

## Distribution of osmophilic and halophilic fungi in combine harvester sorghum dust particles from Egypt

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Fifty-two osmophilic (or osmotolerant) and halophilic (or halotolerant) species and one variety representing 24 genera were encountered from 20 combine harvester sorghum dust samples collected from four Governorates in Upper Egypt, on 50% sucrose – and 15% NaCl-Czapek's agar at 28°C. The results obtained on the two media were basically similar, but numerous fungi could not tolerate 15% NaCl. The most frequent genera were *Aspergillus Eurotium*, *Penicillium* and *Cladosporium*.

### INTRODUCTION

The terms osmophilic (or osmotolerant) and halophilic (or halotolerant) microorganisms are used to describe microorganisms either only or better growing on media containing 20 to 40 percent sugar (sucrose), or an equivalent molar concentration of sodium chloride. Numerous organisms require substantially greater amounts of these compounds for optimum growth and development. Microorganisms (Fungi, Bacteria, *Actinomycetes*) which may be present in large numbers in combine harvester dust particles, are pathogenic to farm workers and animals. They cause a number of diseases such as allergy asthma, infection, pulmonary mycoses and farmer's lung.

In Egypt, a few investigations have been carried out on osmophilic or osmotolerant and halophilic or halotolerant soil fungi (Abdel-Hafez et al. 1977; Moubasher et al. 1985; Abdel-Sater 1987). The present work is aimed to study the distribution, composition, numbers and frequency of osmophilic or osmotolerant and halophilic or halotolerant fungi in combine harvester sorghum dust particles from Egypt.

## MATERIALS AND METHODS

Twenty samples, 0.5 Kg each, of combine harvester sorghum (*Sorghum vulgare* Pers. var. *cernuum* (Ard.) Fiori et Paoli) dust particles (very fine particles of lemmae, paleae and glumes) were collected during November 1986 from four Governorates, commonly cultivated with sorghum plants, in Upper Egypt. The number of samples from each Governorate was as follows: Beni-Suef, 5 samples; Assiut, 4 samples; Sohage, 3 samples; and Qena, 8 samples. Samples were sifted through a 80 mesh sieve to remove large dust particles. All samples were stored at 3-5°C.

Replicates of sorghum (white durra or Egyptian corn) dust particles were milled and a weighed portion of their powder was dried in an oven for 48 h at 80°C, then cooled in a desiccator and re-weighed. The moisture content was expressed as a percentage of the dry-weight. The powder was analysed for the ethanol soluble fraction, diastase soluble fraction, hemicellulose and lignin, as described by Chang (1967).

The fungal flora of combine harvester sorghum dust particles was determined by the dilution plate method (Johnson et al. 1959). Ten plates were used for each sample, of which five plates were poured with 50% sucrose-Czapek's agar and the other five - with 15% NaCl agar in order to isolate osmophilic (or osmotolerant) and halophilic (or halotolerant) fungi, respectively. Then rose-bengal (1/15000) was added as a bacteriostatic (Smith, Dawson 1944). Plates were incubated at 28°C for 1-2 (osmophilic and osmotolerant) and 4-6 (halophilic and halotolerant) weeks. The developing fungi were counted, identified, and their numbers calculated per g dry weight of sorghum dust particles. The colonies of slow-growing fungi, which were about to be overgrown, as well as mycelial fragments of some colonies, were transferred to yeast extract or malt extract agar supplemented with 50% sucrose or 15% NaCl.

## RESULTS AND DISCUSSIONS

The moisture contents of the samples tested widely fluctuated between 3.3 to 12.2%. The highest value was estimated for sample No. 2 from Beba (Beni-Suef Governorate) and the lowest was recorded in sample No. 17 from Deshna (Qena Governorate). It is obvious that the moisture content was less than 5% in eight samples and more than 10% in four samples (Table 1). Chemical analysis of combine harvester sorghum dust particles show clearly that the value of ethanol soluble fraction (which contained sugars, glucosides, essential oils, colouring matter and resinous substances) ranged from 3.6-5% in diastase soluble fraction: 12.1-14.9%, in hemicellulose: 30.2-32.9%; in cellulose: 42.1-46.2; and in lignin 6.4-7.2% of the initial dry weight of the

