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GRAIN SORGHUM-BORNE FUNGI AND THEIR CONTROL 2- RELATIONSHIP BETWEEN FUNGAL FLORA AND STORAGE OF DIFFERENT GRAIN SORGHUM GENOTYPES

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Abstract

Twenty three fungal species were isolated from different grain sorghum genotypes. *Aspergillus flavus, Fusarium oxysporum, F. moniliforme and Gibberella fujikuroi* were of the highest occurrence in the grains of all genotypes examined. Local 29, Selection 1007, Local 162 and Dorado sorghum grain genotypes yielded higher number of fungi than Line C, Giza 15, Local 169 and Local 119, while Local 129, Kuymne, Giza 113, ICSR- 89037 and Giza 54 showed less number of fungi.

Grains were inoculated with spores of each of *A. flavus, F. moniliforme, F. oxysporum* and the results revealed that genotypes Giza 113 and Giza 54 remained essentially free of the three fungi, retained very high level of germinability and showed the lowest mycotoxins accumulation. On the contrary, the highest fungal invasion and decline in germinability occurred in inoculated grains and a high mycotoxins production was observed in Local 29, Selection 1007 and Local 162 genotypes. The remaining sorghum genotypes showed intermediate susceptibility to fungal invasion and a slight reduction in germinability of inoculated grains, as well as lower mycotoxin formation.

Grain coat leachates and grain extracts of thirteen sorghum genotypes exhibited an inhibitory effect on spore germination of *A. flavus, F. moniliforme, and F. oxysporum,* which varied not only by the genotype, but also depended on fungi tested. High inhibition was obtained by grain coat leachates or grain extracts of genotypes Giza 54 and Giza 113.

INTRODUCTION

The interrelationship between seed and seed-borne microorganisms is very complicated and seed-borne fungi affect seeds in various ways. Seeds genotype also influences the physiology of the fungi associated with them as well (Niles, 1976).

One of the major factors of low productivity of grains sorghum (*Sorghum bicolor* (L.) Moench) in Egypt and the world is poor germination and reduced seed-

ling vigour as well as spontaneous heating and various kinds of spoilage due to seed-borne fungi (Fahim *et al.*, 1982 and Arafa *et al.*, 1999).

Alternaria alternata, Alternaria sp, Aspergillus flavus, A. niger, A. glaucus and other Aspergillus sp., Curvularia lunata, Drechslera australiensis, D. rostrata, Fusarium oxysporum, F. solani, F. moniliforme, F. sambucinum, Penicillium sp., Stemphylium sp. and other fungi were isolated from grains of sorghum genotypes during storage as well as in the field (Fahim *et al*., 1982).

A moisture content above 13% in sorghum grains permits invasion by storage fungi such as; *Alternaria, Helminthosporum, Fusarium*, and *Aspergillus* during storage. Other fungi invade grains before harvest (Fahim *et al.*, 1982). Thus, the deteriorationof grains during storage by the activity of fungi has been a problem of economic importance.

Fusarium moniliforme, F. solani, F. oxysporum, Aspergillus flavus associated with sorghum grains were recorded to produce some mycotoxins such as; zearalenone, zearalenols, deoxynivalenol, and aflatoxin (Snijders and Perkowski, 1990). Various substances in grain exudates or extracts have been shown to either stimulate or inhibit spore germination in fungi and/or development of the pathogens and consequently the increase or decrease in disease incidence (Arafa *et al.*, 1999)

The present investigation aimed to evaluate 13 different genotypes of sorghum for 1) the fungal flora associated with each 2) their susceptibility to *Fusarium oxyporum, F.moniliforme*, and *Aspergills flavus invasion* and mycotoxin production and 3) the effect of grain coat leachates or grain extracts on the spore germination of the fungi tested.

MATERIALS AND METHODS

A- Source of sorghum genotypes:

Grain samples, (2 kg each) of the sorghum genotypes Local 169, 29, 129, 119, 162, Giza 15, 54,113, Kuymne, Dorado, Selection 1007, Line C, and ICSR - 89037 were taken randomly from grain lots produced at Shandaweel Res. Station, Sohag, Egypt in 1998/1999 season, and stored for 6 months under commercial storage conditions. Grains of each genotype were kept in a sterile polyethylene bag, sealed and sealed in another bag, to minimize moisture loss. Samples were transferred immediately to the laboratory and kept at 5°C for subsequent determination of fungi, artificial inoculation by fungi tested and mycotoxins production.

B- Estimation of grain-borne fungi:

The incidence of grain-borne fungi was determined by plating 400 grains of each genotype on 1% Glucose - Czapek's agar medium + rose - bengal (0.1 g/l) + streptomycin (30 mgl/l) (Martin, 1950) after being surface-sterilized in 1.25% sodium hypochlorite solution for 1-2 minutes. Plates (five grains/ plate) were incubated either at 28°C for mesophilic fungi or at 45 °C for the thermophilic or thermotolerant fungi for 5-7 days .

