Al-Azhar J. Agric. Res., Vol. 24
PP. 83-99, (December), 1996

PATHOLOGICAL STUDIES ON DETERIORATION OF YELLOW CORN DURING STORAGE AND ITS CONTROL
II. Alfatoxins production and chemical composition of grains.

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ABSTRACT : Fractionation of aflatoxins showed the absence of aflatoxin in the filtrates of all isolated fungi, except the filtrate of yellow corn grains infected with *Aspergillus flavus*. The production of aflatoxins by *A. flavus* was affected by grade of grains (grade 1, 3 and 5) as well as its moisture content (17 and 23% moisture) so the, production was increased from grade one to grade five and from 17% to 23% moisture content within each grade. Also, the concentration of aflatoxin B1 is higher than aflatoxin B2.

The production of aflatoxins were increased by increasing storage temp., grains moisture content and storage period. Grade three showed the highest amounts of both B1 (986 µg/kg) and B2 (219 µ g/kg) followed by grade five (237 µg/kg of B1 and B2, respectively). While the least amount of both B1 and B2 were shown in grade one (32.9 and 5.48 µg/kg).

Protein content was decreased with moisture percentage either low (17%) or high (23%) when stored under low temperature (10°C or 18°C) for storage period long as 90 days compared with the control. While the stored under high temperature (28°C or the room temperature, 25-30°C) for a storage of 30 days decreased the protein content with 17% moisture and increased to the max (100%) with 23% moisture. Reduction in concentration of amino acid proline (in all tested grates) caused when storage the grains with either low moisture (17%) or high moisture (23%) under high temp. (28°C or 25 - 30°C). Total carbohydrates content of grains was slightly increased when stored either higher or low temp. Grading of grains had an effect on total carbohydrates, where grade one showed the least

increase followed by grade three and grade five recorded the highest one, specially with 17% at 10°C or 23% and 28°C.

INTRODUCTION

The The important of reliable quantitative information on aflatoxins arising from the bad condition of corn storage has increased considerably in recent years. Aflatoxins are mycotoxins that has acute chronic and somtime mutant effects. Aflatoxins B1, B2, G1 and G2 are the main toxins produced by *Aspergillus falvus* and *A. parasiticus* (Diener and Davis, 1986). The approximate average compsition of corn grains is 8.2% protein, 3.8% oil, 70.5% carbohydrate and 2.2% crude fiber (AOAC, 1994).

Ozay and Heperkan (1989) studied the occurrence of mould fungi and mycotoxin content in 167 maize grain samples, either imported or locally produced were obtained from various regions and stored houses in turkey. They reported that, aflatoxin (B_1) was found in 46% of the locally produced samples with amounts of about 2-7 µg/kg, while imported ones were aflatoxins free. Tanboon (1989) stated that, both *Aspergillus flavus* and *A. parasiticus* produced aflatoxin in stored maize in Thailand. The natural drying in the field before harvesting followed by mechanical drying after shelling was efficient way in reducing contamination with aflatoxin. Lee and Hagler (1991) showed that, aflatoxins in maize grains were identified as B_1 and B_2 with a concentration ranging from 3-50 µg/kg. Payne (1992) reported that, aflatoxin B_1 produced in corn grains is potentially hepatocarcinogen *Aspergillus flavus* can grow and produce this aflatoxin in corn either in field and storage. Marquez (1993) found that, aflatoxin B_1 was a major contaiminant of different agricultural crops including maize.

Christensen (1970) noticed that, there was a relationship between relative humidity of the store and moisture content of corn grains, that is, when the relative humidity were 75, 80 and 85% the moisture content were 14.9 - 15.9; 15.9 - 16.6; 17.0 - 17.5, respectively. Moisture content of about 14.5% or above increased invasion of seeds with storage fungi. Botast *et al.* (1981) stated that, mould population increased in corn grains stored with 15 and 18% moisture content.

