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HAZARDS RISK ASSESSMENT OF CERTAIN TOXIGENIC FUNGI ON INFECTED CORN GRAINS: WITH SPECAIL REFFRENCES TO CHEMICAL COMPOSITION AND RHEOLOGICAL PROPERTIES.

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ABSTRACT

Fifty corn kernel samples freshly harvested and dried, collected from local markets were tested for the presence of mycotoxins, fungal infection and occurrence of fungal species. Aflatoxin (Afl) and fumonisin (FB_1) producing isolates were identified. The results indicated that the corn samples were free of mycotoxins, while the percentage of infection of kernels was 74%. *Aspergillus flavus* (46.5%) was the dominant species followed by *A. sedowi.* and *Fusarium moniliforme*. The lowest frequency was recorded for *A. niger* .Aflatoxin B_1 (afl B_1) was detected in 22% of corn samples at a mean level of 80µg /Kg. Fifteen samples (30%) were contaminated with Fumonsin B_1 (FB_1) at a mean level of 180µg /Kg. Both Afl B_1 and FB_1 were Co-occurrence in 6 (12%) samples, while 48% of corn grain sample were free of both toxins. 25% of *A.flavus* isolates and 20% of *F. moniliforme* were able to produce mycotoxin at levels ranged from 10-100 µg/Kg Afl and 6.8-280 µg/kg FB_1 respectively. The growth of *A.flavus* and *F. moniliforme* was affect on chemical composition and viscosity of infected corn grains. The changes were depended on the fungal species. Total sugars showed significant increases reached to 25.6%, as well as reducing 21.3% and nonreducing sugars 30%. Moreover, an increase in protein content (7..34% and 17.22%) and fibers (9.7% and 13.03%) were recorded for infected corn with *A. flavus* and *F. moniliforme* respectively. On the other hand, slight decreases in starch (1.54%) and ash contents were recorded. The dough made from infected corn showed higher viscosity, while the temperature at the maximum viscosity and gelation temperature (70.5 ^{o}C) were decreased compared with the control (73.5 ^{o}C). The results concluded that the storage conditions were the main factor to prevent the fungal growth that caused not only changes in chemical composition but also produce large amount of mycotoxins due to contamination corn during storage period.

Keywords: corn, chemical composition, fungi, fumonisin, aflatoxin, rheological properties

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INTRODUCTION

Molds are microorganisms that thrive on common livestock foodstuff. Not only do molds themselves reduce the quality of grains (Lacey *et .al.* 1991), but also the production of mycotoxins impairs growth and reproductive efficiency. The deterioration in the chemical and biochemical grain components of molded corn, wheat and barley are affecting the nutritive values (Aired and Esuruoso ,1987 and Madhyastha *et. al.*, 1993). Farag *et .al* .,(1985) and Jintian *et .al.*, (1997) stated that some physicochemical

changes may occur in starch of molded wheat and corn grains. These changes appeared during baking or frying of starchy products.

Mycological studies of corn have demonstrated the presence of several toxigenic moulds, the major mycotoxin producing genera are *Aspergillus* and *Fusarium,* which are most frequent isolated from corn samples and other cereals (Hirooka *et. al.,* 1996; Castella *et .al.,* 1999a; and Medina and Martenez, 2000). These molds are widespread in nature and produce toxin where conditions are favorable, variety of food and animal feed are often contaminated with aflatoxins and fumonisin . Peanuts, corn , cottonseed, almonds and pecans are frequently contaminated with aflatoxins and fumonisins during growth, harvest, storage and transportation(Girolamo *et. al.,* 2000). Regarding to storage risk , the high frequency occurrence of *Aspergillus* spp. and high level of aflatoxins were detected in stored corn grains with high initial moisture content (16-17%) in a bags a well-ventilated ware-house (Ono *et. al.* ,2002).