Table 1

Moisture content /calculated as percentage of dry weight/, average total count and maximum value /calculated per g dry sorghum dust in every samples/ and number of genera and species isolated from 20 combine harvester sorghum dust samples on 50 % sucrose- and 15 % NaCl-Czapek's agar at 28°C

Samples Nob.	Moisture content %/	50 % sucrose -			15 % NaCl -		
		ATC + SD/MV/	NG	NS	ATC + SD/MV/	NG	NS
1	11.1	20000 ± 6415/27000/	3	12	2283±293/2500/	4	9
2	12.2	30000 ± 3747/34200/	4	10	2550±687/3140/	3	13
3	4.2	8200±4715/13400/	2	8	2283±209/3650/	3	9
4	4.2	13000±600/13600/	2	6	8016±225/8250/	1	4
5	4.5	13400±529/13800/	2	5	7116±1664/8200/	1	3
6	6.9	10266±3523/14000/	2	7	5666±3970/9850/	2	4
7	4.7	4666±2914/7000/	2	8	1566±510/2150/	3	9
8	5.4	8266±7338/16000/	4	9	6516±2456/9300/	4	10
9	8.9	22600±16058/36000/	2	9	5566±982/6700/	2	8
10	6.4	16600±5173/22200/	4	12	4600±1096/5850/	1	6
11	10.2	33200±3616/36600/	5	10	2766±1040/3600/	2	7
12	7.3	12608±2800/14600/	5	10	750±576/1400/	3	6
13	7.5	9200±4386/13000/	6	10	1383±765/1850/	2	7
14	8.3	12666±1527/14000/	6	10	3831±1159/4900/	2	8
15	10.5	15300±4932/20900/	11	19	1033±29/1050/	2	7
16	3.4	5400±721/6000/	7	15	750±150/900/	3	12
17	3.3	5067±2003/7000/	8	17	4150±998/5000/	5	8
18	3.5	5800±1039/7000/	4	12	333±208/500/	2	7
19	4.1	5833±1550/7600/	4	11	3233±539/3850/	4	9
20	4.4	6151±1474/7300/	2	6	3500±1103/4550/	4	14

ATC ± SD/MV/ - average total count in every sample ± standard deviation /the maximum in the brackets/.

NG - number of genera.

NS - number of species.

dust particles. Moubasher et al. (1982) found that the value of the preceding fractions in Egyptian wheat and broad-bean straws were 4.3 and 5.2%, 9.1 and 18.4%, 34.7 and 27.8%, 43.2 and 41.7%, and 8.6 and 6.9%, respectively.

The average total count and maximum value of osmophilic and osmotolerant fungi widely ranged from ranged from 5067-33200; and 6000-36600 colonies per g sorghum dust particles, respectively (Table 1). The highest

Table 2

Number of species, percentage average total count /% ATC; calculated per average gross total count in every samples/ and percentage frequency /% F; calculated per 20 samples/ of genera isolated from 20 combine harvester sorghum dust samples on 50 % sucrose- and 15 % NaCl-Czapek's agar at 28°C

Genera	50 % sucrose-			15 % NaCl-		
	NS	%ATC	%F	NS	%ATC	%F
Alternaria	1	3.63	55	-	-	-
Aspergillus	15 + 1 var.	69.71	100	12	63.2	100
Cephalosporium	-	-	-	1	0.1	10
Circinella	1	0.03	5	-	-	-
Cladosporium	3	6.12	35	1	11.2	45
Curvularia	1	0.01	-	-	-	-
Drechslera	1	0.03	-	-	-	-
Emericella	1	0.01	5	-	-	-
Epicoccum	1	4.11	15	-	-	-
Eurotium	4	5.32	100	4	15.1	100
Exserohilum	1	0.01	5	-	-	-
Fusarium	3	4.1	40	-	-	-
Hemicola	1	0.1	10	-	-	-
Microascus	1	0.01	5	-	-	-
Nucor	1	0.1	20	-	-	-
Nyrothecium	1	0.03	5	-	-	-
Faecilomyces	1	0.01	5	-	-	-
Penicillium	5	6.21	85	4	7.8	75
Rhizomucor	1	0.2	5	-	-	-
Rhizopus	1	0.03	10	-	-	-
Scopulariopsis	1	0.01	5	2	2.6	25
Sepedonium	1	0.03	5	-	-	-
Trichoderma	1	0.01	5	-	-	-
Ulocladium	1	0.03	5	-	-	-
Total	48 + 1 var.	100		24	100	