Wieman *et al.* (1986) reported the significance of temperature and relative humidity on production of aflatoxin by one strain of *Aspergillus flavus* on damaged corn. Zhibiao *et al.* (1989) noticed that, storage fungi and moisture content greatly affected seed germination also moisture content influnced the development of storage fungi. Sauer and Tuite (1986) stated that, the aflatoxin could be produced at temperature ranging from 11-40°C with an optimum temperature ranging from 25-35°C. Ahmed (1971) found that, laboratory storage of corn indicated that, moisture content, high temperature and storage for a long time resulted in a high increase in kernel invasion by mould fungi. Christensen (1972) moticed that, temperature between 10-20°C was suitable for relatively long term storage of maize with high quality and less invasion by storage fungi.

Gangopadhyay and Wyllie (1973) found that, biochemical studies on soybean seeds infected with fungi indicated higher amounts of protein. Osman (1982) found that, infection with *Aspergillus niger* and *A. flavus* in sorghum seeds caused a noticeable decline in protein and free amino acids content probably due to their utilization by both fungi. The grains infection with *A. flavus* and *A. niger* was accompanied by 40.0% and 30.0% loss in free amino acid and protein, respectively. El-Araby (1985) found that, the total protein percentage in infected maize grains with *Fusarium moniliforme* or *Nigrospora oryzae* increased due to prolonging the periods of storage, whereas this percentage was stable in healthy grains for different storage periods. Sauer and Pomerany (1992) showed that, high moisture content of cereal during storage caused a reduction in starch content and an increasing in α-amylase activity. Crude protein was higher in the damaged grains.

The present research was conducted to study the ability of the associated fungi to produce aflatoxins and to evaluate the effect of the aflatoxins production on the chemical composition of the corn grains as protein content, amino acids content and carbohydrates content.

MATERIAL AND METHODS

Aflatoxins production ability of the isolated fungi :

An *Aspergillus flavus* isolate was used in a test for the ability of producing aflatoxin in yellow corn grains. Healthy and insect-free grains were sterilized in an aqueous sodium hypoclorite solution of Clorox 25% for 10 minutes, then washed several times with sterilized distilled water and air dried under aseptic conditions. Sterilized conical flasks (500 ml.), each containing 100 grams of the formerly sterilized grains was separtely inoculated with 12 discs of actively growing day-old, P.D.A. of *A. flavus* culture. Flasks were incubated at 25°C for 10 days. Three replicates were used for each treatment. Uninoculated flalsks served as control (Sabet, 1991). The previously described procedure were repeated with all isolated fungi including *Aspergillus flavus* (Table 1) using a spore suspension (2500-3000 spore/g. grains) instead of discs.

Table (1) : Occurrence percentages of different fungi isolated from yellow corn grains obtained from different imported shipments from USA.

Fungi	Number of colonies/100 grains
Aspergillus flavus	25
Aspergillus terreus	12
Aspergillus niger	8
Pencillium funiculosum	15
Macrophomina phaseolina	2
Alternaria sp.	5
Fusarium moniliforme	10
Fusarium graminearum	8
Fusarium oxysporum	10
Fusarium sp.	2
Rhizopus sp.	3
Unidentified spp.	4

Detection of Aflatoxins produced by *Aspergillus flavus* :

After 10 days from inoculation the formerly treated grains in flasks were ovened at 110°C for 10 minutes, 150 ml of chloroform was added at each flask and boiled under reflex condenser for 5 minutes

with shaking. The chloroform layer was collected and filtered through filter papers No. 1 in the presence of anhydrous sodium sulphate. Second extraction was carried out using 50 ml. of chloroform and vigorously shaken at room temperature for 5 min. The chloroform layer was collected and filtered as mentioned before. The two crude extraction were combined and then concentrated under nitrogen flow in a rotary evaporator at 50 to 70°C treated with charcoal for discoloration of filterate and subjected to further concentration up to 2 ml (Anon., 1975). The samples and standard aflatoxins were spotted on thin layer chromatography (TLC) plates at different concentrations (i.e. 2, 5, 7 and 10 µl. of both extracted sample and standard solution (Sigma Company). The plate was put on a jar containing (96 ml. Diethyl ether +3 ml Methanol +1 ml. distilled water). After 1 hour the plates were examined in an Ultraviolet detector (wavelength 365 mm) for bright greenish-yellow fluorescence which allowed estimation of amounts of aflatoxins in the unknown samples using the method of Calculation (AOAC., 1990).