Mycotoxins are toxic secondary metabolites, have a wide array of chemical structures, and are produced by common field and storage fungi. Most of the isolates of *Aspergillus flavus and A. parasiticus* and *Fusarium moniliforme* produced aflatoxins and fumonisins on corn samples at levels, which varied greatly between, samples (Ali *et. al.,* 1998 and Medina and Martenez, 2000). Because of the hepatotoxic, mutagenic , carcinogenic and teratogenic nature of these toxins, consumption of aflatoxin contaminated food poses a serious health hazard (clavero *et. al.*, 1992 and Brown *et .al.,* 1995). Fumonisins are mycotoxins mainly produced by *Fusarium moniliforme and Fusarium proliferatum,* which have been associated with several animal and human diseases (Riley *et .al.,* 1996). A possible correlation between the consumption of fumonisin contaminated corn and high incidence of esophageal cancer in southern Africa has been suggested (sydenham *et .al.,* 1990).The occurrence of fumonisins in corn and corn based food products have shown high fumonisin contents (?1,000 mg/g) in corn kernels and minimally processed corn , such as , corn grits and corn flour samples has been reported by Castelo *et .al.,* (1998)

In Egypt, corn is an important crop for human and animal consumption as well as the industrial processing of starch, glucose, and syrup. Moreover, corn flour also used for stable bread for human consumption. The aims of the present study were to assessment the hazards of some mycotoxigenic fungi (*Aspergillus flavus* and *Fusarium monilifrome)* , the occurrence of molds on corn and investigate the ability of isolated fungi to produce aflatoxin B_1 and fumonisin B_1. Furthermore, to investigate the biological effect of individually myco toxigenic producing isolates that associated with corn grains especially when stored under improper conditions. This aim can be investigate by determine the aflatoxin and fumonisin levels in stored corn and evaluate the changes in chemical composition and rhiological properties .

MATERIAL AND METHODS

Corn samples

Fifty samples of freshly harvested and dried corn grains were purchased from local markets of Cairo Egypt.

Chemicals:

Aflatoxin B_1 and fumonisin B_1 standards were obtained from Sigma Chemical Co. Ltd (St. Louis, MO, USA). Reagents used were of HPLC grade (Merck Chemicals, Darmstadt, Germany). All other chemicals and reagents were of the highest purity that was commercially available.

Mycological examination:

Isolation of Fungi

Fungi associated with 100 grains, were isolated after surface sterilization with a disinfectant agent. Immersing the corn grains for 3 min. in 3.5 % solution of sodium hypochlorite made disinfecting. After washing 3 times in sterile distilled water, the seeds were dried between sterile filter paper. Isolation of fungi was made by serial dilution according to the method described by Mislivec, (1977) and Ichinone *et al.*,(1983) and by direct plating of five kernels, on potato dextrose agar medium (PDA)in a set of 10 plates. Fungal colonies were picked and finally purified on PDA slants after incubated at 25°C ± 2 °C for 7 days for other investigations. Single spore or hyphal tip techniques were used for purification of different isolates. Identification of isolates were carried out according to Raper and Fennel (1965) and Nelson *et .al.,* (1983). Percentages of infection of corn kernels, occurrence of fungal species were calculated.

Preparation of spore suspension:

Twenty isolates of *Aspergillus flavus* and fifteen of *Fusarium moniliforme* and an identified producing isolate of each Afl B_1 and FB_1 (provided by the Plant Pathology Department, National Research Center) were activated on slants of potato dextrose agar medium for 10 days at 25 °C. The spores were harvested with sterile 0.05% tween 80 to give a final spore concentration of approximately 5×10^5 spores / ml.

Preparation of artificially infected Corn samples:

Fifty grams of corn were adjusted to 20% (for *A. flavus*) and 30% (for *F. moniliforme*) moisture contents. After autoclaving, the samples were inoculated with one ml of spore suspension. Both cultures and the uninoculated samples (served as control) were incubated at 28°C ± 2 °C and 25°C ± 2 °C for *A. flavus* and *F. moniliforme* respectively, for 21 days. The cultures were autoclaved, dried at 50°C for 12h, ground using sample mill grinder and then stored in sealed polyethylene bags at - 20°C until analysis

Chemical analysis:

The moisture content, total sugar, reducing sugar, non reducing sugar, protein, lipid, crude fiber and ash were determined according to the method described in AOAC (1995).

Rheological properties:

Viscoamylograph tests were carried out on corn flour dough samples in a set of three replicates to determine their rheological properties according to the method of AACC(1983) .