value of average total count and the maximum were determined in sample No. 11 (from Sohag Governorate) with high value of moisture (10.2%) and low fungi number (5 genera and 10 species). The lowest counts were recorded in sample No. 17 (from Qena Governorate) with the lowest value of moisture (3.3%), and moderate fungi number (8 genera and 17 species). The widest

fungi spectrum (11 genera and 19 species) was obtained in sample No. 15 with 10.5% moisture moderate value of average total count (15300 colonies) and with maximum value (20900 colonies). The lowest fungi number (2 genera and 5 species) was recorded in sample No. 5 (from Beni-Suef Governorate) with low value of moisture (4.5%) and moderate of average total count (13400 colonies) and the maximum value (13800 colonies). The previous results reveal that there is no correlation between the average total count or maximum value and number of osmophilic and osmotolerant genera and species. But, in several cases the high number of genera and species were estimated in samples with high moisture content and contributed high average total count or maximum value and vice versa (Table 1).

Forty-eight species and 1 variety belonging to 24 genera were collected from 20 combine harvester sorghum dust samples on 50% sucrose-Czapek's agar at 28°C (Table 2, 3). Most of the preceding genera and species were isolated from various substrata in Egypt and some Arab countries on 30-70% sucrose agar plates (Moustafa 1975; Abdel-Hafez et al. 1977, 1983; Moubasher et al. 1979, 1981, 1985; Abdel-Hafez 1982), but all of them were encountered for the first time from combine harvester sorghum dust particles.

*Aspergillus* was the most common genus and occurred in 100% of the samples comprising 69.7% of total fungi. It was represented by 15 species and 1 variety of which *A. niger*, *A. flavus*, var. *columnaris*, *A. sydowi* and *A. ochraceus* were isolated with high frequency. They emerged in 60-100% of the samples constituting 1.4-81.2% of total *Aspergillus* and 1-56.6% of total fungi. These species were also prevalent in soils from Egypt (Abdel-Hafez et al. 1977; Moubasher et al. 1985), Jordan (Moubasher et al. 1981), Kuwait (Moustafa 1975) and Saudi Arabia (Abdel-Hafez 1982) on 30-70% sucrose agar plates. Three species were encountered with moderate frequency; these were *A. terreus*, *A. tamarii* and *A. wentii* which emerged in 25-30% of the samples constituting 0.3-0.65% of total *Aspergillus* and 0.2-0.45% of total fungi. *A. fumigatus*, *A. japonicus*, *A. versicolor*, *A. candidus*, *A. parasiticus*, *A. oryzae* and *A. carneus* were less frequent. These *Aspergillus* species were osmophilic, but to variable extent (Mazen et al. 1981).

*Eurotium* was isolated from all samples encountering 5.3% of total fungi. From species were collected of which *E. amstelodami* (85% of the samples, 90.3% of total *Eurotium* and 4.8% of total fungi) and *E. chevalieri* (25, 8.7 and 0.5%) were the most common; *E. repens* and *E. rubrum* were less frequent. Raper et Fennell (1965) stated that members of *Eurotium* (which includes *E. amstelodami*, *E. chevalieri*, *E. repens*, *E. rubrum*, *E. halophilicus* and other species) are of osmophilic and halophilic nature. The above *Eurotium* species were encountered, but with variable number and frequency, from soils of some Arab countries on osmophilic agar medium (Moustafa

Table 3

Average total count and maximum value /calculated per g dry sorghum dust in every samples/, number and frequency /out of 20 samples/ of fungal genera and species isolated from 20 combine harvester sorghum dust samples on 50 % sucrose- and 15 % NaCl-Czapek's agar at 28° C.

