, Fusarium proliferatum, Fusarium semitectum, Fusarium subglutinans, Fusarium verticillioides, Penicillium* spp., *Rhizopus* sp.) of 23 sorghum seed samples were determined to be new records for Turkey which belong to 11 genera. Our results showed that *Alternaria alternata* was the predominant species among these areas. Higher number of fungi was isolated by using deep-freezing method as compared to agar and blotter methods.

Key words: Sorghum, seed pathology, mycoflora

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is the world's fifth major cereal crop after wheat, rice, maize and barley (Fageria et al., 1997; Ayana and Bekele, 2000; Mullet et al., 2002; Mariscal-Landina et al. 2004). Sorghum, Sudangrass (*Sorghum sudanense* (Piper.) Stapf) and Sorghum x sudangrass hybrid cultivars are important fodder plants grown as the second crop for pasture, silage, green chop and hay in Turkey. Grain sorghum is commonly consumed as foodstuff in developing or less developed countries (Fageria et al., 1997; Kenga et al. 2004) and it is also used as forage and raw material in the industries of developed countries (Delciotti et al. 1998; Kenga et al., 2004). Since sorghum is more tolerant to extreme hot conditions, it has been replaced with corn most regions in the world (Güler et al., 2008).

Sorghum is known to suffer from more than 30 fungal diseases (USDA, 1960). Important seed borne fungal diseases recorded on sorghum are stalk rot *(Aspergillus niger*), target spot (*Bipolaris sorghicola*), stalk rot/anthracnose/red leaf (*Colletotrichum graminicola*), seed rot /stalk rot (*Fusarium verticillioides)*, seedling blight/charcoal rot (*Macrophomina phaseolina*) and covered smut/grain smut (*Sphacelotheca sorghi*) (Richardson, 1990). Seed is the most important input for crop production. Pathogen free healthy seed is urgently needed for desired plant populations and good harvest. Many plant pathogens are seed borne and can cause enormous crop losses. Besides, the mold fungi growing on the seed substratum produce mycotoxins which are hazardous to man and animals (Halt, 1994).

In Turkey, it became necessary to study mycoflora of the sorghum by the fact that seed borne *Bipolaris spicifera (*Syn: *Drechslera tetramera)* was found to cause a disease observed in sorghum growing areas in Sakarya Region (Ünal et al. 2010). Therefore the aim of present study was to reveal seed mycoflora of the sorghum grown in Turkey.

MATERIALS AND METHODS

Totally 23 *Sorghum bicolor* (L.) Moench, *Sorghum sudanense* (Piper) Stapf and *Sorghum bicolor* x *Sorghum sudanense* hybrid samples including 10 varieties (Akdarı, Ogretmenoglu 77, Beydarı, Early sumac, Rox, Leoti, Sugar Graze, Greengo, Gozde 80, Jumbo) were obtained from four sorghum growing areas in Antalya, Adana, Sakarya, and İzmir provinces in Turkey. Out of 23 seed samples, 7 were collected from Antalya, 6 from Sakarya, 6 from Adana and 4 from İzmir. Three different methods recommended by International Seed Testing Association (ISTA) (Anonymous, 1993); Blotter, Deep Freezing and Agar Plate methods were used on each sample. As a pre-treatment, 10g of seed sub-samples were surface sterilized for 1 min with 1% sodium hypochlorite solution (NaOCl).

Blotter Method

Surface sterilised twenty five seeds were placed on three layers of moistened blotters in each Petri dish 10 cm in diameter. The dishes were then incubated at a constant photoperiod (12 h day and 12 h night) for 7 days at 20^{o} C and examined under a stereomicroscope for seed borne mycoflora. Two hundred seeds per sample were tested for blotter method.

Deep Freezing Method

Twenty five seeds per plate were placed on three layers of moistened blotters. Seeds were incubated at a constant photoperiod (12 h day and 12 h night) at 20^{o} C for a day and frozen at -20 ^{o}C for 24h. The plates were then kept at 22 ± 1 ^{o} C for 5 days. Two hundred seeds per each sample were tested for deep freezing method.