Determination of total protein :

Protein was determined by Kjeldahl method according to (AOAC., 1994).

Determination of amino acids content :

Amino acids were determined by using a Beckman amino acid analyzer (Model 7300) according to the method described by Moore *et al.* (1958).

Determination of total carbohydrates :

Carbonhydrate was determined using the phenol-sulphuric acid method described by Dubois *et al.* (1956).

Determination of Aflatoxins :

Aflatoxins were determined according to AOAC., (1994).

Adjustment of moisture content of corn grains :

Moisture content of grains in each used grade was determined by Motamco apparatus (serial No. K 3668, U.S.A.). Moisture content of corn grains was adjusted to the required moisture level (17% and 23%)

by adding calculated volumes of sterilized distilled water to the tested quantity of grains. The required volume of water needed for each moisture content level was calculated according to the following equation, Approved Method of American Association of cereal chemists (Anon., 1962).

$$S = \frac{\text{Required moisturecontent} - \text{Initial moisturecontent}(10\% - 12\%)}{100 - \text{Required moisturecontent}} \times 100$$

where :

S = The volume of water required for 100 grams of corn grains to reach the desired level of moisture content.

RESULTS AND DISCUSSION

Data in Table (2) showed that, theproduction of aflatoxins by *A. flavus* was affected by the grade of grains as well as its moisture content so, the intensity of the fluorescent substances increased from grade one to grade five and from 17% to 23% moisture content within each grade, in case of spore suspension method. The concentration of aflatoxins (B1 and B2) produced were estimated in case of fungal methods. The concentration of aflatoxin B1 is higher than that aflatoxin B2 in all tested cases. Grade one exhibited the least concentration of both B1 and B2 while grade five showed the highest concentration of both B1 and B2. These results are in agreement with those stated by (Diner and Davis, 1986; Pyne, 1992; Boller and Schroeder, 1974; Lee and Hagler, 1991; Abramson *et al.*, 1992 and Marquez, 1993).

No production of aflatoxin (B1 or B2) under low storage temperature, 10°C with 17% or 23% moisture content and 18°C with 17% moisture content for 90 days storage period in all tested grades (Table 3). The production of aflatoxin was initiated at 18°C with 23% moisture content after a long period of 90 days in all tested grades. Amount of aflatoxin (B1 and B2) was increased by increasing temperature, moisture content and storage period. Grade 3 recorded the highest concentration of both B1 (968.3 µg/kg) and B2 (219 µg/kg) and grade 1 showed the least one (37.2 and 12.4 µg/kg) of B1 and B2 respectively. These results are in the same line with those recorded by

Epstein *et al.* (1970); Boller and Schroeder (1974); Sauer and Tuite (1986); Lee and Hagler (1991); Abrmson *et al.* (1992); and Marquez (1993).

Data presented in Table (4) indicated that the protein content of inoculated grains was slight variously affected by storage conditions. The protein content with low moisture i.e. (17%) or high (23%) was decreased when stored under low temperature (10°C or 18°C) for a storage period as long as 90 days while grains with 23% moisture content was increased when stored under high temperature (28°C or the room temperature, 25-30°C) for 30 days. These results are in agreement with the results previously recorded by Gangopadhyay and Wyllie (1973); and El-Araby (1985).

