

PATHOLOGICAL STUDIES ON DETERIORATION OF YELLOW CORN DURING STORAGE AND ITS CONTROL

I. Associated fungi, percentage of infection and its control.

Nawal, Abdel-Monem Esia*; Abdel-Reheem, S.K.;
Badr, A.Y.*; and Abo-Elela, M.E.****

* Dep. Agric. Botany, Fac. Agric., Moshohor, Zagazig Univ.

** Centrl Lab. For Food and Feed, ARC, Giza.

ABSTRACT : The most dominant fungi which were found to be associated with yellow grains could be arranged descendingly, according to the occurrence percentage, as follows : *Aspergillus flavus*, *Penicillium funiculosum*, *Asp. terreus*, *F. moniliforme*, *F. oxysporum*, *F. graminearum*, *Asp. niger*, *Alternaria sp.*, *Rizopus sp.*, *Macrophomina phaseolina* and *Fusarium sp.*

The percentage of infection of yellow corn grains caused by *A. flavus* was significantly increased by increasing the storage temperature degree, grain moisture content, time of storage and percentage of broken Kernels (grade of the grains).

The tested fungicide, Rovral 50% and Topsin M 70% were successively decreased the percentage of infection of yellow corn grains caused by *A. flavus*. Each fungicide at any tested rate significantly decreased grain invasion. The efficiency of each fungicide was significantly increased by increasing its concentration. The usage of both fungicides at the rate of 1000 ppm caused the least percentage of grain infection, specially after storage period of 15 days.

The infection percentage was influenced by application of the tested preservatives, thiourea and 8-hydroxyquinoline. All tested doses of thiourea or 8-hydroxyquinoline decreased percentage of grain infection. Generally, thiourea was superior (33.9%) in this respect when compared with either 8-hydroxyquinoline (53%) or the untreated grains (96.65%).

INTRODUCTION

The spectacular increases in production of corn are due to its two valuable components the food oil and the feed meal. Corn oil is the world's leading vegetable oil and accounts about 20-30% of all fats oils in the world (Anon, 1994). Corn as a feed meal is the second major component that has commercial value. It is used mainly as a foodstuff for animals and poultry wealth. Hassan and Selim (1982) stated that, the corn samples were associated with 168 isolates of fungi belonged to 11 genera among which 18 isolates were of *Aspergillus*, 11 of *Fusarium*, 13 of *Mucor* or *Rhizopus* and 10 of hyphomycetes. Garcia (1984) isolated and identified 122 isolates of *Pencillium* either from commercial corn samples or corn stored at experimental conditions. *Pencillia* isolates can grow and deteriorate shelled corn stored in the American midwest. Hsia *et al.* (1988) isolated and identified *Fusarium scrip* from maize grains stored under natural conditions in mid north of China. Chatterjee (1989) stated that, *Aspergillus flavus* and *A. glaucus*, were the dominant storage fungi, which produced aflatoxin and caused loss of seed germinability. Munoz *et al.* (1990) studied the presence of pathogenic fungi and mycotoxin in maize and found that, ten different fungi were present specially *Fusarium moniliforme* and *F. poae*. Sayer (1991) showed that, *Fusarium* infection of either grown or stored maize in the waikato region of new Zeland, *Fusarium crook wellens*, *F. graminereum* and *F. semitactum* were the most common species isolated from leaf pieces, field kernels, or stored maize. Yasin *et al.* (1992) found that, the predominant storage molds of corn were *Pencillium*, *Aspergillus*, *Fusarium*, and *Cladosporium*. Botast *et al.* (1981) stated that, mould population increased in corn grains stored with 15 and 18% moisture content. Sauer and Tuite (1986) reported that, growth of *Aspergillus flavus* was inhibited at relative humidities less than 85% or 16% moisture content of stored maize. Zhibiao *et al.* (1989) noticed that, storage fungi and moisture content greatly affected seed germination also moisture content influenced the development of storage fungi.

Wilcke *et al.* (1992) concluded that, the use of rovr al the rate of corn grain detrioration when measured as CO₂ production. Yasin *et al.* (1992) showed that, corn grains with high moisture content treated

with potassium sorbate or potassium sorbate with propylene glycol stored for along period more than untreated ones. Mahdy (1994) used Rovral 50% and Topsin M 70% among five fungicides in the two growing winter seasons and on some traits wheat and infection with fungi. Each with two different concentration (500 and 1000 ppm). The studied fungicides significantly affected percentage of infection in the two winter seasons.