Agar Plate Method

The seeds were plated on potato dextrose agar (PDA), 10 seeds per Petri dish and dishes were incubated at 24 ^{o}C for 7 days. One hundred seeds from each sample were tested for agar plate method.

Identification of fungal species

Fungi grown on seeds were identified by using morphological criteria of Ellis (1971), Hanlin (1990) and Burgess et al. (1994). In order to identify *Fusarium* spp. subcultures were made on Carnation Leaf Agar (CLA) and Potato Dextrose Agar (PDA) and incubated at 25^{o}C for 5–7 days. Final identification was made following Leslie and Summerell (2006).

RESULTS

The results of mycological tests indicated that a total number of 11 genera and 19 species of fungi; *Absidia* sp, *Acremoniella* sp., *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Aspergillus* sp., *Cladosporium* sp., *Curvularia lunata, Drechslera tetramera* (Syn; *Bipolaris spicifera*)*, Epicoccum* sp., *Fusarium avenaceum, Fusarium nygamai, Fusarium proliferatum, Fusarium semitectum, Fusarium subglutinans, Fusarium verticillioides, Penicillium* spp., *Rhizopus* sp. were isolated from sorghum seeds. All of the 19 species isolated from sorghum seeds are new records for Turkey.

The results of the seed tests indicated that 17 fungal species belonging to 9 genera (*Alternaria alternata, Aspergillus flavus, Aspergillus niger, Aspergillus* sp., *Cladosporium* sp., *Curvularia lunata, Drechslera tetramera* (Syn; *Bipolaris spicifera*)*, Epicoccum* sp., *Fusarium avenaceum, F. nygamai, F. proliferatum, F. semitectum, F. subglutinans, F. verticillioides, Penicillium* spp., *Rhizopus* sp.), and 16 fungal species in 10 genera (*Absidia* sp, *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Aspergillus* sp., *Cladosporium* sp., *Curvularia lunata, Drechslera tetramera* (Syn; *Bipolaris spicifera*)*, Epicoccum* sp.*, Fusarium semitectum, F. subglutinans, F. verticillioides, Penicillium* spp.*, Rhizopus* sp.) were identified by blotter and agar plate methods respectively. On the other hand deep freezing method allowed us to identify all 19 fungal species 11 genera clearly.

The seed test performed on total 23 seed samples which were provided from four different regions where grain sorghum is intensively cultivated, showed that the highest number of fungi was obtained from Antalya with 17 fungal species (Table 1) followed by Adana and Sakarya Regions with 11 fungal species (Table 2 and 3) and finally Izmir Region with 12 fungal species (Table 4). The common fungal species, which were identified by three seed tests performed on 7 sorghum seeds of Antalya Region *Alternaria alternata*, *Rhizopus* sp., *Fusarium semitectum, F. subglutinans* and *F. verticillioides* (Table 1). The common species identified from the seed samples of Sakarya were *Alternaria alternata* ve *Drechslera tetramera* (Table 3). *Alternaria alternata* was the most common fungal species identified from the seeds provided from all regions (Table 2 and 4).

Table 1. Numbers and percentages of fungi in infected seeds of sorghum in Antalya region studied by three different methods.