Data in Table (5) clearly show that, in grade one grains with 17% moisture content, high temperature (25-30°C) and low protein, amino acids content as well as the concentrations of proline, lysine and arginine were reduced, but the concentration of the other amino acids were increased, especially isoleucine (55%), serine (43.8) and phenylalanine (30.4%) compared with control. Infected grains with 17% moisture content and stored at 25-30°C showed reduction in the total amino acids content in grades 1 and 5. The Infested grains of different grades increased their content of free amino acids when stored for 30 days with 17% moisture content of grades 1 and 5, and with 23% moisture content of grade 3. Exceptions were detected with proline, lysine and arginine in grade 1 and glutamic, proline in grade 5. It is clear that, the change observed in amino acids content were found to be correlated with the change of total protein content (except at 28° C). These results are in conformance with those obtained by Osman (1982).

Total carbohydrate was increased (compared with control) in all tested grade (1, 2 and 3), storage temperatures (10, 18, 28 and room temperature 25-30°C) and moisture content after storage for 90 days (Table 6). Carbohydrate content of grains was highly affected when stored with high moisture content (23%) under high temperature (28-30°C) and less affected with low temperature. Grading had a slight

Table (2) : Detection of aflatoxins B1 by *A. falvus* grown in yellow corn grains medium at 25°C for 10 days.

Fungi	Grade	Spore Suspension Method			Disc Method (Mycelial)	
		Seed moisture %				
		17%	20%	23%	B1	B2
	G1	-	+	+	++	+
Aspergillus flavus	G3	+	+	++	+++	++
	G5	++	++	+++	++++	++

- : Not Toxin production.
+ : Concentration of Toxin production (B1).
G1, G3, G5 : Seeds grades.

Table (3) : Effect of inoculating yellow corn grains with *Aspergillus flavus* under different grain grads, temperature, moisture content percentages and storage intervals on production of aflatoxin.

Tempera-ture		10°C		15°C		28°C				Room Temperature (25-30.2°C)			
Moisture		17%	23%	17%	23%	17%		23%		17%		23%	
Days		90	90	90	90	15	30	15	30	15	30	15	30
G1	B1	-	-	-	32.9	-	27.4	32.87	37.2	27.4	16.43	-	-
	B2	-	-	-	5.48	-	13.7	-	13.4	-	-	-	-
G3	B1	-	-	-	174.7	4.93	10.27	821.9	986.3	30.7	54.8	38.49	64.8
	B2	-	-	-	-	-	-	136.9	219.0	-	27.4	25.1	27.0
G5	B1	-	-	-	98.6	-	13.7	-	273.9	-	-	219.2	240.2
	B2	-	-	-	43.8	-	-	-	54.79	-	-	-	-
Control		-	-	-	-	-	-	-	-	-	-	-	-

G1, G3, G5 : Grades of seeds.
- : No Toxin Production.

Table (4) : Effect of infesting yellow corn grains with *Aspergillus flavus* under different grain grades, temperatures, moisture content percentages and storage intervals on the total protein percentage.

Temperature	10°C				18°C				28°C						Room Temperature (25-30.2°C)					
Moisture content	17%		23%		17%		23%		17%			23%			17%			23%		
Days of storage	Zero time	90 days	Zero time	90 days	Zero time	90 days	Zero time	90 days	Zero time	15 days	30 days	Zero time	15 days	30 days	Zero time	15 days	30 days	Zero time	15 days	30 days
Treated G1	8.0	7.4	7.5	7.2	8.0	7.5	7.5	7.3	8.0	8.1	7.3	7.5	8.1	8.4	8.0	7.8	7.5	7.5	7.0	7.8
Control G1	8.0	8.1	7.8	7.8	8.0	8.0	7.5	7.6	8.0	8.2	7.9	7.5	7.6	7.7	8.0	8.0	8.0	7.5	7.4	7.4
Treated G3	7.9	7.5	7.8	7.4	7.9	7.8	7.8	7.1	7.9	8.2	7.4	7.8	8.0	8.8	7.9	7.6	7.2	7.8	7.4	8.6
Control G3	7.9	7.7	7.8	7.8	7.9	8.0	7.8	7.5	7.9	8.0	7.9	7.8	7.8	8.0	7.9	8.0	8.0	7.8	7.4	7.3
Treated G5	7.8	7.5	7.5	7.3	7.8	7.4	7.5	7.2	7.8	7.7	7.3	7.5	8.2	8.5	7.8	7.9	7.3	7.5	7.8	8.3
Control G5	7.8	7.7	7.5	7.5	7.8	8.0	7.5	7.4	7.8	7.9	8.0	7.5	7.8	7.6	7.8	8.0	8.0	7.5	7.4	7.3

G1, G3, G5 : Grades of seeds.

