The Effect of Storage Time on the Mycoflora of Maize Grains at Ambient Conditions

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ABSTRACT

Maize grains stored under ambient laboratory conditions were sampled monthly over a five months period. Isolations of fungi yielded six different genera. Four genera, namely, *Aspergillus, Drechslera, Penicillium* and *Rhizopus* are a new addition to the list of seed-borne fungi on maize in Libya. In comparison to the blotter method, a high significant increase of infected grains percentage was found using agar medium "G-18". *A. flavus*-infected grains increased significantly after three months of storage and up to the fifth month; whilst those infected by *A. niger* remained fairly unchanged. There was a high significant difference in infection by *A. flavus* between maize grains brought from Ajelat and those from Tajoura. The effect of grain origin, surface treatment and storage time on the degree of infection was also investigated.

Key words: Mycoflora, *Zea mays* L., maize.

INTRODUCTION

Maize, *Zea mays* L., is a very important staple cereal crop in Africa. Being a seasonal crop, it is stored for long periods as dry grains for human consumption and as a feed component for poultry and live-stock. However, a substantial amount in storage is subject to insect attack and microbial spoilage causing 30% loss of annual harvest (2, 3). Maize is infected by important pathogens, many of which are seed-borne (12). Although the role of storage fungi in grain quality deterioration is well documented in developed countries (4), there is a paucity of information regarding these fungi on maize grains in Africa and the role that such fungi play. Moubasher et al. (11) isolated and identified thirty four species of fungi belonging to thirteeen genera on stored maize in Egypt. Members of the genus *Aspergillus* were most predominant followed by *Penicillium*. In Nigeria, Broadbent et al. (3) provided an extensive list of fungi associated with maize. Later, Oyeniran (14, 15) added to the list eleven different fungi belonging to the genera *Aspergillus* and *Penicillium*.

Danquash (6) isolated twenty two seed-borne fungi on maize in Ghana including only one *Aspergillus* sp. and one *Penicillium* sp.

The objective of this investigation was to update the list of the storage fungi associated with maize grains kept under ambient conditions and to acquire information on the infection patterns of individual fungi.

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MATERIALS AND METHODS

Four maize grain samples were brought from four different locations in Libya (Zeletin, Tajoura, Zawia, and Ajelat). Each sample was stored separately in a large plastic container under ambient laboratory conditions (75% ± 5 RH and 30 ± 2 °C). Grains were sampled monthly for five months, each time portions of 500gm of grains were taken from top, middle and bottom of the plastic container and pooled together. Each sample was dry examined by the naked eye and under low magnification of stereo-microscope. The mycoflora on grains were assessed using the following testing methods:

I. Blotter method: Using the modified method of Tempe (16) and Limonard (10). Ten seeds were plated in a petri dish of 9 cm in diameter containing three well moistened blotters. The plates were incubated at 30 ± 2 °C under 12 hours of alternating cycles of near ultraviolet light (NUV) and darkness for seven days. This method was used as a comparison test to the agar plate method using Zeletin sample only.

II. Agar plate method: A modified Dichloran-Glycerol (DG-18) medium developed by Hocking and Pitt (8) for primary isolation of *Aspergillus, Fusarium* and *Penicillium* had been used. This modified Glycerol (G-18) medium contains the following ingredients: peptone 5.0 gm, glucose 10.0 gn, $K_2H_2PO_4$ 1.0 gm, $MgSO_4 \cdot 2H_2O$ 0.5 gm, agar 15.0 gm, Chloramphenicol 0.1 gm, distilled water 1000.0 ml, and glycerol 220.0 ml. All the ingredients were dissolved in distilled water first and then chloramphenicol and glycerol were added before autoclaving. The grains were plated on the G-18 medium at the rate of 5 seeds per dish and incubated at 30 ± 2 °C under 12 hours alternating cycles of NUV and darkness for seven days.