Place of collection / Fungi isolated	Blotter method				Agar plate method				Deep freezing method			
ANTALYA	NIF	%P	NIS	I%± SD	NIF	%P	NIS	I%± SD	NIF	%P	NIS	I%± SD
Absidia sp.	-	-	-	-	8	1,14	1	-	5	2,5	1	-
Acremoniella sp.	-	-	-	-	-	-	-	-	10	5	1	-
Alternaria alternata	465	33,21	6	39.58±23.9	254	36,28	6	42.33±35.7	274	19,57	6	22.83±13.0
Aspergillus flavus	146	10,42	5	14.60±10.7	60	8,57	6	10.00±6.8	98	7	5	9.80±10.0
Aspergillus niger	98	7	5	9.80±11.6	139	19,85	6	23.7±16.2	50	3,57	5	5.00±2.9
Aspergillus sp.	18	1,28	4	2.5±1.2	-	-	-	-	-	-	-	-
Curvularia lunata	15	1,07	4	2.7±0.8	20	2,85	2	10.00±0.0	8	0,57	2	2.00±0.0
Drechslera tetramera	22	1,57	4	2.75±2.4	-	-	-	-	20	1,42	3	3.17±1.9
Epicoccum sp.	15	1,07	3	2.50±2.2	-	-	-	-	44	3,14	1	-
Fusarium avenaceum	12	0,85	3	2.00±1.0	-	-	-	-	10	0,71	1	-
Fusarium nygamai	48	3,42	3	8.00±5.2	-	-	-	-	15	1,07	1	-
Fusarium proliferatum	77	5,5	3	12.83±8.6	12	1,71	1	-	56	4	2	14.00±1.4
Fusarium semitectum	161	11,5	6	13.42±20.2	40	5,71	3	13.33±5.8	153	10,92	6	12.75±11.4
Fusarium subglutinans	258	18,42	6	21.50±11.9	63	9	6	10.50±10.0	173	12,35	6	14.42±8.5
Fusarium verticillioides	180	12,85	4	22.50±7.5	78	11,14	3	26.00±19.3	112	8	5	11.20±10.8
Penicillium spp.	78	5,57	6	6.50±9.3	92	13,14	6	15.33±15.7	90	6,42	5	9.00±9.8
Rhizopus sp.	221	15,78	4	27.63±29.3	78	11,14	2	39.00±1.4	70	5	2	17.50±20.5

NIF = Numbers of infected grains with fungi in seven varieties, % percentage =%P
NIS= Numbers of infected samples out of seven tested.
I% = Percentange of infected seed, ± SD = Standard deviation

Table 2. Numbers and percentages of fungi in infected seeds of sorghum in Adana region studied by three different methods.

Place of collection / Fungi isolated	Blotter method				Agar plate method				Deep freezing method			
ADANA	NIF	%P	NIS	I%± SD	NIF	%P	NIS	I%± SD	NIF	%P	NIS	I%± SD
Alternaria alternata	298	24,83	5	59.60±45.5	199	33,1	5	39.80±27.7	502	41,8	5	50.20±30.3
Aspergillus flavus	-	-	-	-	10	1,66	1	-	30	2,5	1	-
Aspergillus niger	24	2	2	6.00±5.7	14	2,33	2	7.00±0.0	9	0,75	3	1.50±0.5
Aspergillus sp.	-	-	-	-	10	1,66	1	-	-	-	-	-
Cladosporium sp.	-	-	-	-	2	0,33	1	-	4	0,33	1	-
Drechslera tetramera	10	0,83	1	-	-	-	-	-	5	0,41	1	-
Epicoccum sp.	-	-	-	-	15	2,5	1	-	-	-	-	-
Fusarium semitectum	8	0,66	1	-	3	0,5	1	-	11	0,91	2	2.75±2.5
Fusarium subglutinans	35	2,91	2	8.75±1.0	39	6,5	3	13.00±8.5	21	1,75	3	3.50±2.3
Fusarium verticillioides	10	0,83	2	2.50±0.7	25	4,16	4	6.25±2.2	13	1,08	1	-
Penicillium spp.	30	2,5	1	-	50	8,33	2	25.00±21.2	40	3,33	2	10.00±7.1

NIF = Numbers of infected grains with fungi in seven varieties, % percentage =%P
NIS= Numbers of infected samples out of seven tested.
I% = Percentange of infected seed, ± SD = Standard deviation

Table 3. Numbers and percentages of fungi in infected seeds of sorghum in Sakarya region studied by three different methods.

Place of collection / Fungi isolated	Blotter method				Agar plate method				Deep freezing method			
SAKARYA	NIF	%P	NIS	I%± SD	NIF	%P	NIS	I%± SD	NIF	%P	NIS	I%± SD
Alternaria alternata	256	21,33	5	25.60±16.2	149	24,83	6	24.83±10.5	278	23,16	6	23.17±12.9
Aspergillus flavus	36	3	4	4.50±1.5	16	2,66	2	8.00±4.2	63	5,25	4	7.88±3.4
Aspergillus niger	12	1	1	-	10	1,66	2	5.00±2.8	44	3,66	3	7.33±6.2
Aspergillus sp.	-	-	-	-	1	0,16	1	-	4	0,33	1	-
Cladosporium sp.	-	-	-	-	-	-	-	-	16	1,33	2	4.00±0.7
Drechslera tetramera	102	8,5	5	10.20±8.1	31	5,16	4	7.75±6.3	151	12,58	5	15.10±0.1
Fusarium semitectum	6	0,5	2	1.50±1.4	3	0,5	1	-	4	0,33	1	-
Fusarium subglutinans	3	0,25	1	-	9	1,5	2	4.50±3.5	5	0,41	1	-
Fusarium verticillioides	-	-	-	-	-	-	-	-	22	1,83	2	5.50±5.7
Penicillium spp.	18	1,5	3	3.00±0.5	54	9	4	13.50±9.3	45	3,75	4	5.63±4.6
Rhizopus sp.	-	-	-	-	10	1,66	1	-	15	1,25	3	2.50±0.9