Genera and species	50 % sucrose-		15 % NaCl-	
	ATC ± SD/NV/	NCI/OR	ATC ± SD/NV/	NCI/OR
<i>Aspergillus</i> /total count/	17994±20600/200600/	20 H	42895±5100/48300/	20 H
<i>A. niger</i> Van Tieghem	146133±16685/159400/	20 H	4250 ±976/5200/	13 H
<i>A. flavus</i> Link	6333 ±832/7000/	18 H	4616 ±2860/7700/	12 H
<i>A. flavus</i> var. <i>columnaris</i> Raper et Fennel	2566 ±1289/4000/	15 H		
<i>A. sydowi</i> /Bain, et Sart./ Thom et Church.	16200 ±3666/19400/	13 H	2333 ±1612/4050/	7 H
<i>A. ochraceus</i> Wilhelm	5233 ±1662/7000/	12 H	18666±3213/22300/	17 H
<i>A. terreus</i> Thom	566 ±378/1100/	6 M	566 ±340/950/	9 M
<i>A. tamarii</i> Kita	1166 ±1150/2300/	5 M	83 ±104/200/	4 L
<i>A. wentii</i> Wehner	633 ±493/1200/	5 M	133 ±57/200/	7 M
<i>A. fumigatus</i> Freudenius	233 ±321/600/	3 L	9250±±1250/10500/	4 L
<i>A. japonicus</i> Saito	166 ±208/400/	2 R		
<i>A. versicolor</i> /Vuill./ Tiraboschi	600 ±600/1200/	2 R		
<i>A. candidus</i> Link	33 ±57/100/	1 R	16 ±28/50/	1 R
<i>A. parasiticus</i> Speare	33 ±57/100/	1 R		
<i>A. oryzae</i> /Ahlb./ Cohn	33 ±57/100/	1 R	2966 ±57/2900/	6 M
<i>A. carneus</i> /V. Tiegh./ Blochwitz	33 ±57/100/	1 R	100 ±86/150/	4 L
<i>A. terricola</i> /Marchal/	33 ±57/100/	1 R	16 ±28/50/	1 R
<i>Bacterium</i> /Total count/	13732 ±4600/19800/	20 H	10215±1400/12200/	20 H
<i>B. pasteurianus</i> Wangin	12400 ±3051/15400/	17 H	5016 ±927/5900/	16 H
<i>B. chevallieri</i> Wangin	1200 ±950/2000/	5 M	33 ±57/100/	1 R
<i>B. roseni</i> De Bary	66 ±115/200/	2 R		
<i>B. rubrum</i> Konig, Spieckermann et Bremer	66 ±115/200/	1 R	300 ±346/700/	6 M

*E. halophilicum* Christensen, Papavizas et Benjamin

*Emicella nidulans* / Eidam/ Vuillemin

Penicillium /total count/

*P. oxalicum* Currie

*P. chrysogenum* Thom

*P. citrinum* Thom

*P. janczewski* Zaleski

*P. funiculosum* Thom

*P. janthinellum* Biourge

Penicillium spp.

*Alternaria alternata* /Fr./ Keisler

Fusarium /total count/

*F. moniliforme* Sheldon

*F. oxysporum* Shelecht.: Fr.

*F. solani* /Mart./ Sacc.

Cladosporium /total count/

*C. herbarum* /Pers./ Link: S.P. Gray

*C. macrocarpum* Zreans

*C. sphaerospermum* Tenzig

Mucor /total count/

*M. racemosus* Fresenius

Mucor sp.

*Epicoceum purpurascens* Zhrenb.: Schlecht.