Pure cultures of the developing fungi were obtained by hyphal tip transfers.

Stock cultures were kept on 1% Glucose-Czapek's agar slants in a refrigerator for further studies.

The isolated fungi were identified according to Barnett and Hunter (1972) and Booth (1977).

C- Artificial infection of the grains tested:

One hundred gm of healthy grains from each genotype of sorghum were surface-disinfested with 2% aqueous solution of calcium hypochlorite as described by Seenappa *et al.*, (1981). The disinfested grains were then transferred to 250 ml sterile Erlenmeyer flasks and moistened to reach a moisture content of 25% by adding sterile distilled water according to Lutey and Christensen (1963). Each flask was inoculated with 2 ml. of concentrated spore suspension from 10 day-old PDA cuture of the fungi *Aspergills flavus, Fusarium oxysporum, and F.moniliforme.*

After inoculation, the flasks were gently shaken to distribute the inoculum evenly. The inoculated and uninoculated treatments were stored at 85% RH (26±2 °C) for 30 days in a humidity chamber as described by Seenappa *et al.* (1981). At the end of the incubation period, the growth of fungi was visually assessed on each grain genotype and grains were examined for germination and mycotoxin presence.

Grain moisture contents (M.C.):

These were determined by the two-stage air oven method (Qasem and Christensen, 1958) on a wet-weight basis.

Percentage of grain invaded by fungi tested and their germinability:

Fifty Kernels of each sample genotype were shaken in 2 % Na OCl for 1 minute, rinsed in sterile distilled water, and placed either on Czapek's agar +

500ppm rose bengal + 100ppm streptomycin sulfate to detect A. flavus, and on potato-sucrose agar (PSA) + 450ppm rose bengal + 80ppm streptomycin sulfate to detect *F. oxysporum and F. moniliforme*. Another 50 grains were placed between moist paper towels at temperature 26±2°C in a plastic box; seedlings were counted daily for 7 days, with any grain producing a root or coleoptile being counted as germinated.

Mycotoxin analysis:

Fifty gm samples each of inoculated and noninoculated grains of every genotype were screened for the presence of mycotoxins. Extraction of grinded grains was made according to Abdel-Gawed and Zohri (1993). Clean-up of the crude extracts was made according to AOAC (1980). The detection of mycotoxins by chromatographic analysis of the chloroform extracts was wade on precoated silica gel plate type 60F254 (Merck) for the presence of Aflatoxin, Zearalenone, Zearalenols, Deoxynivalenol and Moniliformin according to standard procedures (Gimeno, 1979 and Bottalico *et al.*, 1989).

D- Effect of grain coat leachates or grain extracts of thirteen sorghum genotypes on spore germination of *A. flavus, F. oxysporum, and F. moniliforme:*

Grain coat leachates of the thirteen sorghum genotypes tested were prepared by placing 10 gm of surface sterilized grains of each in sterilized Petri dishes (10 cm in diameter) each containing 20 ml. of distilled water and incubated in a grain germinator at 20°C (I.S.T.A., 1959). After 24 h, grain coat leachates were collected by washing the incubated grains with distilled water and made up to a final volume of 100 ml.

The grain extracts were prepared by crushing 10 gm of surface-disinfested (1.25% NaOCl for 2 mintes) grains in 100 ml of sterilized distilled water. The crushed grains were filtered and their final volume were adjusted to 100 ml. Grain coat leachates and grain extracts were sterilized by Seitz filter before use. Spores of fungi tested were suspended in the grain coat leachates, grain extracts or distilled water, which served as a control, Hanging drop technique (Claudius and Mehrotra, 1973) was used to determine the percentage of spore germination. Three replicates were used per treatment and the mean values of the replicates were recorded. Percent spore germination was recorded after an incubation period of 6-8 h at 25±2°C.

RESULTS

Grain samples, 2 Kg each, of each of the tested sorghum genotypes were visually examined after being stored for 6 months. Discolored grains were sorted out and categorized according to the type of discoloration into 5 categories.

Data in table (1) show that the percentage of different categories of discolored sorghum grains differed from one variety to another. The lowest percentage of all discoloration was found in giza 54 (3.40 %) , ICSR- 89037(3.86%), Kuymne (4.56 %) and Giza 113 (4.83 %) , whereas, the highest percent of discoloration was 24.98 %, 17.87 % and 16.48 % in the Local 29, Line C and Selection 1007 , respectively.

Grains having black points and red points were the most commonly encountered in various genotypes tested, then pink, brownish and deep red discoloration followed (Table 1).

Table 1. Percentage of naturally discolored grains in stored sorghum genotypes under commercial conditions for six months.