Mycotoxins assay

Fumonisin Analysis

Corn grain samples were ground in coffee mill and 25 g of the ground material were extracted magnetic stirring for 30 min with 50 ml of methanol : water (3+1) , followed by centrifugation at 500 x g at 4 ^{O}C for 10 min. the supernatant was filtered through a Watman No:4 filter paper, and a 10 ml portion was applied to a Bond-Elute Sax cartridge which had been conditioned with methanol : water (3:1). Subsequently, the cartridge was washed successively with 8 ml of methanol: water (3:1) and methanol (3ml), finally the toxins were eluted with 14 ml of 0.5% acetic acid in methanol. The elute was evaporated to dryness under vacuum, redisolved in 2ml of methanol and re-evaporated to dryness under a nitrogen stream

Derivatization and HPLC analysis

The purified samples was disolved in 200 ml methanol, 50µlof sample solution derivatizatied with 200µl of α Phthaldialdehyde (OPA) solution as reported by Sydenham et al., (1996) and mixed for 30 (s) with vortex mixer. Exactly 3 min after addition of OPA, 20-µl derivatizatied solution was injected into the HPLC system. The reversed phase column was a spherisorb ODS-2 10µm (25x 0.4cm0 preceded by a C18 guard column (Waters Chromatography Division, Millipore corporation ,Mildford MA). The mobile phase was mixture of methanol: sodium dihydrogenphosphate (77:23) adjusted to pH 3.35. Flow rate was 1ml/min. The fluoresence detector was set at 335 nm excitation and 440 nm emission. Final concentration in corn grain was calculated on dry weight.

Aflatoxin determination:

The ground samples (50 gm) of both corn grains or fermented corn samples were extracted using BF method as described in AOAC (1995). Identification of aflatoxin was carried out according to the method outlined by Kpodo *et al* (1995) The concentrations of Afl B_1 were determined using Waters HPLC, (Ex. 365, Em. 450 nm). Millennium software program was applied for calculations, Nova Pak C18 column (3.4 X 150 mm, 4u), Mobile phase A: Acetonitrile: H_2O, 15: 85 (V/V) Mobile phase B : (Methanol 100%).

Statistical analysis:

All recent data were subjected to statistical analyses using the General Linear Models Procedure of the Statistical Analyses System (*SAS* Institute, Inc., 1982).The significance of the differences among treatment groups with variable means was determined by Waller - Duncan K-ritio T test , Waller and Duncan (1969). All statements of significance were based on a probability level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Mycological studies

Fungal flora associated with Corn grain Fungal flora associated with freshly harvested and dried corn grains, frequency, percentage of fungal infection and aflatoxin and fumonisin contaminants were determined. Data in Table (1) showed that the percentage of kernels contamination with fungi was 74%. A total of 130 fungal isolates belong to *Aspergillus* and *Fusarium* genera were recovered from all corn samples. The most dominant fungi was *A. flavus* (Fig 1) follcwed by *A. sedowi* and *F. moniliforme* which were isolated as the same percentage of occurrence. The lowest frequency was recorded for *A. niger*. These results are in good harmony with those reported by Castella et *.al.* (1999), they recorded *Aspergillus spp*, *Pencillium spp* and *Fusarium spp* in higher frequencies on corn and corn based feed and cereal. Among *Fusarium spp.*, the isolates of *F. moniliforme* (47.4 %) was the predominant species while *F. proliforatum* (5.3%) were isolated at lower frequency. Also, Battacharya and Raha (2002) stated that different species of *Aspergillus (A. flavus, A. niger, A* .terreus and *A.*ruber) were dominant fungi on stored corn grain followed by *Rhizopus ,Fusarium, Penicillium* and *Alternaria*. Whereas, Ono *et..al.*, (2002) found that *Fusarium spp, Penicillium spp* prevailed at the freshly harvested stage while *Aspergillus spp.* were detected at lower frequencies .The presence of *A. flavus* and *F. moniliforme* frequently together on corn kernels after harvest were supported by Lacey *et .al., (*1991). They reported that *Aspergillus* and *Fusarium* genera are known as commnon field and storage fungi and several investigators (Brown *et .al.,*1999 and Medina *et .al.*,2000) added that A. *flavus* and *F. moniliforme* were the most prevalent fungi on corn samples collected from different tropical region.