Table (5) : Effect of infesting yellow corn grains with *Aspergillus flavus* under different grains grades, temperatures, moisture content percentages and storage intervals on amino acids content (%).

Amino acids	G1 T4 M1 (36.0%)*			G3 T3 M2 (100%)*			G5 T4 M1 (55.2%)*		
	Zero time	After 30 D.	% Change	Zero time	After 30 D.	% Change	Zero time	After 30 D.	% Change
Aspartic acid	0.44	0.54	22.7	0.43	0.67	55.8	0.47	0.5	6.3
Thereonine	0.23	0.28	21.7	0.23	0.32	39.1	0.26	0.26	Zero
Serine	0.32	0.46	43.8	0.31	0.54	74.1	0.34	0.39	14.7
Glutamic acid	1.36	1.41	5.2	1.31	1.45	10.6	1.4	1.32	-26.3
Proline	1.42	0.56	-60.6	1.41	0.6	-57.4	1.58	0.0	-100
Glycine	0.25	0.3	20	0.24	0.35	45.8	0.27	0.28	3.7
Alanine	0.49	0.59	20.4	0.28	0.61	117.8	0.51	0.52	1.9
Valine	0.26	0.33	26.9	0.26	0.35	34.6	0.27	0.32	18.56
Isoleucine	0.18	0.28	55.5	0.18	0.37	105.5	0.19	0.27	42.1
Leucine	0.8	0.95	18.8	0.79	1.11	40.5	0.83	0.83	Zero
Tyrosine	0.19	0.23	21.0	0.17	0.28	64.7	0.19	0.3	57.8
Phenylalanine	0.23	0.3	30.4	0.3	0.34	-13.3	0.31	0.33	6.4
Histidine	0.17	0.18	5.9	0.17	0.22	29.4	0.18	0.19	5.5
Lysine	0.21	0.19	-9.5	0.2	0.23	15	0.23	5.25	8.6
Arginine	0.33	0.22	-33.3	0.23	0.31	34.7	0.24	0.31	29.1

Where :

G1 Grade 1
G3 Grade 3
G5 Grade 5
D. After 30 days stored.
*. % ofinfection.
T3 Temperature 28°C
T4 Room temperature 25 to 30.2°C
M1 Moisture content 17%
M2 Moistute content 23%.

Table (6) : Effect of inoculating yellow corn grains with *Aspergillus flavus* under different grain grades, temperature moisture content percentages andstorage intervals on total carbohydrate % as glucose.

Temperature	10°C				18°C				28°C				Room Temperature (25-30.2°C)			
Moisture	17%		23%		17%		23%		17%		23%		17%		23%	
Stored days	Zero time	90 days	Zero time	90 days	Zero time	90 days	Zero time	90 days	Zero time	30 days	Zero time	30 days	Zero time	30 days	Zero time	30 days
Treated -1	69.5	70.7	67.7	72.4	69.5	71.0	67.7	74.2	69.5	73.2	67.7	79.7	69.5	72.2	67.7	79.9
Control	69.5	69.4	67.7	69.0	69.5	69.9	67.7	70.0	69.5	69.8	67.6	67.9	69.5	69.5	67.7	69.1
Treated-3	69.8	71.0	68.4	71.0	69.8	71.8	68.4	76.6	69.9	68.4	68.4	69.8	69.8	74.2	68.4	78.2
Control	69.8	70.0	68.4	68.8	69.8	68.4	68.4	69.8	69.8	68.4	68.4	68.4	69.8	69.7	68.4	69.0
Treated-5	70.2	72.8	68.0	71.3	70.2	71.9	68.0	71.8	70.2	73.1	68.0	80.4	70.2	72.1	68.0	76.1
Control	70.2	70.8	68.0	68.9	70.2	70.6	68.0	68.9	70.2	69.9	68.0	69.5	70.2	70.7	68.0	69.1

