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# Seed Mycoflora of *Sorghum bicolor* in Iraq

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ABSTRACT. Seeds of 9 cultivars of Sorghum sp. (*Sorghum bicolor* (L) Moench) were screened for their mycoflora. The seeds were associated with 38 species of fungi belonging to 22 genera. *Alternaria alternata, Aspergillus fumigatus, A. niger, A. flavus, Curvularia lunata, Drechslera spicifera, D. australiensis* and *Fusarium moniliforme* were the most frequent species. The blotter method detected more species than the agar plate method. From 29 to 92.6% of the seeds were infected with fungi, and their percentage germination ranged from 21 to 85.6%. The species composition and percentage germination of *Sorghum* seeds differed among cultivars. *Fusarium moniliforme* and *Aspergillus fumigatus* were recovered from seed embryos. Discoloured seeds had a higher incidence of fungi and lower percentage germination compared to clean seeds.

*Sorghum bicolor*(L.) Moench is cultivated widely in the middle and southern parts of Iraq (Missan and Kaddisia Governorate) and the grain is used principally as animal feed. Previous mycological surveys on this plant in Iraq have been mainly concerned with plant pathogens causing rust, smut and damping-off diseases (Mathur 1968, Hussain 1974, and Al-Jibbouri and Al-Sohaily 1981). However, the mycoflora of *Sorghum* grain have received increased attention during the last two decades by many workers in Asia, Africa and America (Verma and Khan 1965, Junejo and Malik 1967, Niles 1976, Bhadrallah and Ram Rao 1979, Rai and Gupta 1978, Pettit and Taber 1978, El-Shafie and Webster 1981, and El-Kady *et al.* 1982).

It is now becoming clear that saprophytic fungi associated with seed or grain are of economic importance causing deterioration of seed, reduced germinability and the production of chemicals (Mycotoxins) that are toxic to man and animals (Coady 1965, Neergard 1977, Pettit and Taber 1978, El-Shafie and Webster 1981, William and Rao 1981, and Bhat and Rukmini 1980). Therefore, this study was

undertaken to better understand and catalogue the mycoflora of *Sorghum* seed in Iraq and to assess seed quality from several sources.

## Materials and Methods

Seed of five cultivars of *Sorghum bicolor viz.* G-516, G-522, G-642, Brefes, and Kafer were obtained from Seed Section, Ministry of Agriculture, Abu-Ghraib, Baghdad, and four unidentified varieties were collected from the local markets in Basrah Governorate. The former five cultivars were collected immediately after harvest in 1981.

A working sample of each cultivar was obtained according to the international rules of seed testing (ISTA 1966). Two isolation methods were used in this study.

### *Blotter Method*

Three hundred seeds were taken randomly from each working sample of each cultivar. Seeds were surface disinfected with 1% sodium hypochlorite in a beaker for 10 min. Surface disinfected seeds were placed on water soaked blotters in sterilized aluminum trays as described by El-Shafie and Webster 1981. One hundred seeds were placed in each tray. The trays were covered by autoclavable cellophane sheets and the *Sorghum* seeds in the trays were incubated for 7-10 days at 25°C and a regime of 12 hr light and 12 hr darkness.

### *Agar Plate Method*

Three hundred surface disinfected seeds from each cultivar were used for the agar plate method. Fifteen seeds were placed on corn meal agar (CMA) in petri dishes (diam. 15 cm). The medium was supplemented with 50 mg/l chloramphenicol before autoclaving, to restrict bacterial growth. Seeds were incubated at 25°C as described before.

Seeds were examined individualy under a dissecting microscope. Percentage frequency of occurrence for each fungus and percentage contamination of seeds by fungi were recorded. Representative strains of each fungus sporulating on *Sorghum* were isolated and grown on CMA medium in petri dishes for identification.

### *Isolation of Fungi*

One hundred clean and one hundred discoloured seeds from each cultivar were chosen. Clean and discoloured seeds were treated with sodium hypochlorite (1%) and incubated separately in aluminum trays for 7-10 days as described before.

The percentage frequency of occurrence of the most common fungi in the seeds and percentage contamination and germination were calculated.