Genotypes	%, grain discoloration[(a)]					
	Black point	Red point	Pink	Slight black	Deep red	Total
Local 169	5.21	- -	3.14	1.94	- -	10.29
Local 29	11.64	- -	7.23	6.11	- -	24.98
Giza 15	- -	3.45	2.27	2.09	- -	7.81
Local 129	6.12	- -	1.91	4.96	- -	12.99
Kuymne	1.82	- -	2.74	- -	- -	4.56
Selection 1007	- -	12.66	- -	- -	3.82	16.48
Dorado	3.04	2.91	- -	1.03	- -	6.98
Giza 54	1.2	- -	1.14	1.06	- -	3.4
Local 119	- -	3.2	4.33	1.46	- -	8.99
Local 162	3.42	6.94	- -	1.49	2.14	13.99
ICSR-89037	1.34	1.31	- -	1.21	- -	3.86
Line C	5.48	6.64	2.33	- -	3.42	17.87
Giza 113	1	2.09	- -	- -	1.84	4.93
Total	40.27	39.2	25.09	21.35	11.22	

(a) Percentage of discolored grains based on 2 kg kernels were estimated by visual inspection of each genotype tested.

The results in Table (2) show that twenty-three fungal species were isolated from the grains of thirteen sorghum genotypes; however, the latter varied as to the number of fungi associated with each.

Local 29 and 162 genotypes yielded 20 out of the 23 isolated fungi, whereas selection 1007 gave 19 fungi. Eighteen fungi were isolated from each of Do-

rado and Line C genotype, while 14 fungi were isolated from Local 169, Giza 15 and Local 119. The genotype ICSR 89037 yielded only 7 out of the 23 fungi encountered. Nine fungi were isolated from Giza 113 and Giza 54 grains.

Specifically *Aspergillus flavus, Alternaria alternata, Fusarium oxysporum* and *F. moniliforme* were isolated from all genotypes tested. Results indicate that some fungi were isolated from certain genotypes but not from others such as, *Cladosporium sphaprospormum, Epicoccum nigrum* and *Nigrospora sphaerica* from four *genotypes, Aspergillus condidus, Aspergillus tamarii, Alternaria temuissima, Cochliobolus lunatus, C. spicifer, Curvularia ovoidae,* and *Emericalla nidulans* from five, *Gibberella acuminata* and *G. pulicaris* from seven, *Aspergillus glaucus* group and *Curvularia lunata* from eight, *Penicillium chrysogenum* from ten, *Fusarium solani* and *Gibberella fujikuroi* from eleven genotypes. However, *Aspergillus niger* and *Rhizopus stonolifer* were isolated from all genotypes except ICSR-89037 genotype.

Table (3) shows the behavior of 13 genotypes with respect to their liability to infection with the tested fungi; *fusarium oxysporum, F. momiliforme and Aspergillus flavus* expressed as percent invasion and compared with the non-inoculated control. It is obvious that the genotypes local 162, Selection 1007, local 29 and line C exhibited much higher level of invasion by the different fungi compared with the other genotypes ranging between 20 and 74 %. The invasion by *A.flavus* was lower than the other two fungi particularly in genotypes showing higher susceptibility to infection. Grain invasion with *F. maniliforme* was mostly higher than with *F.oxyspoorum or A.flavus* reaching 74 % in local 162 followed by Selection 1007 at 50 %. Grains of Giza 113 and Giza 54 showed very little infection with the fungi tested, ranging between 1 % and 6 % depending on the fungus and the genotype.

With respect to germinability of grains before and after storage for 30 days without being inoculated, it was obvious that the change in this parameter was very limited. Certain genotypes exhibited somewhat reduced germinability (77 %) even before storage such as in local 29 and Selection 1007 and 82 % in local 162. This reduced germinability could be a reflection of a relatively higher infection level(s) with the different fungi to start with. However, when grains were artifically inoculated with the fungi under consideration, different degrees of reduction in germination were experienced. The magnitude of such reductions varied with the fungus and the genotype as well as the extent of fungal invasion of the grains . The higher the invasion, the greater the reduction in germinability such as in local 162 (52 %). Selection 1007 (59 %), local 29 (49 %), LC (69 %) and Dorado (66 %). These lower germinations associated with inoculation with *F.moniliforme* corresponded with the high invasion levels experienced with such

Table 2. Fungi isolated from grains of thirteen sorghum genotypes after storage for six months under commercial conditions.