Table (1): Aflatoxin and fumcnisin contaminants in corn grain samples

Parameters	N=50		Mycotoxin concentration	
	No of positive samples	%	Mean µg/kg	Range µg/Kg
Aflatoxin	11	22	80	10-150
Fumonisin	15	30	180	50-310
Contam.	26	52		
Co-occurrence $AflB_1$ +FB_1	6	12	45 + 160	10-80/ 50-280
Uncontamenated	24	48		
Fungal infected	37	74		

Knowledge about kernel mycoflora during storage can predict the post –harvest deterioration, oscillation in Taxonomic groups, and further mycotoxin hazard. Concentration, mean and incidence in positive samples for mycotoxins are showed in Table (1), afl B_1 was detected in 11(22%) of the 50 corn samples at a mean level of 80µg /Kg (maximum 150 µg /Kg). Fifteen samples (30%) were contaminated with F B_1at a mean level of 180µg /Kg (maximum 310µg/Kg). Table (1) also showed that both Afl B_1 and FB_1 were Co-occurrence in 6 (12%) samples. These levels were lower than those reported by Ali *et. al.,* (1998),they detected Afl B_1 in 69% of samples and FB_1 in all samples at mean level 895µg /Kg .Also Castell *et.al.,* (1999) were detected FB_1 in79% of samples with average level of 3.3 mg/g . All samples of freshly harvested corn samples were positive for fumonisin as reported by Ono *et. al.,* (2002) and the mean concentration was 9.9 mg/g-1 ranged between 0.74 to 22.6 mg/g-1 .The variation between results were depending on the region , and quality of the grains. These fungi damage to grains and accumulation of the secondary metabolites (mycotoxins) . Because of The improper storage of grains have led to levels of these toxins which exceed the levels of concern (Bennet and Richard ,1996) , high frequencies of *Aspergillus* (storage fungi can infected at the preharvest stage) and high level of aflatoxins were detected in stored corn grains with high initial moisture content (16-17%) while *Fusarium spp* (field fungi) can remain in stored grains and fumonisin levels did not change during storage (Ono *et. al.* ,2002) .Data in Table (1) also showed that 60% of corn grain sample were fee of both toxins while some of these samples contaminated with producing toxin fungi. The results of the presence of toxigenic fungi while the samples are mycotoxins free mean that may or may not be a correlation between the levels of mycotoxins and percentage of infections as previously reported by Bacon and Nelson (1994 and Sahab, *et. al.* (1999).Recently ,Atalla *et. al.,* (2003) reported that grain moisture content and relative humidity are the major affecting factor during cereal storage .

The ability of isolates for producing mycotoxins

The corn samples were inoculated with both isolates of *A. flavus* or *F.moniliforme* and then Incubated to the suitable conditions for each species to determined the ability of isolates for producing AflB_1 or FB_1, respectively. Data in Table (2) showed that 25% and 20% of the isolates have the ability of producing AflB_1 in amount ranged between 10 to100 µg/kg and FB_1level ranged between 6.8 to 280µg/kg. This may be explained that the production of mycotoxins is related to regional temperature and moisture content as well as the improper storage conditions. these results are lower than those reported by Castella *et al* (1999b) , they found that all isolates of *F . Proliforatum* (9 isolates) and 94% of *F. moniliform* (119 isolates) are able to produce fumonisin B_1 at level reached to 30.94 mg/g.. Our results are in accordance with those recorded by Yoshizawa *et.al.*,1996 and Katta *et .al.* , (1997) recorded that storage conditions for corn and corn based food and feed in different countries consider the determinant factors in the growth of

fungi and toxins production espcially moisture content which consider the critical control point of stored corn .

Table (2): Natural occurrence of *A. flavus* and *F. moniliforme* and its capacity for aflatoxin and FB_1 production

Species	No. of isolates	No. of tested isolates	No. of positive isolates	Range [µg/kg]
A. flavus	54	20	5 (25%)	10-100 (mean =(55)
F. moniliforme	35	15	3 (20%)	6.8-280 (mean = (107.5)