### *Location of Fungi in the Seeds*

Twenty seeds from each cultivar were taken randomly and mixed together to form a working sample. Seeds were soaked individually in sterilized water for 18 hr and dissected aseptically into three parts as described by Mathur *et al.* (1975). Seed parts were surface disinfected with 1% sodium hypochlorite solution for 5 min and plated separately on CMA medium in petri dishes. The dishes were incubated at 25°C for 7 days. The percentage frequency of occurrence of fungi in different parts of the seeds was recorded.

## Results and Discussion

A total of 38 species of fungi was isolated from seeds of the nine cultivars (Table 1). Among the common genera were *Aspergillus* (6 species) and *Drechslera* (5 species). Only two species of *Drechslera* were common; *Drechslera australiensis* (6 samples) and *D. spicifera* (5 samples). *Alternaria spp., Cladosporium spp., Fusarium spp., Ulocladium spp.,* and *Nigrospora spp.,* were represented by two species each. Other genera were represented by single species (Table 1).

The most common species were *Aspergillus fumigatus, A. niger,* and *Alternaria alternata* which were isolated from almost every sample. The percentage frequencies recorded on blotter method for these three species were 83.3, 31.9, and 20.3% respectively. *Aspergillus flavus* and *Fusarium moniliforme* were isolated from 7 samples. Their percentage frequencies as determined by the blotter method were 31.9 and 26.0 respectively. *Curvularia lunata* and *Aspergillus candidus* were isolated from five samples while *Aspergillus ochraceous, Chaetomium sp., Chaetomidium sp.,* and *Aspergillus fumigatus* (thermotolerant strains) were recovered from four samples. With few exceptions the remainder of the species were recovered from one or two samples and with low percentage frequencies.

Thirty four species were recovered from *Sorghum* grains using the blotter method, and 16 species of these were also isolated by the agar plate method. In addition, higher percentage frequencies of species such as *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. ochraceus, Curvularia lunata, Drechslera australiensis, D. spicifera, Fusarium moniliforme* and *Penicillium sp.* were recorded by the blotter method compared to the agar plate method.

**Table 1.** Percentage frequency of occurrence of fungi on *Sorghum* seeds determined by blotter and agar plate methods, number of samples infected and cultivar source.

| Fungi | % Frequency of fungi[x] | | No. of samp. infected | Cultivar[y] |
|---|---|---|---|---|
| | BM | AM | | |
| *Alternaria alternata* (Fr.) Keissir | 20.3 | 6.3 | 9 | 1 - 9 |
| *A. tenuissma* (Kunz ex Pers.) Wiltshire | 0.6 | – | 1 | 5 |
| *Aspergillus flavus* Fres | 31.9 | 9.6 | 7 | 1,3,4,5,7,8,9 |
| *A. fumigatus* Link | 83.3 | 34.3 | 9 | 1 - 9 |
| *A. niger* V. Tieghen | 31.9 | 34.2 | 9 | 1 - 9 |
| *A. orchraceus* Wilhelm | 12.3 | 3.6 | 4 | 4,5,7,8 |
| *A. terreus* Thom | 1.6 | – | 2 | 4,5 |
| *Aureobasidium pullulans* (DeBary) Arnaud | 0.9 | – | 2 | 1,6 |
| *Cladosporium cladosporioides* (Fres.) de Vries | 4.6 | – | 3 | 6,8,9 |
| *C. herbarum* (Pers.) Link | 4.3 | – | 2 | 7,8 |
| *Chaetomium sp.* | 6.6 | – | 4 | 4,6,7,8 |
| *Chaetomidium sp.* | 4.6 | 5.3 | 4 | 1,2,3,4 |
| *Curvularia lunata* (Walker) Boedijn | 21.6 | 7.3 | 5 | 1,2,3,6,8 |
| *Drechslera australiensis* (Bugn.) Subram. & Jain | 6.3 | 4.6 | 6 | 1,2,4,5,7,9 |
| *D. halodes* (Drechsler) Subram. & Jain | 1.6 | 1.0 | 2 | 4,5 |
| *D. hawaiiensis* (Bugn.) Subram. & Jain | 1.3 | – | 1 | 4 |
| *D. rostrata* (Drechsler) Rich. & Fraser | 1.6 | 2.3 | 2 | 5,6 |
| *D. spicifera* (Bain) Von Arx | 10.9 | 2.0 | 5 | 3,4,6,7,9 |
| *Fusarium moniliforme* Sheldon | 26.0 | 10.6 | 7 | 1,3,4,5,6,7,9 |
| *F. oxysporum* Schlecht | 0.6 | – | 1 | 8 |
| *Microascus sp.* | 1.6 | – | 1 | 8 |
| *Nigrospora sphaerica* (Sacc.) Mason | 2.9 | – | 1 | 8 |
| *N.* State of *Khuskia oryzae* Hudson | 1.0 | – | 1 | 6 |
| *Penicillium sp.* | 15.9 | 7.0 | 3 | 6,7,8 |
| *Phytophthora sp.* | 1.3 | – | 1 | 8 |
| *Rhizopus sp.* | 0.3 | 4.6 | 3 | 7,8,9 |
| *Stachybotrys sp.* | 1.3 | – | 1 | 7 |
| *Stemphyliomma terricola* Manoharachary & Ram Rao | 3.0 | – | 2 | 2,3 |
| *Stemphylium* state of *Pleospora herbarum* (Pers. ex Fr.) Rabenh. | 1.6 | – | 1 | 9 |
| *Trichoderma sp.* | 3.3 | – | 1 | 9 |
| *Trichotecium roseum* (Pers.) Link & Gray | 1.6 | – | 2 | 7,8 |
| *Ulocladium atrum* Preuss | 2.3 | – | 1 | 5 |
| *U. botrytis* Preuss | 1.3 | – | 1 | 6 |
| *Black mycelium* | – | 1.0 | 1 | 5 |
| *White mycelium* | 14.6 | 7.6 | 9 | 1 - 9 |
| [z]*Aspergillus candidu* Link ex Fries | – | 37.5 | 5 | 1,5,6,7,8 |
| [z]*A. fumigatus* Fres. | – | 50.0 | 4 | 4,7,8,9 |
| [z]*Thermomyces sp.* | – | 5.0 | 2 | 3,9 |