Fungi	Genotypes/Frequency (%) of the isolated fungus												
	Local 169	Local 29	Giza 15	Local 129	Kuymne	Selection 1007	Giza 113	Dorado	Giza 54	Local 119	Local 162	Icsr-89037	Line C
Alternaria alternata (Fries: FR.) Link	1.25	12.75	2.5	3.25	2.5	10.25	0.5	4	0.5	1.25	5.5	2.5	16.5
A. tenuissima (Kunze : Pers.) Wiltshire	- -	1.25	- -	- -	- -	4	- -	2.5	- -	- -	3.5	- -	0.5
Aspergillus flavus (Link : TR.)	3.5	30.75	4.75	2	3.5	11.75	1.25	5.5	2	2.5	7	2	12.75
A. candidus (Link) Fr.	- -	6.5	3.5	- -	- -	8.25	- -	- -	- -	- -	4.25	0.5	- -
A. niger (Vantieghem)	6.5	15.25	2.5	3.5	- -	13.75	1.5	1.25	2.5	5.5	6.75	1.5	6.75
A. glaucus group	- -	3.5	0.5	4.25	2	5.25	- -	5.25	1.25	- -	2.5	- -	8
A. tamarii (Kita)	- -	- -	1.5	- -	- -	1.25	- -	2	- -	- -	4	- -	5.5
Cladosporium sphaerospermum (Penzig)	0.5	- -	- -	- -	- -	2	- -	2.5	- -	2.75	- -	- -	- -
Cochliobolus lunata (Nelson & Haasis)	1.25	7	- -	- -	- -	1.5	- -	- -	- -	1.25	- -	- -	1.5
C. spicifer (Nelson)	- -	1.25	- -	- -	- -	- -	2	2	- -	- -	3.5	- -	4.75
Curvularia ovoidae (Hiroe & Watan. Munt.)	- -	2	0.5	1.25	2.5	- -	- -	- -	- -	- -	- -	- -	2.75
C. lunata (wakker & Boedijn.)	2	2.5	- -	- -	- -	3.25	3	- -	- -	2	2.75	- -	1.25
Emericalla nidulans (Eidom) Vuillemin	- -	0.5	- -	- -	- -	- -	- -	1.5	- -	3	1.25	- -	0.5
Epicoccum nigrum (Link) Fr.	- -	- -	- -	- -	- -	1.25	- -	0.15	- -	- -	4.75	- -	0.75
Fusarium oxysporum (Schlecht : FR.)	9.75	25.25	12.5	6	1.25	9.75	2.5	3	1.5	6.75	8.25	5.25	4.25
F. solani (Mart.) Sacc.	5.5	7	6.75	2	1.5	6	- -	1.5	0.25	5.25	7	- -	16.6
F. moniliforme (Scheldon.)	6	18	13.25	5	4	13.25	3.5	5.5	2.75	9.75	9.75	6.5	20.25
Gibberella acuminata Wollenw.	4	6.75	1.5	- -	1.5	- -	- -	2	- -	1.8	6	- -	- -
G. fujikuroi (Sawada) Wollenw.	5.5	11	2	4	3	9	1.25	4.75	- -	2.75	8.25	- -	18
G. pulicaris (Fries) Sacc.	4.25	5.25	- -	- -	2	4	- -	3	- -	- -	7.75	0.75	- -
Nigrospora sphaeica (Sacc.) Masom	- -	2	- -	- -	1.25	2	- -	- -	- -	- -	1.5	- -	- -
Penicillium chrysogenum (Thom)	1.5	4.25	5.5	2.5	- -	5.5	- -	5.5	3.25	4.25	6.5	- -	5.25
Rhizopus stolonifer (Ehrenb : FR.) Vuill.	3.5	4.75	4	2.75	3.5	6.75	1.5	6.75	2	5.5	5.5	- -	3

genotypes with respect to that fungus.

Higher levels of germinability were retained by those genotypes showing low infection % such as in Giza 113, Giza 54 and Kuymne, where it varies from 88 % with *F. moniliforme* in Kuymne to 95 % in Giza 113. Other genotypes such as Giza 15, local 129 and ICSR- 89037 invaded still at a relatively low level suffered a little as far as losing germinability and could be considered good genetic material.

Table 3. Infection of thirteen sorghum genotypes grain by *Fusarium oxysporum*, *F. moniliforme*, and *Aspergillus flavus* and their germinability after storage for 30 days at 85% RH 22-25°C.

Genotypes grains	Treatment	Invasion(a)			germinability				
					*Control		Inoculated by		
		Fusarium oxysporum (%)	Aspergillus flavus (%)	Fusarium moniliforme (%)	Before storage	After storage	F. oxysporum (%)	A. flavus (%)	F. moniliforme (%)
Line C	Inoculated	36	19	32	90	89	70	76	69
	control	5	4	2					
Local 169	Inoculated	16	8	14	92	90	77	78	71
	control	2	2	1					
Local 29	Inoculated	45	26	66	77	70	60	50	49
	control	8	6	12					
Giza 15	Inoculated	15	7	12	89	86	76	80	81
	control	1	2	0					
Local 129	Inoculated	12	5	4	94	92	85	84	80
	control	1	2	0					
Kuymne	Inoculated	10	6	4	97	95	90	91	88
	control	0	1	0					
Selection 1007	Inoculated	50	20	55	77	72	61	54	59
	control	6	4	10					
Giza 113	Inoculated	1	2	3	98	96	94	92	95
	control	0	1	0					
Dorado	Inoculated	24	11	26	92	89	71	74	66
	control	1	2	0					
Giza 54	Inoculated	4	6	2	96	95	93	90	94
	control	0	2	0					
Local 119	Inoculated	18	10	13	88	84	70	79	70
	control	0	3	1					
Local 162	Inoculated	46	30	74	82	78	60	58	52
	control	14	8	16					
ICSR-89037	Inoculated	8	8	5	94	90	87	85	82
	control	2	3	0					

(a) Mean of four replicate lots of inoculated grains and one replicate lot of control grains.