The present study was conducted to isolate and identify the associated fungi and to evaluate the effectiveness of some factors that affect corn damage during storage as well as the application of some fungicide and/or preservatives as control.

MATERIAL AND METHODS

Isolation and identification of fungi associated with yellow corn grains :

Samples used in this study were obtained from different yellow corn imported shipments from U.S.A. Samples were graded in Feed Grains Inspection Lab., Central Laboratory for Food and Feed, Agricultural Research Center, Giza, Egypt. Corn grains were disinfested by immersing in 5% sodium hypochlorite solution for 3 minutes-washed thoroughly in three changes of sterilized water and dried between sterilized filter paper. Grains were aseptically transferred to ready plates of potato dextrose agar (P.D.A.) (Christense, 1957). Plates were then incubated at 25°C observations were daily recorded up to the 7th day. The emerged fungi were counted, isolated on P.D.A. plates and purified using the single spore technique (Hansen, 1926) and/or the hyphal tip technique (Riker and Riker, 1936). Representative samples of at least 100 grains of each grade were usually used for the isolation of the associated fungi. Pure cultures were maintained on P.D.A. slants at 25°C for 15 days and stored at 8°C in a refrigerator. Identification of the associated fungi was carried out by Dr. Samy Moustafa in Plant Pathology Institute, Agricultural Research Center, Giza, Egypt.

Factors affected on stored yellow corn grains infection with *Aspergillus flavus* :

A. Grading of grains :

Grains were graded by using Carter Day Grading Machine (Dockage Tester Serial No. 1712 U.S.A.) in Feed Grain Inspection Lab, Agricultural Research Center, Giza, Egypt. Three grades (1, 3 and 5) were tested in this study, its characteristic shown in Table (1).

Table (1) : Characteristics of tested yellow corn grain grades.

Grade	Minimum test weight (Pounds/bushel)	% of broken kernels
U.S. No. 1	56.0	2.0
U.S. No. 3	52.0	4.0
U.S. No. 5	46.0	7.0

* (Federal Grain Inspection Service, U.S.A. Dept. of Agriculture 1995).

B. 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).

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

where :

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

C. Preparation of spore suspension :

Spore suspension was prepared from pure cultures of *Aspergillus flavus* (15 days old) grown on P.D.A. plates. Plates were flooded with 10 ml of sterilized distilled water and brushed thoroughly for 1-2

minutes. The suspension was filtered by cloth filter to remove the mycelia residues. Number of spores/ml. was counted in the spore suspension by using a Spencer hemocytometer, which was been found to be about 625×10^3 . The spore suspension has been added to tested grains in a volume to gave a final density of approximately 2500-3000 spore/1 gram of corn grains, as described by (Osman, 1982).

D. Inoculation :

Grain samples of each tested grade (1, 3 and 5) formerly prepared with known moisture content were inoculated with a spore suspension of *A. flavus* (2500-3000 spore/g. grains) and finally the desired moisture content levels (17 and 23%) of corn grains were performed. The inoculated grains were subjected to storage under different temperature degrees (10, 18, 28 and natural one about 25-30.2°C) for different periods (15, 30, 60. and 90 days). Controls (non inoculated) for each total treatments (inoculated) were conducted. A randomized complete block design with three replicates was carried out, under Lab. conditions.

Percentage of grain infection :

Hundred grams of previously inoculated grains and non inoculated in each grade handly were examined. The resulted infected grains were weighed and the percentage of infection was calculated according to the following equation (El-Araby, 1985).

$$\text{Percentage of infection} = \frac{\text{Infected grains}}{\text{Total number of grains}} \times 100$$

Determined percentages of infection were transformed to the arcsin before carrying out the statistical analysis. Data obtained were subjected to the proper analysis of variance (Snedecor and Cochran, 1980).

Control experiments :

Rovral 50% and Topsin M 70% fungicides were used as recommended by Mahdy (1994). 8-hydroxyquinoline and thiourea preservatives, were used as recommended by Ahmed, (1971).