Changes in chemical components

Corn grains are characterized by high carbohydrates content. The main changes in carbohydrates due to fungal infection was observed in total sugar (Table 3). The increases were 25.6 % and 10.24 % as resulting of the growth of *A.flavus* and *F.moniliforme*, respectively. The increases reached to 21.3% and 30.1% in reducing and non-reducing sugars with *A.flavus* ,respectively. Similar increases were recorded in reducing and non-reducing sugars was reported by Boyacioglu and Hettiarachchy (1995). In contrast, the starch content showed slightly decreases in the infected corn with both fungi. The increasing amounts of total and reducing sugars are due probably to the breakdown of starch endosperm and to release of sugars caused by the degradation of cell walls from the outer layers of the kernels. The decreases in lipid content (Table 3) ranged between 3.18% and 2.38% for corn infected with *A.flavus* and *F.moniliforme* respectively. These decreases may be due to fungal activity or to the deterioration in lipids as a result of the improper storage conditions (temperature and moisture content) as reported by Madhyastha *et .al.* (1993) and Bacon and Nelson (1994). These results are in accordance with those reported by Aziz *et .al.* (2002) they added that level of lipids of maize decrease gradually whereas fungal lipase activity increased markedly by increasing the storage period of artificially inoculated corn grains with *A.flavus* and these changes are related to the production of aflatoxin B_1. The increases in crude fiber reached to 13.03%, these increases may be due to the break down of the cellulose and hemicellulose of the cell walls. Data also showed a significantly decreases in ash content because of fungal growth.

Table (3): Changes of chemical composition *(g/100 g dry weight) of infected corn grain induced by *A. flavus* and *F. moniliforme* (means ± SE)

Treatments	Total Sugar	Reducing Sugar	Non R. sugar g/100 (% change)	Starch g/100 (% change)	Protein g/100 (% change)	Lipids g/100 (% change)	Fiber g/100 (% change)	Ash g/100 (% change)
Control	3.71 ± 0.02[a] (0.0)	1.88 ± 0.08[a] (0.0)	1.83 ± 0.13[a] (0.0)	72.72 ± 0.01[a] (0.0)	6.68 ± 0.09[a] (0.0)	6.29 ± 0.13[a] (0.0)	3.3 ± 0.01[a] (0.0)	1.08 ± 0.06[a] (0.0)
Contaminated with *A. flavus*	4.66 ± 0.12[b] (+25.60)	2.28 ± 0.10[b] (+21.30)	2.38 ± 0.07[b] (+30.10)	72.16 ± 0.03[a] (-0.77)	7.17 ± 0.01[b] (+7.34)	6.09 ± 0.00[b] (-3.18)	3.62 ± 0.06[b] (+9.70)	0.89 ± 0.04[b] (-17.6)
Contamin. With *F moniliforme*	4.09 ± 0.09[c] (+10.24)	2.04 ± 0.12[c] (+8.51)	2.05 ± 0.13[c] (+12.80)	71.60 ± 0.04[a] (-1.54)	7.83 ± 0.06[c] (+17.22)	6.14 ± 0.09[a] (-2.38)	3.73 ± 0.12[b] (+13.03)	1.04 ± 0.11[a] (-3.70)

In each column, means with the same letter are not significantly different ($P \leq 0.05$).
Values represent the means of three replications.
Values in practical represent % of changes.
*** moisture content =14%**

Regarding to protein changes, the infection with either *A.flavus* or *F.moniliforme* caused increases in protein contents. These increases (reached to 17.2%) may be related to fungus protein since, the hyphae from fungal growth in liquid culture contain about 42% crude protein as reported by Hamilton and Trenholm (1984) and Farag *et .al.* (1985)..These increases in protein content supported our results for increasing the viscosity of infected corn flour dough. Boyacioglu and Hettiarachchy (1995) and Madhyastha *et .al.* (1993) also reported that the changes in chemical composition of infected samples were indicated the interference between the fungal components and crop constituents and depending on the fungal genus. In the same way a gradual loss of carbohydrate and a small increase in protein contents were recorded by Bhattacharya and Raha,(2000) in both maize (starchy) , groundnut (oily) and soybean seeds (proteinaceous) during storage

Rheological properties of infected corn dough

Dough were made from the infected corn samples with either *A. flavus* or *F.moniliforme* .The rheological properties were studied using viscoamylograph tests to study the effect of the fungal growth on some properties with special references of viscosity. The obtained results (Table 4) indicated that the growth of *A. flavus* or *F.moniliforme* led to increases of maximum and minimum viscosity compared with control samples (non-infected). These increases may be due to the severe changes of chemical components of contaminated flour samples (i.e increase in protein content...) as resulting of the fungal growth. These reasons lead to decrease in gelation temperature (70.5 ^{o}C) and the temperature at maximum viscosity) for contaminated dough depended on the fungal species since, the contaminated dough with *A. flavus* showed an increases in maximum and minimum viscosity. The changes in physiochemical properties had affect on the tendency to absorb water or hydrophoisty and affect on gelling and thickening function in food systems.The results in our investigation are in accordance with those reported by Bekheit, (1993) who stated that the increase in flour protein content lead to increase in rhiological properties .