effect on the total carbohydrate. An increase in total carbohydrate content can be interpreted on the basis that *A. flavus* may split fat and oil of corn grains by its Lipase into free fatty acids and glycerol which used as an available source of carbon for metabolizing fungal cell constituents including fungal carbohydrates as explained by (Leasage *et al.*, 1991).

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ACKNOWLEDGMENT

The authors wish to express their deep thanks and sincere appreciation to **Prof. Dr. Akila S. Hamza,** General Director of Cental Lab. for Food & Feed (CLFF), A.R.C., and **Prof. Dr. Abdo Mohamed,** Pathology Division Dept. of Agric. Botany, Moshtohor for their interest and sincere help. The authors also thank the stuff member of both, Central Lab. for Food & Feed (CLFF), A.R.C., and the Fungi and Plant Pathology Div., Fac. of Agric., Moshtohor. Gertitude is extended also to **Dr. Samy Moustafa,** Plant Pathology Institute, A.R.E.

مجلة الأزهر للبحوث الزراعية
العدد (٢٤) ديسمبر ١٩٩٦

دراسات على أسباب تدهور بعض الحبوب أثناء التخزين ومقاومتها
(٢) إنتاج الأفلاتوكسينات والتركيب الكيماوى للحبوب

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الملخص العربى

أجرى هذا البحث لدراسة مقدرة الفطريات المعزولة والمعروفة بقدرتها على إنتاج الأفلاتوكسينات والتى يمكن ترتيبها تنازلياً على أساس النسبة المئوية للظهور فى العينات المختلفة التى تم فحصها كالآتى : أسبرجلس فلافس، بنسليوم فونيكيوليوزوم، أسبرجلس نيريس، فيوزاريوم مونيليفورم، فيوزاريوم أوكسيسبوريم، فيوزاريوم حرامينياريم، أسبرجلس نيجر، نوع من الترنارياء، نوع من الدريزوبس، ماكروفومينا فاصولينا ونوع آخر من فيوزاريوم وتأثير العدوى الصناعية لحبوب الذرة الصفراء عند تلوثها بالفطر أسبرجلس فلافس على محتوى الحبوب المصابة من البروتين الكلى، والأحماض الأمينية والمحتوى الكلى من الكربوهيدرات وكذلك على إنتاج الأفلاتوكسينات.

ويمكن تلخيص النتائج المتحصل عليها فى الآتى :-

١- تأثر إنتاج الأفلاتوكسينات بواسطة الفطر أسبرجلس فلافس برتبة الحبوب ومحتواها من الرطوبة.

٢- قل المحتوى البروتينى لحبوب الذرة ذات المحتوى الرطوبى ١٧٪ أو ٢٣٪ عندما خزنت على درجات منخفضة كما زاد المحتوى البروتينى للحبوب المصابة ذات المحتوى الرطوبى ٢٣٪ وذلك بعد فترة تخزين قدرها ٣٠ يوماً.

٣- تخزين الحبوب المصابة (محتوى رطوبى ١٧٪ أو ٢٣٪) تحت درجات حرارة عالية أدى غلى نقص فى تركيز الحامض الأمينى برولين كما نقص تركيز كل من الليسين والأرجنين بينما ظل البعض الآخر مثل الثريونين والليوسين دون تغيير.

٤- زيادة المحتوى الكلى من الكربوهيدرات عندما خزنت الحبوب المصابة على درجات حرارة عالية (٢٨م أو ٢٥م - ٣٠م). كما لوحظ أن هناك تأثير لرتبة الحبوب على المحتوى الكلى من الكربوهيدرات.