x BM: Blotter method, AM: Agar plate method, frequency represents occurrence from 300 seeds of each cultivar.
y Sample number: G-516:1; G-522:2; G-642:3; Brefes:4; Kafer:5; Unidentified local cultivars from 6-9.
z Thermophilic or thermotolerant strains (isolated on agar plate method at 45°C).

Other fungi such as *Alternaria tenuissima, Aspergillus terreus, Aureobasidium pullulans, Cladosporium cladosporioides, C. herbarum, Chaetomium sp., Drechslera hawaiiensis, Fusarium oxysporum, Microascus sp., Nigrospora* state of *Khuskia oryzae, N. sphaerea, Phytophthora sp., Stachybotrys sp., Stemphyliomma terricola, Stemphylium* state of *Pleospora herbarum, Trichoderma sp., Trichothecium roseum, Ulocladium atrum* and *U. botrytis* were isolated only by the blotter method.

It is evident from Table 1, that the number of fungi recovered from unidentified local cultivars (sample numbers 6-9) were more than those isolated from identified cultivars, with the exception of Kafer cultivar (Number 5). This may be explained by the fact that the identified cultivars were collected immediately after harvest, and hence, they were infested by field fungi only. On the other hand, some unidentified cultivars, whose harvest dates were not known, were collected from local markets, and so might be harbouring both field and storage fungi. Similar findings have been made by Rai and Gupta (1978) who recorded 45 and 57 fungal species from *Sorghum* grain in postharvest and storage conditions respectively.

*Stemphyliomma terricola* and *Chaetomidium sp.*, have been identified for the first time on *Sorghum* grain, while the remaining fungi have been previously reported by others. However, the majority of the species recorded in this study are new records for *Sorghum* grain in Iraq.