* Non - inoculated

Data in Table (4) show the moisture content of the grains before and after storage at 85 % rh for 30 days and the concentration of mycotoxins in the grains after such a storage. It is obvious that grains of the different genotypes showed a range of M.C % of 11.5 -13.0% at the onset and 12.4 -14.2 % after storage.

The mycotoxin contents were determined in the controls after storage. Some genotypes such as L 29, Giza 113, Dorado, Giza 54, Kuymne and ICSR-89037 did not show the presence of any toxins, while others showed the presence of aflatoxin and /or Zearalenone at different contents ranging from 10 to 30 mg / Kg grain. In such cases, the accumulation of mycotoxins differ from the result of previous infection with toxigenic fungi.

When stored grains were previously subjected to artificial inoculation with the fungi under consideration, their moisture content exhibited some increase at the end of the storage period. With respect to toxin contents in the different varieties, inoculation led to the production and occurrence of different mycotoxins at different levels depending on the variety and the fungus. Considering *F.oxysporum,* MF was detected at different levels ranging from 0 in Giza 113 and Giza 54 to 100 mg / kg in Selection 1007. Certain genotypes showed low content e.g 15 mg / Kg in ICSR 89037, 20 mg / kg in Kuymne and 25 mg / kg in Dorado. The absence or the low content probably reflects the extent of infection by *F.oxysporum* which reach 85 % in selection 1007. With respect to *A.flavus*, no aflatoxin could be detected in Giza 113 or Giza 54. Relatively low aflatoxin ranging between 15 and 25 mg / kg were found in certain genotypes such as Dorado and ICSR - 89037, respectively. The aflatoxin reached 105 mg / kg grain in local 29 which showed 30 mg / kg grain in the control.

As to *F.moniliforme*, the genotypes Giza 113, Giza 54 ICSR - 89037 and Dorado exhibited the lower content of ZON, ranging from 15 to 20 mg / kg grains. Some show the presence of more than one of the mycotoxin ZON, ZOL and DON

Generally, the less invaded genotypes contained no or little amounts of toxins which are the results of fungal infection and activity.

Table 4. Moisture content and detected mycotoxins in control and inoculated sorghum grains after storage for 30 days at 85% RH at 26±2°C.

Genotypes of sorghum grains	Control			Infected by;					
	Moisture content(a) (M.C,%)		Mycotoxins detected Mg/Kg grains	F. oxysporum		A. flavus		F. moniliforme	
	Initial	Final		(M.C) %	Mycotoxins detected Mg/Kg grains	(M.C) %	Mycotoxins detected Mg/Kg grains	(M.C) %	Mycotoxins detected Mg/Kg grains
Line C	12.5[b]	13.9[c]	Afkatoxin 25 ZON 30	13.2	MF 65	13.5	Aflatoxin 95	13.7	ZON 75 ZOL 62
Local 169	13	14	Aflatoxin 20	13.6	MF 50	13.8	Aflatoxin 60	13.4	ZON 55 DON 60
Local 29	12.7	13.8	Afkatoxin 30 ZON 15	13.8	MF 95	14	Aflatoxin 105	13.9	ZON 90 DON 110
Giza 15	12.9	13.1	Aflatoxin 15	13.1	MF 30	13.3	Aflatoxin 45	13.2	ZON 35
Local 129	13	13.3	0	13.3	MF 45	13.4	Aflatoxin 35	13.4	ZON 40
Kuymne	12.4	12.9	0	13	MF 20	13.2	Aflatoxin 20	13.1	ZON 25
Selection 1007	13.1	14.1	Afkatoxin 15 ZON 20	14.5	MF 100	14.8	Aflatoxin 85	14.3	ZON 90 DON 80
Giza 113	12	12.7	0	13.2	0	13.1	0	13.2	ZON 15
Dorado	11.5	12.4	0	13	MF 25	13.4	Aflatoxin 15	13.3	ZON 20
Giza 54	11.9	12.8	0	13.4	0	13.2	0	13.1	ZON 15
Local 119	12.6	13.8	ZON 20	14.2	MF 50	14.6	Aflatoxin 44	14.5	ZON 65 ZOL 40
Local 162	13	14.2	Afkatoxin 10 ZON 15	14.8	MF 85	14.9	Aflatoxin 90	14.4	ZOL 95
ICSR-89037	13.1	13.5	0		MF 15	13.8	Aflatoxin 25	13.4	ZON 20

(a) Fresh. Weight basis. (b) Moisture content at 0 (c) Moisture content after storage period.