RESULTS AND DISCUSSION

Results of occurrence and percentage of the associated fungi are presented in Table (2). Identification trials showed that, the isolated fungi belong to 6 genera and 11 species. Among the so-called "field fungi", *Fusarium moniliforme* and *F. oxysporum* were the most dominant fungi which occurring at higher percentages, followed by *Fusarium graminearum*, *Alternaria sp.*, *Fusarium sp.* and *Macrophomina phaseolina*, the latter two fungi showed the lowest frequency percentages. On the other hand, *Aspergillus flavus* was found in higher occurrence percentage as storage fungus, followed by *Penicillium funiculosum*, *Aspergillus terreus*, *A. niger* and *Rhizopus sp.*

Generally, in this study, the most dominant fungi associated with yellow corn grains could be arranged descendingly, according to the occurrence percentage, as follows : *Aspergillus flavus*, *Penicillium funiculosum*, *Aspergillus terreus*, *Fusarium moniliforme*, *F. oxysporum*, *F. graminearum*, *Aspergillus niger*, *Alternaria sp.*, *Rhizopus sp.*, *Macrophomina phaseolina* and *Fusarium sp.*

Table (2) : Occurrence percentages of different fungi isolated from yellow corn grains obtained from different imported shipments from USA.

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

This result is in accordance with those recorded by many investigators who isolated and identified one or more of the formerly mentioned fungi as corn grains-associated fungi (Mohamed *et al.*, 1967; Christensen and Kaufman, 1969; Ahmed, 1971; Mazhara, 1974; Hassan and Selim, 1982; Barry, 1986; Carrillo *et al.*, 1989; Sayer, 1991 and Yasin *et al.*, 1992).

Results in Table (3) clearly show that, there were significant differences, in the percentages of grain infection, between the different tested grades. Grade one showed the least percentage of infection (26.6%) followed by grade three (32.5%) while grade five recorded the highest one (40.6%).

The percentages of infection were significantly affected by the tested storage temperatures either within a particular grade or between the tested grades and hence the percentage of infection has significantly increased by increasing the temperature degrees within the grade, specially grade five in which 10°C recorded the least percentage of infection (21.2%) followed by 18°C (41.3%), 28°C (48.5%) and the room temperature (about 25 - 30.2°C) under commercial storage conditions showed the highest percentage of infection (51.4%). Both grades one or three, 10°C showed the least percentage of infection (8.4%) and (10.5%) followed by 18°C (26.3%) and (30.7%), the room temperature (under room storage conditions, about 25 - 30.2°C) (37.1%) and (49.3%) and 28°C indicated the highest percentage of infection (34.5%) and (39.6%), respectively. All tested temperature degrees in grade one recorded the least significant percentages of infection followed by analogous ones of grade three and then these of grade five.

Data showed that, the increase in grain moisture content percentages from 17% to 23% has significantly increased the percentage of grain infection under all tested and all tested grades. It is of great importance to mention that, 17% moisture content percentage under grade three and temperature degrees 10°C, 18°C and the normal one recorded the least significant percentages of infection, 5.9%, 7.2% and 24.4%, respectively, while 17% moisture content under grade five

and room temperature, showed the lowest percentage of infection (27.7%). Comparing with the analogous ones in other tested grades. On the other hand, 23% moisture content under all tested temperature showed the least significant percentages of infection in grade one (10.4%, 36.3%, 49.3% and 53.2%, respectively), followed by grade three (15.8%, 49.9%, 55.0% and 76.1%, respectively) and grade five indicated the highest ones (34.4%, 61.5%, 68.8% and 75.1%, respectively).

Results in Table (3) and indicated that, the percentages of infection has significantly increased by prolonging storage intervals (15, 30, 60 and 90 days). The effect of storage periods was varied according to grade, temperature degrees and moisture content percentages. In grade one contain 17% moisture was obtained after 90 days storage period as 13.3% and 37.3% at temperature degrees of 10°C and 18°C but 30 days 46.3% and 36.3% at 28°C and room temperature (about, 25 - 30.2°C). While under 23% moisture content the maximum percentages of infection were obtained after 90 days storage period in case of 10°C (23.7%) and 18°C (70.3%) and after 30 days in case of 28°C (119%) and the room temperature (about 25 - 30.2°C), (100%). Grade three showed the same trend as grade one and hence the obtained percentages of infection were 18.8% and 30.3% after 90 days storage period at 17% moisture content and under different temperature degrees (10°C and 18°C), while these percentages were 25 - 30.2°C and 100% after 30 days 23% moisture content and at 10°C and 18°C, respectively, but these percentages were 100% after 30 days in either 28°C or the normal one 25 - 30.2°C. In case of grade five, the maximum percentages of infection which were 22.5% and 38.5% after 90 days storage period at 17% moisture content and at 10 and 18, while these infection percentages were 100% after 90 days at 23% moisture content and under 10°C storage temperature, but these percentages were reached 100% after 30 days under the other tested temperature degrees. The percentage of infection or the damage of yellow corn grains caused by *Aspergillus flavus* was increased compared with control treatment by increasing the storage temperature from 10 to 28°C, grain moisture content percentage, time of storage and percentage of broken kernels (seed). These result is in accordance