In both methods, untreated grains as well as treated ones were tested. Grains were surface sterilized with a solution containing 10% ethanol and 2% soidum hypochlorite (pH, 10.5) for 10 minutes and rinsed twice with sterilized distilled water. Four hundred grains were used for each treatment. After incubation, numbers and kinds of fungi growing on each grain were recorded. Isolated fungi were purified and maintained on potato dextrose agar (PDA), and G-18 media.

Data were analyzed using three factors ANOVA, in a completely randomized design, regarding location as a main plot, grain treatment and storage period as sub-subplots.

RESULTS AND DISCUSSION

Dry examination of maize grain samples showed that few grains had slight purple discoloration, and some were not fully developed. No insect damage was observed. The different fungi isolated from grains in this investigation belonged to six different genera as shown in table 1.

Molds which grew on untreated grains were the same as those found on the pre-treated ones using the blotter method. They were thus seed-borne. This observation is consistent with those of Lichtwardt and co-workers (9) and Odamtten (13) for maize. Four new genera have been identified and are considered a new addition to the list of seed-borne fungi on maize in Libya. These genera are *Aspergillus, Drechslera,*

Penicillium, and *Rhizopus*. Abughnia and Faraj (1) had only listed *Alternaria*, and *Fusarium* on maize in Libya.

Molds isolated on the solid medium "G-18" were mostly the same as those obtained by the blotter method. *Alternria alternata* and *Drechslera* sp. were not detected with G-18 (Table 1). The percentage of maize grains infected by *Aspergillus flavus* and *A. niger* increased significantly using G-18 agar as compared to the blotter method. Using the agar method, a significant decrease of *A. niger* was observed in the case of treated grains in comparison to the untreated ones. No significant difference was observed using the blotter method. Furthermore, in both methods the incidence of *A. flavus* did not differ significantly between treated and untreated grains (Table 1).

Table 1 – Comparison between blotter and agar method on the percentage incidence of seed-borne fungi of maize.

	Blotter		Agar	
Fungi	**Untreated**	**Treated**	**Untreated**	**Treated**
Alternaria alternata	0.40	1.40	0.00	0.00
Aspergillus flavus	2.25	1.00	9.50	11.50
Aspergillus niger	3.75	3.25	98.75	95.20
Aspergillus spp.	0.00	0.00	0.00	0.75
Penicillium spp.	63.50	54.75	4.50	40.50
Drechslera sp.	0.20	0.20	0.00	0.00
Fusarium spp.	3.25	7.30	2.00	2.00
Rhizopus stolonifer	4.00	3.50	5.00	0.25

LSD Value *"A. flavus"* = 3.42 at 0.05, and 5.18 at 0.01.

LSD Value *"A. niger"* = 2.18 at 0.05, and 3.30 at 0.01.

A distinct pattern of infection was exhibited by the storage fungi during five months period. Data analyzed following three factors, in completely randomized design, showed no significant increase in the incidence of *A. flavus* during the first three months; but a highly significant increase was observed in the last Two months (Table 2).

The average of infected grains with *A. niger*, however, remained unchanged over five months period (Table 3). These patterns of growth during that period were similar to those of Odamtten (13).

The total average number of *A. flavus* developed on surface sterilized grains was much less than that on untreated grains (Table 2) because the sodium hypochlorite treatment reduced external conidia on the grains.

Also there was a difference in the average percentage of infected grains depending on their origin. The sample from Ajelat was significant and highly

significant compared to samples from Zawia and Tajoura, respectively. However, no difference was recorded between samples from Zawia and Tajoura. (see Table 2).

Table 2 – Effect of storage period on infection counts of *Aspergillus flavus* in untreated and treated maize grains from three locations.

		Average percent of infection*					
Location	**Treatment****	1	2	3	4	5	**Location average**
Tajoura	Treated	44.00	27.25	31.75	35.50	32.50	
	Untreated	44.75	39.25	41.00	46.25	41.00	38.33
Zawia	Treated	65.50	28.50	27.00	34.00	48.75	
	Untreated	36.25	37.00	35.25	42.00	61.25	41.60
Ajelat	Treated	18.25	41.00	39.50	40.50	66.75	
	Untreated	26.00	49.00	47.00	68.00	61.75	45.78
Monthly average		39.13	37.00	36.92	44.38	52.08	

* Average of four replicates, 100 grains/replicate.