MF: Moniliformin. ZON: Zearalenone. ZOL: Zearalenols. DON: Deoxynivalenol

Table 5. Effect of grain extract and grain coat leachates of sorghum genotypes on spore germination of *F. oxysporum*, *A. flavus* and *F. moniliforme* in vitro after 6-8 h. at 25±2°C.

Genotypes	Grain extract and grain exudate	*F. oxcysporum*		*F. moniliforme*		*A. flavus*	
		Germinated spores number	%, of the control	Germinated spores	%, of the control	Germinated spores	%, of the control
Local 169	Grain extract	13	54.16	16	57.14	15	50.00
	Grain leachates	12	50.00	13	46.42	14	46.66
Local 29	Grain extract	20	83.33	24	85.71	21	70.00
	Grain leachates	18	75.00	20	71.42	18	60.00
Giza 15	Grain extract	13	54.16	18	64.28	15	50.00
	Grain leachates	11	45.83	16	57.14	14	46.66
Local 129	Grain extract	14	58.33	19	67.85	17	56.66
	Grain leachates	11	45.83	16	57.14	15	50.00
Kuymne	Grain extract	11	45.83	13	46.42	12	40.00
	Grain leachates	9	37.50	12	42.82	11	36.66
Selection 1007	Grain extract	19	79.16	22	78.57	23	76.66
	Grain leachates	16	66.66	20	71.42	21	70.00
Giza 113	Grain extract	9	37.50	10	35.71	7	23.33
	Grain leachates	7	29.16	8	28.57	6	20.00
Dorado	Grain extract	10	41.66	11	39.28	12	40.00
	Grain leachates	9	37.50	10	35.71	10	33.33
Giza 54	Grain extract	6	25.00	8	28.57	5	16.66
	Grain leachates	5	20.00	7	25.00	3	10.00
Local 119	Grain extract	13	54.16	14	50.00	16	53.33
	Grain leachates	12	50.00	12	42.85	13	43.33
Local 162	Grain extract	19	79.16	20	71.42	25	83.33
	Grain leachates	17	70.83	17	60.71	22	73.33
ICSR-89037	Grain extract	9	37.50	14	50.00	14	46.66
	Grain leachates	8	33.33	13	46.42	12	40.00
Line C	Grain extract	21	87.50	26	92.85	27	90.00
	Grain leachates	23	95.83	25	89.28	24	80.00
Control	Distilld water	24	100	28	100	30	100

Grain coat leachates or grains extracts of various sorghum genotypes were found to inhibit spore germination of the three fungi tested. The magnitude of inhibition varied not only with the sorghum grain genotypes but also with the fungal isolates (Table 5). Grain extracts and grain coat leachates of genotype Giza 54 were the most inhibitive to spore germination of the three fungi tested, which gave 25% and 20% with *F.oxysporum*, 28.57 and 25% with *F.moniliforme* and 16.66% and 10% with *A.flavus*, respectively. On the other hand, grain coat leachates and grains extracts of sorghum genotypes Line C, Local 162, Selection 1007, and Local 29 were the least effective and induced less than 20%, 40%, 34%, and 40% inhibition, respectively in the three fungi tested. However, coat leachtes or extracts of sorghum grains genotypes ICSR- 89037, Drado and Kuymne were moderately effectve and caused between 67% to 54% inhibition to the three fungi. Coat leachtes or extracts of the remaining sorghum grain genotypes clearly reduced spore germination by 33% to 58% on the three fungi tested.

DISCUSSION

After six months storage of sorghum grain genotypes, it is common to find increasing levels of discolored grains. Total infection in samples could be determined from external symptoms such as discoloration, shrivelling, and mold on the grain surface (Spilker *et al.*, 1981). Excessive fungal growth in the field or during storage can result in black point and dark discoloration of the grain by *Alternaria alternata* or other fungi (Arafa *et al.*, 1999) and shrivelling and discoloring of the grain by *Phomopsis sp.* and *Aspergillus spp.* (Clear *et al.*,1989). However, the reddish discoloration of wheat grain by Fusarium spp, was also reported by Bechtel *et al.*, (1985) that by visual inspections of wheat samples one can obtain a rough idea of the degree of infection and mycotoxin levels.

Extensive survey on thirteen sorghum genotypes was conducted to determine the occurrence and the frequency of various fungi associated with stored sorghum grains. Twenty-three fungal species were isolated. *Asperillus flavus, Fusarium oxysporum, F. moniliforme and Gibberella fujikuroi* were the most freguently reported in the grains of all genotypes tested. Such results are in agreement with those reported by Fahim *et al.*, (1982), who found one or more of these fungi associated with sorghum grains.