Table (3) : Effect of some fungicides andpreservatives on infection percentage of yellow corn grains (grade 3 and with 23% moisture content) inoculated with *Aspergillus flavus* and stored under the normal temperature from 25.0 - 30.2°C for 15 and 30 days.

Fungicides									
Topsin M				Control		Rovertal 50%			
500 ppm.		1000 ppm.				500 ppm.		1000 ppm.	
15 D	30 D	15 D	30 D	15 D	30 D	15 D	30 D	15 D	30 D
25.4	29.3	12.7	24.3	93.3	100	32.7	48.3	19.7	30.9
27.35		18.5		96.65		40.5		25.3	
22.9				96.65		32.9			

For fungicides	= 3.78
For concentration	= 3.78
For days	= 3.78

Preservatives									
Topsin M				Control		Thiourea			
4000 ppm.		5000 ppm.				6000 ppm.		7000 ppm.	
15 D	30 D	15 D	30 D	15 D	30 D	15 D	30 D	15 D	30 D
47.0	55.0	46.0	70.0	93.3	100	11.6	39.5	42.0	42.5
51.0		52.5		93.3	100	25.55		42.25	
53.0				96.65		33.9			

For days	= 7.08
For concentration	= N.S.
For interaction	= N.S.

For days	= N.S.
For concentration	= 3.89
For interaction	= N.S.

with that stated by Qasem and Christensen (1960) who found that, mechanical damage in seeds, cracks, breaks or scratches in the pericarp or seed coat, are conditions that substantially favour invasion of storage fungi and increasing in mycelial growth and damage with increasing degree of kernel injury. These results also are in harmony with the previously recorded ones (Botast *et al.*, 1981). The increase in moisture content from 17% to 23% has significantly increased the percentage of grain infection under all tested temperature degrees or grades. These results are in accordance with those stated by (Sauer and Tuite, 1986; Siriacha *et al.*, 1989 and Zhibiao *et al.*, 1989).

The percentages of infection were significantly increased by prolonging storage period (15, 30, 60 and 90 days). These results are in harmony with the previously recorded results (Qasem and Christensen, 1969). Also all the possible interactions, between storage temperature degrees, moisture content percentages of grains, storage periods and percentages of broken kernels (grades), were significantly affected the percentage of grain infection and hence the obtained percentages of infection have significantly varied from grade to another and within each grade according to temperature degree, moisture content and storage period, so the least significant percentage of infection (0.0%) was observed in grade 1 at 10°C with 17% moisture content after 15 days storage period, while the highest one (100%) was obtained in grade 5 at 28°C with 23% moisture content after a storage period of 30 days. Percentage of infection was significantly increased by increasing each of storage temperature, moisture content, storage period and percentage of broken kernels (grade). These results are in accordance with those recorded by (Perez *et al.*, 1982 and Siriacha *et al.*, 1989).

With regard to the fungicides, results presented in Table (4) and indicated that, the two fungicides have fully decreased percentage of infection. Each fungicide at any tested rate significantly decreased grain invasion in comparison with the control. The efficiency of each fungicide was significantly increased with the increase of its concentrations. The use of both fungicides at the higher rates (1000 p.p.m.) caused the least percentage of grain infection specially after a storage period of 15 days. In general, Topsin M 70% at the rate of 1000 p.p.m. showed the least significant percentages of grain infection

Table (4) : Effect of some fungicides and preservatives on infection percentage of yellow corn grains (grade 3 and with 23% moisture content) inoculated with *Aspergillus flavus* and stored under thenormal temperature from 25.0 - 30.2°C for 15 and 30 days.