NIF = Numbers of infected grains with fungi in seven varieties, % percentage =%P
NIS= Numbers of infected samples out of seven tested.
I% = Percentange of infected seed, ± SD = Standard deviation

Table 4. Numbers and percentages of fungi in infected seeds of sorghum in İzmir region studied by three different methods.

Place of collection / Fungi isolated	Blotter method				Agar plate method				Deep freezing method			
İZMİR	NIF	%P	NIS	I%± SD	NIF	%P	NIS	I%± SD	NIF	%P	NIS	I%± SD
Alternaria alternata	182	22,75	3	30.33±18.4	18	4,5	2	12.14±5.5	71	8,87	2	17.75±20.9
Aspergillus flavus	10	1,25	1	-	5	1,25	2	2.4±0.5	21	2,62	2	3.50±0.5
Aspergillus niger	50	6,25	4	6.25 ± 2.6	16	4	3	4.71±2.1	21	2,62	2	3.50±1.5
Aspergillus sp.	13	1,62	2	3.25 ± 2.5	47	11,75	3	11.84±20.1	2	0,25	1	-
Cladosporium sp.	42	5,25	2	10.50 ± 2.8	-	-	-	-	12	1,5	2	3.00±0.7
Curvularia lunata	15	1,87	2	3.75±0.4	8	2	1	-	16	2	2	4.00±0.7
Epicoccum sp.	3	0,37	1	-	-	-	-	-	5	0,625	1	-
Fusarium semitectum	17	2,12	2	4.24±1.1	12	3	2	5.06±4.3	15	1,87	3	2.17±0.8
Fusarium subglutinans	10	1,25	2	2.50±2.1	7	1,75	3	2.02±0.8	6	0,75	1	-
Fusarium verticillioides	5	0,62	1	-	17	4,25	3	4.78±3.7	10	1,25	1	-
Penicillium spp.	28	3,5	3	4.67±0.8	13	3,25	3	3.44±3.2	20	2,5	3	3.33±2.3
Rhizopus sp.	87	10,87	3	14.50±3.8	30	7,5	3	8.44±4.0	192	24	4	24.00±18.9

NIF = Numbers of infected grains with fungi in seven varieties, % percentage =%P
NIS= Numbers of infected samples out of seven tested.
I% = Percentange of infected seed, ± SD = Standard deviation

Overall results indicated that *Fusarium* was dominant species in Antalya while *Drechslera tetramera* was commonly identified from Sakarya region. *Aspergillus niger* and *Fusarium verticillioides* which are seed-born and cause stalk rot and seed rot diseases were found to be common on the seeds collected from Antalya Region. Important sorghum seed diseases, target spot (*Bipolaris sorghicola*), stalk rot/anthracnose/red leaf (*Colletotrichum graminicola*), seedling blight/charcoal rot (*Macrophomina phaseolina*) and covered smut/grain smut (*Sphacelotheca sorghi*), (Richardson, 1990) were not detected in the present study. Among 10 different sorghum seed species, the highest number of different fungal species was identified from Early sumac providing 11 fungal species. However the least fungal infection was observed in Greengo (only 3 species) provided from Sakarya.