*Exserohilum rostratum* /Brechler/ Leonard et Suggs

anamorph of *Setsphaeria rostrata* Leonard

*Drechslera spicifera* /Rainier/ Von Arx anamorph of

*Cochliobolus speciferus* Nelson

*Humicola grisea* Traaen

*Rhizopus stolonifer* Ehrenb.: Fr. Lindt

33	±57/100/	1 R	4866	±869/5800/	10 H
16032	±7552/23700/	17 H	5316	±1271/4100/	15 H
6833	±2025/9100/	14 H	1000	±1250/2600/	5 M
1233	±802/2000/	7 H	483	±115/550/	6 M
3800	±3616/7200/	3 L	2350	±1670/4150/	10 H
800	±916/1800/	2 R			
66	±115/200/	1 R	400	±608/1100/	4 L
3300	±1100/4400/	4 L	1083	±1300/2700/	3 L
9366	±3946/13900/	11 H			
10466	±1914/12500/	8 M			
10200	±1609/11900/	7 M			
200	±346/600/	1 R			
66	±115/200/	1 R			
15798	±7901/23900/	7 M	7616	±1533/8600/	3 H
14166	±7778/22300/	7 H	7616	±1533/8600/	9 M
66	±115/200/	1 R			
1566	±404/2000/	1 R			
266	±202/600/	4 L			
200	±346/600/	2 R			
66	±115/200/	1 R			
10600	±2500/13100/	3 L			
33	±57/100/	1 R			
66	±115/200/	1 R			
200	±346/600/	2 R			
800	±800/1600/	2 R			

<i>Circinella mucosa</i> / Sorok. / Berl. et De Toni	66	$\pm 115/200/$	1	R	
<i>Curularia lunata</i> / Wäcker / Boedijon anamorph of	33	$\pm 57/100/$	1	R	
<i>Cochliobolus lunatus</i> Nelson et Haasls	66	$\pm 115/200/$	1	R	
<i>Myrothecium verrucaria</i> / Alb. et Sch. / Ditmar	33	$\pm 57/100/$	1	R	
<i>Paecilomyces variotii</i> Bainier	400	$\pm 692/1200/$	1	R	
<i>Rhizocorpus pusillum</i> / Mindt / Schipper	33	$\pm 57/100/$	1	R	1766 $\pm 500/2250/$
<i>Scopulariopsis</i> / total count/	33	$\pm 57/100/$	1	R	
<i>S. brevicornis</i> / Sacc. / Rainier					250 $\pm 50/300/$
<i>S. candida</i> / Oueguen / Vuillemin					1516 $\pm 450/1950/$
<i>S. halophilica</i> Tubaki					
<i>Sepedonium Chrysoepermum</i> / Bulliard / Fr.	66	$\pm 115/200/$	1	R	
<i>Trichoderma viride</i> / Pers. ex Gray	33	$\pm 57/100/$	1	R	
<i>Ulocladium atrum</i> Freese	66	$\pm 115/200/$	1	R	
<i>Cephalosporium</i> sp.					83 $\pm 76/150/$
<i>Micromascus cingereus</i> / Trille-Cell. et Gaudin / Gursi	33	$\pm 57/100/$	1	R	
Gross total count	258215	$\pm 21043/281400/$			67891 $\pm 5553/73750/$

ATC  $\pm$  SD/NV/-average total count in every sample  $\pm$  standard deviation/in the maximum bracketin, in all cases the minimum value being zero/.

OK - frequency

H - High between 10-20 cases /out of 20 samples/.

L - low 3 or 4 cases.

R - rare 1 or 2 cases.



1975; Abdel-Hafez et al. 1977 et 1983; Abdel-Hafez 1982; Moubasher et al. 1981 et 1985).

*Penicillium* was isolated from 17 samples (out of 20) contributing 6.2% of total fungi. It was represented by 5 species of which *P. oxalicum* (70% of the samples, 42.6% of total *Penicillium* and 2.6% of total fungi) and *P. chrysogenum* (35, 7.7 and 0.5%) prevailed; *P. citrinum*, *P. janczewskii* and *P. funiculosum* were less common in combine harvester sorghum dust particles. Moubasher et al. (1985) found that *P. chrysogenum*, *P. citrinum* and *P. corylophilum* were the most encountered *Penicillium* species in soils of Wadi Bir-El-Ain (Egypt) on 40% sucrose agar plates at 28°C. *P. notatum*, *P. citrinum* and *P. chrysogenum* or *P. albidum* were the most common *Penicillium* species from soils of Saudi Arabia and Syria on osmophilic agar medium (Abdel-Hafez 1982; Abdel-Hafez et al. 1983). Mazen et al. (1981) rated *P. notatum* and *P. citrinum* as fairly osmophilic (best growth at 30-40% sucrose); and *P. chrysogenum*, *P. funiculosum* and *P. albidum* as indifferently osmophilic (almost equal growth at all concentrations of sucrose; 30, 40, 50, 60 and 70%).