Sorghum grains of genotypes Local 29, Selection 1007, Local 162 gave the highest number of isolated fungi followed by genotypes Giza 15, Local 169, Local 119 and Local 129; however, genotypes ICSR-89037, Kuymne, Giza 113 and Giza 54 were the least infected with the isolated fungi.

It was obvious that the occurrence and frequency of the isolated fungi dif-

fered among genotypes and was probably due to the moisture content and nutritional aspects of sorghum grains, length of storage, susceptibility of each genotype to the fungal infection that occurs in the field or during storage, and also due to the chemical structure of grains and the internal changes in the grain constituents during fungal invasion (Fahim *et al.*, 1982; and Zohri 1993).

Many investigators pointed out that there is a correlation between moisture content of grains and their liability to invasion by storage fungi. A moisture content above 13.0% in grains permits invasion by storage fungi which may be accompanied or followed by mycotoxins production (Arafa *et al.*, 1999).

Inoculation trials with the three dominant fungi proved that they differed in their ability to induce grain deterioration. *Fusarium oxysporum and F. moniliforme* were more deleterious as they profoundly affected grain germination and caused the highest percentage of grain infection as compared with *A. flavus*. The amount of fungal growth and mycotoxin production differed from one genotype to another. Giza 54 and Giza 113 were resistant to fungal invasion and mycotoxins production by the three fungi tested. On the contray, the most susceptible genotypes to the establishment of tested fungi *(A.flavus.,F.oxysporum and F.moniliforme)* and mycotoxins accumulation were Local 29, Selection 1007, Line C and Local 162, but to different degrees. The remaining genotypes showed intermediate responses.

Similar observations were reported by Arafa *et al.* (1999) on six genotypes of durum wheat where mycotoxin accumulation was dependant on the genotype of durum wheat, also they found that grains accessions inoculated with spores of *A. restrictus* or *F. oxysporum* revealed that two genotypes Stn/Gote and Sohag 2, remained essentially free from both fungi tested, retained very high germinability and showed the lowest amount of toxins accumulation. However, the reverse effects were obtained from genotypes Crs/Ple's/Teal's 6811 and Beni-Suef of durum wheat.

In our study on sorghum genotypes, the amount of aflatoxin produced on different cultivars varied between 20 and 105 µg/Kg grains. Similar low levels of aflatoxin were also obtained when leguminous crops were artifially infected by toxigenic fungi. El-Kady *et al.*, (1991) examined 100 cultivars and lines of broad bean seeds to determine varietal differences which may support or prevent aflatoxin production and found that 11 cultivars/lines were highly resistant to fungal invasion and aflatoxin formation, while 9 cultivars/lines showed partial resistance.

Grain coat leachates or grain extracts of various sorghum cultivars were

found to inhibit a number of fungi tested. The magnitude of inhibition varied not only with the cultivars, but also with fungal genera.

The inhibition of spore germination may be due to the presence of anti-fungal substances in the grain coat leachates and in the grain extracts. These constituents affect also grain sucesptibility to various fungi; consequently the production of mycotoxins by the respective fungi. The presence of antimicrobial substances in the seed coats and seed has been reported by a number of workers (Amal, 1993 and Arafa *et al.*, 1999). It has been suggested that these substances act as defensive agents against seed infection by pathogens (Chandra *et al.*, 1985).

REFERENCES

1. Abdel-Gawad, K.M. and A.A. Zohri. 1993. Fungal Flora and mycotoxins of six kinds of nut seeds for human consumption in Saudi Arabia. Mycopathologia, 124: 55-64.
2. AOAC. 1980. Association of official Analytical Chemists. Official methods of analysis. 13th ed., Washington, DC 429 pp.
3. Arafa, M.K.M., T.M. Abd El-Rahman, A.A. Zohri, A.M.Tammam, S.A. Ali and M.B. Tawfek. 1999. Studies of seed-borne pathogens on some durum wheat genotypes in Upper Egypt. Bull. Fac. Sci., Assiut Univ. 28: (1-D), p.p. 19-33.
4. Barnett, H.L and B.B. Hunter. 1972. Illustrated genera of imperfect fungi. 3rd ed. Burgess Publishing Co. Minnesota 241 pp.
5. Bechtel, D.B., L.A. Kaleikau, R.L. Gaines and L.M. Seitz. 1985. The effects of *Fusarium graiminearum* infection on wheat Kernels. Cereal Cham. 62(3): 191-197.
6. Booth, C. 1977. Fusarium. Laboratory Guide to the identification of the major species. C.M.I. Kew, Surrey, England, 608 p.
7. Bottalico, A., A. Logrieco, and A.Visconti. 1989. Fusarium species and their mycotoxins in infected corn in Italy. Mycopathologia, 107: 85-92.
8. Chandra, S., M.N. Narang and R.K. Srivastava. 1985. Studies on mycoflora of oil seeds in India. Interrelationship between mycoflora and seed. Seed Sci., Technol., 13: 543-549.
9. Claudius, G.R. and R.S. Mehrotra. 1973. Root exudates from lentil seedlings in relation to wilt disease. Plant and Soil, 38:315-320
10. El-Kady, I.A., S.S. El-Maraghy and A.A. Zohri. 1991. Mycotoxin production on different cultivars and lines of broad bean seede in Egypt. Mycopathologia, 113: 165-169.
11. Eraky, Amal. 1993. Studies on seed-borne disease of wheat in Upper Egypt. M. Sci. Thesis Plant Path., Dept., Fac., Agric., Assiut Univ. Egypt.
12. Fahim, M.M., F.M. Barakat, M.F. Attia and H.Y. Aly. 1982. Fungi associated with stored sorghum grains and their role in grain deterioration. Egypt. J. Phytopathol., 14 (1-2): 39-49.