DISCUSSION

Of the three methods compared in the present study, the deep freezing method yielded the highest number of fungi (11 genera and 19 species; *Absidia* sp, *Acremoniella* sp., *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Aspergillus* sp., *Cladosporium* sp., *Curvularia lunata, Drechslera tetramera, Epicoccum* sp., *Fusarium avenaceum, Fusarium nygamai, Fusarium proliferatum, Fusarium semitectum, Fusarium subglutinans, Fusarium verticillioides, Penicillium* spp., *Rhizopus* sp.). The deep freeze method seemed to be more effective for the detection of deep seated as well as slow growing seed borne fungi like *Drechslera* spp., *Fusarium* spp., *Penicillium* spp., *Alternaria alternata* (Niaz and Dawar 2009). Previous studies on sorghum seeds also revealed that deep freezing method was most suitable for detection of *Fusarium* species (Mathur et al. 1975; Nahar et al. 2005). In the present study, *Fusarium* species and *Drechslera tetramera* were isolated in higher percentages both by blotter and deep freezing method. On the other hand, in this study, saprobic fungi; *Aspergillus flavus, Aspergillus niger, Aspergillus* sp. and *Curvularia lunata* were isolated in higher percentages by agar plate method. The agar plate method was found to be the most suitable method for the isolation of saprobic fungi. Mathur and Neergaard, (1970) and Khan et al. (1988) preferred agar plate method rather than blotter method for the isolation of *Curvularia* spp. and *Drechslera* spp., from seeds of rice. However, in the present study, *Drechslera tetramera* and *Fusarium* species were isolated in higher percentage by deep freezing method. *Alternaria alternata* was identified in highest rates with all methods used in the study.

A lot of fungi isolated in the present study are known to produce mycotoxins. Mycotoxins have been implicated as having toxic effects on animals and human being and they can cause severe damage to liver, kidney and nervous system even in low dosages (Rodricks 1976).

Fusarium species (*F. avenaceum, F. nygamai, F. proliferatum, F. semitectum, F. subglutinans, F. verticillioides*) obtained from sorghum seeds in this study are known to produce mycotoxins deoxyninalenol (DON) zearalenone, fusaric acid and trichothecene. (ApSimon et al., 1990; Miller, 1995; Sweeney and Dobson, 1998; Abbas et al., 1999; Benneth and Klich, 2003; Desjardins, 2006), *Aspergillus flavus* produces aflotoxin, B1, B2, G1 and G2. *Alternaria alternata* produces alternariols. These toxins are very toxic and carcinogenic. They may cause liver cancer in human and livestock animals and especially loss of weight in cattle, pigs and poultry resulting in economic losses for the farmers (Diener and Davis, 1969; Purchase, 1974; Pesta and Bonday, 1990).

To the best of our knowledge, this is the first report on the mycoflora of Sorghum seed grown in Turkey. Pathogenic fungal species (*A. alternaria, Drechslera tetramera, Fusarium* spp.) were recovered in significant rates. Various fungus species which were commonly determined in this study, such as *Alternaria alternata, Aspergillus* spp. and *Fusarium* spp. are also known as mycotoxin producers and dangerous for human and animal health.

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ÖZET

TÜRKİYE'DE SORGUMDA TOHUM KAYNAKLI MİKOFLORANIN BELİRLENMESİ

Türkiyenin farklı bölgelerinden toplanan 23 sorgum tohum örneğine ait mikoflora ISTA tarafından önerilen agar, deep-freezing ve blotter yöntemleri kullanılarak araştırılmıştır. 23 sorgum tohum örneğinin 11 genusa ait 19 türü (*Absidia* sp, *Acremoniella* sp., *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Aspergillus* sp., *Cladosporium* sp., *Curvularia lunata, Drechslera tetramera, Epicoccum* sp., *Fusarium avenaceum, Fusarium nygamai, Fusarium proliferatum, Fusarium semitectum, Fusarium subglutinans, Fusarium verticillioides, Penicillium* spp., *Rhizopus* sp.) Türkiye için yeni kayıt olarak belirlenmiştir. Elde edilen sonuçlar *Alternaria alternata*'nın en yaygın tür olduğunu göstermiştir. Deep-freezing yöntemi ile izole edilen fungus sayısının diğer yöntemler kullanılarak elde edilen fungus sayısından daha yüksek olduğu belirlenmiştir.

Anahtar kelimeler: Sorgum, tohum patolojisi, mikoflora

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