*Alternaria*, represented only by *A. alternata*, was encountered in 55% of the samples comprising approximately 3.6% of total fungi. This species were isolated previously, but with variable number and frequency, from Egyptian soils and barley grains (Abdel-Hafez et al. 1977; Moubasher et al. 1979 et 1985), as well as from soils of some Arab countries (Moustafa 1975; Moubasher et al. 1981; Abdel-Hafez 1982; Abdel-Hafez et al. 1983) on 30-70% sucrose agar plates.

*Fusarium* (4 species) and *Cladosporium* (3 species) occurred with moderate frequency in 40 and 35% of the samples constituting 4.1 and 6.1% of total fungi, respectively. *F. moniliforme* (35% of the samples and 3.95% of total fungi) being the most common member of the former genus and *C. herbarum* (35 and 5.5%) the most prevalent of the latter. Abdel-Hafez (1982) isolated these two species with low frequency from desert soils of Saudi Arabia on 40% sucrose agar plates at 28°C. The remaining genera and species were less frequent and accounted collectively for 5% of total fungi (Table 2).

Twenty-four halophilic and halotolerant species which belonged to 6 genera were collected on 15% NaCl—Czapek's agar at 28°C, i.e. less than on 50% sucrose agar (24 genera and 48 species). This means that numerous fungal genera (75% of total genera) and species (50% of total species) could not withstand 15% NaCl. The average total count and maximum value of halophilic and halotolerant fungi widely fluctuated between 333-8016, and 500-9850 colonies per g dry dust particles, respectively. The results show clearly that there is no correlation between the counts of these fungi and number of genera and species. However, some samples which contained high

numbers of halophilic and halotolerant fungi there were wide spectra of genera and species and vice versa (Table 1).

The results obtained on 15% NaCl agar plates were basically similar to those on 50% sucrose with the most encountered genera being *Aspergillus* (12 species), *Eurotium* (4 species) and *Penicillium* (4 species). They occurred in 100%, 100 and 75% of the samples giving rise to 63.2, 15.1 and 7.8% of total fungi, respectively. The highest numbers and frequency were displayed by *A. niger*, *A. flavus*, *A. sydowi*, *A. ochraceus*, *A. terreus*, *A. wentii*, *A. oryzae*, *E. amstelodami*, *E. rubrum*, *E. halophilicus*, *P. oxalicum*, *P. chrysogenum* and *P. citrinum*. Moubasher et al. (1985) isolated 27 genera and 99 species from desert soils of Wadi Bir-El-Ain (Egypt) on 5% NaCl-Czapek's agar, of which *A. niger*, *A. fumigatus*, *A. glaucus* group (represented by *A. amstelodami*, *A. chevalieri*, *A. ruber*, *A. athecus* and *A. montevidensis*), *A. terreus*, *A. sydowi*, *P. chrysogenum*, *P. citrinum*, *P. corylophilum* and *U. botrytis* prevailed. Most species were also common in Saudi Arabian desert soils on 5% NaCl agar plates at 28°C (Abdel-Hafez 1981). *Cladosporium* (*C. herbarum*) and *Scopulariopsis* (*S. candida* and *S. halophilica*) were isolated with moderate frequency they were encountered in 45% and 25% of the samples comprising 11.2% and 2.6% of total fungi, respectively. The remaining species were less frequent (Table 3). Abdel-Sater (1987) listed *E. amstelodami*, *E. chevalieri*, *E. repens*, *E. rubrum*, *E. halophilicus* and *S. halophilica* as highly halophilic (they grow on 5-25% NaCl, but exhibited very restricted growth or no growth on Czapek's medium free from NaCl); *A. flavus*, *A. nidulans*, *A. ochraceus*, *A. sydowi*, *A. terreus*, *A. wentii*, *P. chrysogenum*, *P. citrinum*, *P. oxalicum* and *S. candida* as fairly (moderately) halophilic (they grow on 5 to 15 or 20% NaCl with the best growth at 10 or 15% NaCl); and *A. candidus*, *A. niger*, *A. oryzae*, *A. tamarii* and *C. herbarum* as weakly halophilic (they grow on 5 to 10 or 15% NaCl with the best growth at 5% NaCl).

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