** Total average of untreated grains = 45.07.

Total average of treated grains = 38.72.

LSD Value "monthly storage" = 4.46 at 0.05 and 5.93 at 0.01.

"treatment" = 2.79 at 0.05 and 4.01 at 0.01.

"locations" = 3.51 at 0.05 and 5.04 at 0.01.

"infection" = 10.44 at 0.05.

From ANOVA analysis (Table 2), there are significant differences in interaction between locations, surface treatment and storage time, e.g. the average percentage of grains infected by *A. flavus* showed an LSD value of 10.44 at 0.05. Storing maize grains from Ajelat for five months gave a higher percentage of grain infection compared with other locations. This trend was evident in the last four months of storage when comparison between treated and untreated was made.

Table 3 shows that the average percentage of grains infected by *A. niger* depends on the grain origin and surface sterilization treatment; whereas no significant effect was observed due to storage time.

This study was carried out at a temperature of 30 ± 2 °C and relative humidity of $75\% \pm 5$ which supported the growth pf both *A. flavus* and *A. niger;* but *A. niger* was predominant. This suggests that the average percentage of mycoflora-infected grains present in any grain stock at a particular time would depend on the type of seed microflora, geographical location, prevailing weather conditions in the local area and postharvest storage conditions.

Table 3 – Effect of storage period on infection counts of *Aspergillus niger* in untreated and treated maize grains from three locations.

		Average percent of infection*					
Location	**Treatment****	1	2	3	4	5	**Location** average
Tajoura	Treated	93.25	74.25	88.25	98.00	98.25	
	Untreated	87.50	78.00	87.00	97.00	96.75	89.88
Zawia	Treated	99.75	100.00	99.75	100.00	100.00	
	Untreated	96.00	100.00	99.25	100.00	100.00	99.48
Ajelat	Treated	94.50	97.25	99.25	96.50	93.50	
	Untreated	96.75	97.75	98.75	73.25	89.75	93.78
Monthly average		94.63	91.21	95.38	98.38	93.71	

*Average of four replicates, 100 grains/replicate.

**Total average of untreated grains = 95.57.

Total average of treated grains = 93.18.

LSD Value "treatment" = 1.86 at 0.05.

"locations" = 1.31 at 0.05 and 1.89 at 0.01.

Production of aflatoxins by *A. flavus* and its toxic effects on human and animal health are well-documented (5, 7). Effects of certain strains of *A. niger* on growth and aflatoxin formation by *A. flavus* were also documented (17). However, such information regarding the toxigenic fungi, and the concentration of toxins that would render maize toxic for human and animal consumption in libya is not available.

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تأثير وقت التخزين على الحمل الفطري لحبوب الذرة تحت الظروف المختبرية

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المستخلص

جمعت عينات شهرية من حبوب الذرة المخزنة تحت ظروف المختبر لمدة خمسة أشهر. وأظهرت نتائج العزل وجود فطريات تنتمي إلى ستة أجناس مختلفة. حيث تعتبر أربعة منها وهي *Rhizopus, Penicillium, Drechslera, Aspergillus* إضافة جديدة لقائمة الفطريات المحمولة على حبوب الذرة في ليبيا. كما أظهر العزل باستخدام أجار G-18 مقارنة بطريقة النشاف زيادة معنوية عالية في نسبة الحبوب المصابة، وازدادت نسبة الحبوب المصابة معنوياً بالفطر *A. flavus* بعد ثلاثة أشهر ولمدة خمسة أشهر من التخزين، بينما بقيت تلك الحبوب المصابة بالفطر *A. niger* ثابتة نوعاً. ووجدت زيادة معنوية عالية في نسبة الإصابة بالفطر *A. flavus* لحبوب الذرة المجمعة من العجيلات مقارنة بتلك الحبوب المجمعة من تاجوراء. كما بحث تأثير كل من اصل الحبوب، التعقيم السطحي للحبوب ووقت التخزين على شدة الإصابة.