Treatment	Conc. (ppm)	Storage Period		Mean
		15 days	30 days	
Topsin	500	25.4	29.3	27.35
	1000	12.7	24.3	18.50
Rovral	500	32.7	48.3	40.5
	1000	19.7	30.9	25.3
8-hydroxyquindine	4000	47.0	55.0	51.0
	5000	46.0	70.0	58.0
Thiourea	6000	11.6	39.5	25.55
	7000	42.0	42.5	42.25
Control	-	93.3	100.0	96.65
Mean		36.7	48.87	

L.S.D. at 5% for :

Treatment (T) = 5.3

Period (S) = 2.1

Interaction T x S = N.S.

either after 15 (12.7%) or 30 days (24.3%) storage periods followed by Rovral 50% at the rate of 1000 p.p.m. (19.7 and 30.9% after 15 and 30 days storage periods, respectively) in comparison with the control (93.3 and 100% after 15 and 30 days storage periods, respectively). Thiourea at 6000 ppm and Topsin at 1000 ppm were the best for superessing infection with *A. flavus* in stored corn grains after 15 days storage. However, Topsin either at 500 or 1000 ppm produced the minimum infection after storage period of 30 days. In general, any of the tested chemical (fungicides or preservitives) exhibited significant reduction in percentage of infection either after 15 days or 30 days storage compared with control (untreated) treatment. With respect to the preservatives, data clearly showed that, all the tested doses of Thiourea or 8-hydroxyquinoline decreased grain invasion comparing with control. Thiourea was superior (33.9%) in this respect when compared with either 8-hydroxyquinoline (53.0%) or the untreated grains (96.65%). These results are in harmony with those stated by Abdel-Hamid (1991), Wilcke *et al.* (1992) and Mahdy (1994).

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دراسات مرضيه على تدهور حبوب الأذرة الصفراء أثناء التخزين ومقاومتها (أ) الفطريات المرتبطة بحبوب الذرة - نسبة الإصابة والمقاومة

نوال عبد المنعم عيسى*، أبو اليزيد إمام بدر*، سيد كامل عبد الرحيم*
محمد فتحى ابو العلا**

* قسم النبات الزراعى - فرع الفطر - كلية زراعة مشهور - جامعة الزقازيق.
** المعمل المركزى للأغذية والأعلاف - مركز البحوث الزراعية - الجيزة.

الملخص العربى

أجرى هذا البحث لدراسة الفطريات المرتبطة بحبو الذرة الصفراء، تأثير العدوى الصناعية لحبوب الذرة الصفراء عند تلوثها بالفطر أسبرجلس فلاكس (ذلك تحت ظروف مختلفة تتضمن رتب الحبوب، درجات حرارة التخزين، النسب المئوية للمحتوى الرطوبى وفترات التخزين) على النسبة المئوية للإصابة وفاعلية بعض المبيدات الفطرية والمواد الحافظة ضد إصابة الحبوب المخزونة بالفطر اسبرجلس فلاكس.

ويمكن تلخيص النتائج المتحصل عليها فى هذا البحث كما يلى :-

١- الفطريات التى وجدت مرتبطة بحبوب الذرة الصفراء يمكن ترتيبها تنازلياً على أساس النسبة المئوية للظهور فى العينات التى تم فحصها كالأتى : أسبرجلس فلاكس، نيسليوم فونيكوليوزوم، أسبرجلس نيريس، فيوزاريوم مونيليفورم، فيوزاريم اوكسيسبوريم، فيوزاريم جراميناريم، أسبرجلس نيجر، نوع من التراتاريا، نوع من اليزوبس، ماكروغومينا فاصيولينا ونوع من فيوزاريم.

٢- النسبة المئوية لإصابة حبوب الذرة الصفراء بالفطر أسبرجلس فلاكس زادت بزيادة معنوية بزيادة كل من درجة حرارة التخزين، فترة التخزين والنسبة المئوية للحبوب المكسورة (رتب الحبوب) خاصة عند ارتفاع الرطوبة فى الحبوب.

- ٣- أدى إستخدام المبيدات (روفرال ٥٠٪، توبسن إم ٧٠٪) إلى خفض النسبة المئوية لإصابة حبوب الذرة المخزنة بالفطر أسبرجلس فلافس وكل التركيزات المستخدمة من أى من المبيدين أدت إلى خفض معنوى فى نسبة إصابة الحبوب. كما لوحظ أن فاعلية أى من المبيدين تزداد زيادة معنوية بزيادة التركيز المستخدم.
- ٤- أدى إستخدام المواد الحافظة التى تم إختيارها (ثيويوريا و ٨ هيدروكسى كينولين) إلى خفض النسبة المئوية للإصابة.