The degree of fungal contamination ranged from 29 to 92.6% of seeds infected, and the germination percentage of the seeds ranged from 21.0 to 85.6% (Table 2). Two *Sorghum* cultivars *viz.* G-516 and G-522 showed the lowest fungal contamination (29.0 and 38.0%) and their percentage germination of their seeds were 85.6 and 82.6% respectively. Loss in viability of contaminated *Sorghum* grain has been reported by many workers. Arif and Ahmed (1969) showed that fungi such as *Fusarium sp., Aspergillus sp., Penicillium sp.*, and *Helminthosporium sp.*, were isolated from *Sorghum* grain with reduced germination. Rao and Williams (1977) reported a viability loss of up to 100% in *Sorghum* grains heavily contaminated with *Fusarium* and *Curvularia spp.*

Fungal species composition and percentage fungal contamination varied among cultivars. Such variation may be attributed to the differences in geographical locality of cultivation, storage conditions, or to differences in the physiochemical natures of *Sorghum* cultivars. Similar suggestions have been made by Verma and Khan (1965), Vaidehi and Ram Rao (1977), Junejo and Malik (1967) and El-Shafie and Webster (1981).

**Table 2.** Percentage seed germination and percentage contamination by fungi from nine *Sorghum* samples

| Sample No. | *Sorghum* cultivar | seed germination[z] % | Contamination by fungi[z] % |
|---|---|---|---|
| 1 | G-516 | 85.6 | 29.0 |
| 2 | G-522 | 82.6 | 38.6 |
| 3 | G-642 | 57.3 | 91.0 |
| 4 | Brefes | 62.0 | 85.6 |
| 5 | Kafer | 69.0 | 82.0 |
| 6 | Unidentified local cultivar 1 | 61.0 | 89.6 |
| 7 | Unidentified local cultivar 2 | 66.0 | 91.3 |
| 8 | Unidentified local cultivar 3 | 35.3 | 92.6 |
| 9 | Unidentified local cultivar 4 | 21.0 | 72.0 |

z Percentage germination and percent contamination are based on 600 seeds of each cultivar.

Table 3 shows the percentage contamination by fungi and percentage germination in the clean and discoloured seeds. The percentage contamination by fungi of discoloured seeds ranged between 70-99% and the percentage germination of the same seeds ranged between 33-78%. The results show that seed

**Table 3.** Percentage seed germination and percentage contamination by fungi of clean and discoloured *Sorghum* seeds from nine cultivars

| Sample No. | *Sorghum* cultivar | Clean seeds | | Discoloured seeds[z] | |
|---|---|---|---|---|---|
| | | germination % | contamination % | germination % | contamination % |
| 1 | G-516 | 81.0 | 23.0 | 62.0 | 90.0 |
| 2 | G-522 | 87.0 | 66.0 | 68.0 | 99.0 |
| 3 | G-642 | 70.0 | 67.0 | 41.0 | 97.0 |
| 4 | Brefes | 93.0 | 67.0 | 54.0 | 99.0 |
| 5 | Kafer | 88.0 | 15.0 | 58.0 | 70.0 |
| 6 | Unidentified local cultivar 1 | 97.0 | 60.0 | 73.0 | 100.0 |
| 7 | Unidentified local cultivar 2 | 95.0 | 63.0 | 78.0 | 97.0 |
| 8 | Unidentified local cultivar 3 | 96.0 | 55.0 | 33.0 | 97.0 |
| 9 | Unidentified local cultivar 4 | 84.0 | 30.0 | 50.0 | 80.0 |

z Percentage germination and percent contamination are based on 100 seeds of both clean and discoloured seeds from each cultivar.

discoloration is associated with high incidence of fungi and a lower percentage of germination. Similar results were found by Tripathi (1974), Lopez and Christensen (1963) and El-Shafie and Webster (1981). The percentage frequencies of fungi in discoloured and clean seeds of all varieties are shown in Table 4. The major fungi associated with discoloured seeds were *Aspergillus fumigatus, Curvularia lunata, Aspergillus flavus, Fusarium moniliforme, Drechslera australiensis, D. spicifera, Aspergillus ochraceus* and *Alternaria alternata* and with percentage frequencies of 10.2, 8.0, 6.6, 4.3, 4.1, 3.3, 3.3 and 2.8% respectively.

**Table 4.** Percentage frequency of occurrence of the most common fungi on clean and discoloured *Sorgham* seeds

| Fungi | Clean seeds[z] | Discoloured seeds[z] |
|---|---|---|
| *Alternaria alternata* | 0.3 | 2.8 |
| *Aspergillus flavus* | – | 6.6 |
| *A. fumigatus* | 5.8 | 10.2 |
| *A. ochraceus* | – | 3.3 |
| *Curvularia lunata* | 1.0 | 8.0 |
| *Drechslera australiensis* | – | 4.1 |
| *D. spicifera* | 1.0 | 3.3 |
| *Fusarium moniliforme* | 0.7 | 4.3 |

z Percentage frequency is based on 900 seeds, 100 seeds from each a 9 different *Sorghum* cultivars.