13. Gimeno, A. 1979. Thin layer chromatographic determination of aflatoxins, ochratoxin, steriymatocystin, Zearalenone, citrinin, T-2 toxin, diacetoyyscirpenol, penicillic acid patulin and penitrem. A. J. AOAC. 62: 579-585.

14. I.S.T.A. 1959. International rules of seed testing Proc. Int. Seed testing Assoc., Wageningen (Holland), 24 (3).

15. Martin. J.P. 1950. Use of acid, rose bengal and styeptomycin in the plate method for estimating soil fungi. Soil. Soc., 69: 215-233.

16. Niles, E.V. 1976. The mycoflora of sorghum stored in underground pits in Ethiopia. Trop. Sci., 18,115.

17. Qasem, S.A., and C.M. Christensen. 1958. Influence of moisture content, temperature, and time on the deterioration of stored corn by fungi. Phytopathology, 48: 544-549.

18. Seenappa, M., C.L. Keswani and M. Matiko. 1981. *Aspergillus infection* and aflatoxin production in beans in Tanzaia. Int. Biodeteror. Bull. 17: 79-82.

19. Snijders, C.H. and J. Perkowski. 1990. Effect of head blight caused by *Fusarium culmorum* on toxin content and weight of wheat kernels. Phytopathology., 80: 566-570

20. Zohir, A.A. 1993. Studies on some cowpea cultivars. II-Suitability for *Aspergillus flavus* growth and aflatoxin production. Qatar Univ. Sci. J. 13(1): 57-62.

الفطريات المحمولة على حبوب الذرة الرفيعة ومقاومتها
٢- العلاقة بين الفطريات والتخزين لطرز وراثيه مختلفة من حبوب الذرة الرفيعة

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عزل ثلاثة و عشرون نوعاً من الفطريات من حبوب ثلاثة عشر طرزا وراثيا من الذرة الرفيعة و كانت أكثر الفطريات كثافة وتكرارا في العزل هي : أسبر جلس فلافس ، فيوزاريوم مونيلفورم ، جبريلا فوجيكوروى.

احتوت حبوب الطرز الوراثية محلى ٢٩ ، مختار ١٠٠٧ ،محلى ١٦٢ ، دورادو على أعلى عدد من الفطريات المعزولة ، تلي ذلك الطرز الوراثية سلالة سى ، جيزة ١٥ ، محلى ١٦٩، محلى ١١٩، بينما الأصناف محلى ١٢٩، كيومين، جيزة ١١٣، ICSR-89037، جيزة ٥٤ كانت الأقل عدداً في الفطريات المعزولة منها.

تم الحقن الصناعي لحبوب ثلاثة عشر طرزا وراثيا من حبوب الذرة الرفيعة بجراثيم الفطريات الأكثر تكراراً عند العزل وهى:

A. flavus, F. moniliforme, F. oxysporum

وكانت النتائج كما يلي:

الطرز الوراثية جيزة ٥٤، جيزة ١١٣ لم تتأثر حبوبها كثيراً بهذه الفطريات ، وأعطت أعلى نسبه إنبات، وأقل تركيز للسموم الفطرية الناتجة ، بينما أعطت الطرز الوراثية محلى ٢٩، مختار ١٠٠٧ محلى ١٦٢ عكس التأثيرات السابقة، أما بقية الطرز الوراثية أظهرت درجات متوسطة في تأثرها من حيث مهاجمة الفطريات وانخفاض الأنبات وتكون السموم الفطرية.

تباينت مستخلصات و رواشح غلاف حبوب الطرز الوراثية من الذرة الرفيعة المختبرة فى تأثيرها التثبيطى بالنسبه لانبات الجراثيم وكذا تأثيرها على الفطريات الثلاث محل الدراسه ، ولقد أعطت الطرز الوراثية جيزة ١١٣ ، جيزة ٥٤ أعلى نسبة تثبيط للفطريات المختبرة.