El-Shafie and Webster (1981) attributed the seed discolouration in cultivar Mayo to *Drechslera spicifera, D. rostrata* and *Curvularia lunata,* while in cultivar Daber seed discolouration was mainly due to *Phoma sorghina.* However, *Fusarium* and *Curvularia spp.* were reported by Castor (1977) as the principal fungi causing grain discolouration and reduction in viability on *Sorghum* in Texas (USA).

The percentage frequency of the most prevalent fungi recovered from different parts of the seed are listed in Table 5. The majority of these fungi were recovered from the pericarp or pericarp and endosperm of the seed. However, two species, namely *Aspergillus fumigatus* and *Fusarium moniliforme,* were detected in the embryo in addition to the pericarp and endosperm. William and Rao (1981) pointed out that infection occurred soon after flowering and through the developing grain. Mathur *et al.* (1975) detected *Fusarium moniliforme* in embryos of *Sorghum* seed samples. Castor (1977) has suggested that the fungus can destroy the embryo indirectly by interfering with translocation from endosperm to embryo during germination.

**Table 5.** Percentage frequency of occurrence of fungi isolated from different locations in *Sorgham* seeds

| Fungi | Pericarp | Endosperm | Embryo[z] |
|---|---|---|---|
| *Alternaria alternata* | 3.0 | – | – |
| *Aspergillus flavus* | 2.4 | 0.7 | – |
| *A. fumigatus* | 3.4 | 6.0 | 0.3 |
| *A. niger* | 1.4 | 1.7 | – |
| *A. ochraceus* | 0.4 | – | – |
| *Curvularia lunata* | 3.2 | 0.8 | – |
| *Drechslera australiensis* | 1.1 | – | – |
| *D. spicifera* | 1.7 | 0.7 | – |
| *Fusarium moniliforme* | 3.5 | 0.3 | 0.1 |

z Percentage frequency of occurrence from 180 seeds representing a mixture of 20 seeds of 9 *Sorghum* cultivars.

We have shown that there is a large number of fungal species associated with *Sorghum* grain in Iraq. Greatest numbers of fungi were recovered from grain purchased from local markets. This grain also exhibited reduced germination. Discoloured seed from cultivars examined had larger numbers of fungi and greatest reduction in germination compared to clean seed.

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# المجموعة الفطرية لحبوب الذرة البيضاء في العراق

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تضمنت الدراسة مسح للفطريات المصاحبة لتسعة أصناف من حبوب الذرة البيضاء في العراق. تم التعرف على ٣٨ نوعاً «تعود إلى ٢٢ جنساً» من الفطريات. وكانت الفطريات السائدة تعود إلى الأنواع:

*A. niger, Aspergillus fumigatus, Alternaria alternata Drechslera spicifera, Curvularia lunata, A. flavus, Fusarium moniliforme, D. australiensis.*

أوضحت الدراسة بأن استعمال طريقة الورق النشاف في العزل أحسن بكثير من طريقة الأطباق الحاوية على الوسط الغذائي، حيث تم عزل ٢٨ نوعاً بالطريقة الأولى و١٧ نوعاً بالطريقة الثانية. تراوحت النسبة المئوية من تلوث الحبوب بالفطريات ما بين ٢٩ ـ ٦,٩٢٪، والنسبة المئوية للإنبات ما بين ٢١ ـ ٦,٨٥٪. وجد اختلافاً "واضحاً" في التركيب النوعي للفطريات وكذلك النسبة المئوية للإنبات من صنف إلى آخر من أصناف الذرة البيضاء.

وجد أن الحبوب المبقعة أكثر تلوثاً بالفطريات وأقل نسبة في الإنبات من الحبوب النظيفة. ودرس كذلك موقع تواجد الفطريات من جسم الحبه (الغلاف، السويداء، الجنين) وقد تم عزل الفطرين *F. moniliforme, A. fumigatus* من الجنين.