Table (4): Changes in Rheological Properties of infected * corn grains induced by *A. flavus* and *F. moniliforme*

Treatments	Gelation temp. [° C]	Minimum viscosity [B. U.]	Temp. at max. viscosity [° C]	Maximum viscosity [B. U.]
Control	73.5	1095	95.5	1110
Contaminated with *A. flavus*	70.5	1620	91.5	1720
Contaminated with *F. moniliforme*	70.5	1440	88.5	1700

* Moisture content =14%
Values represent the means of three replications.

CONCLUSION

In conclusion, both fungal and mycotoxin contamination are a primary concern in the effort to reduce economic losses and minimize potential risks to human and animal health. Moreover, the growth of these fungi on corn grains affected the chemical composition. The storage of corn is the critical point lead to contaminant corn with aflatoxin and fumonisin since storage fungi can infect corn grains at pre-harvest stage . Therefore, adequate postharvest management is advantageous for assuring the quality of stored grains.

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تقييم مخاطر تواجد بعض ا لفطريات المفرزة للسموم على الذره الملقحه : مع التمثيل بالتغير فى التركيب الكيماوي والصفات الريولوجيه

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يحتل الذره اهميه كبيره نظرا لدخوله فى العديد من الصناعات الغذائية كذلك لاستعماله كغذاء للحيوان- تعتبر الفطريات من اهم الملوثات التى تصيب الذر أثناء الزراعة و الحصاد و التخزين و خاصة الفطريات المفرزه للسموم الفطريه وتسبب التلوث فقد فى المحصول وانخفاض فى الجودة هدفت الدر اسه تحديد دور كل من الفطرين الأكثر انتشارا فى تدهور الذره المصاب أثناء التخزين وبصفه خاصة الفطريات المفرزه للسموم الفطريه.

تم تجميع عدد خمسين عينة من الذرة حديثه الحصاد والتجفيف والتخزين وقدر تركيز الأفلاتوكسين و الفيومونزين.

وقدرت نسبة الاصابه بالفطريات وعدد الفطريات المصاحبة- وتم تعريف الفطريات كذلك قدرت قدرة العزلات من جنس اسبر جلس فلافس و والفيوزاريوم مونيليفورم على إفراز الافلاتوكسين والفيومونزين.

دلت النتائج على أن نسبة الأصابه بالفطريات٧٤% و وبتعريف الفطريات لوحظ تواجد اسبرجلس فلافس بنسبة ٤٦% يليها اسبرجلس نيجر والفيوزاريوم مونيليفورم وقد تراوحت نسبة تواجدهما ٢٠% كذلك تم عزل اسبرجلس سيدوى بنسبه مخفضه.

درست مدى قدرة العزلات على افراز السموم وقد وجد أن ٢٥% من عزلات الأسبرجلس فلافس و ٢٠% من فيوزاريوم مونيليفورم كان لها قدره على إفراز السموم الفطرية .كانت التركيزات المفرزه من الأفلاتوكسين ١٠ –١٠٠ ميكروجرام /كيلوجرام و من الفيومونزين ٨،٦ –٢٨٠ ميكروجرام /كجم . دلت النتائج ان نسبة٢٢% من العينات كانت ملوثة بالأفلاتوكسين B1تتراوح التركيزات بين ١٠, ١٥٠ ميكروجرام /كجم الأفلاتوكسين. بينما ٣٠%من العينات كانت ملوثة بالفيوموزين وصل اعلى تركيز الى٣١٠ ميكروجرام/كجم وكانت نسبه العينات التى تواجد فيها الفيومونزين والأفلاتوكسين معا ١٢% داخل حدود التركيزات السابقه . وان٤٨%من العينات كانت خاليه من الأفلاتوكسين و الفيومونزين .

تم حقن الذره بأحد الفطريات المفرزه للأفلاتوكسين وأحد الفطريات المفرزه للفيومونزين كلا على حده للحصول على ذره ملوثة بتركيز مرتفع من السموم لأجراء التحاليل الكيمائيه والاستدلال على التغيرات فى التركيب الكيماني للذره الناتجه عن نمو الفطريات فى الذرة.

تم تسجيل زياده فى السكريات الكليه بمقدار ٢٥،٦ % بالنسبه للعينات الملوثه بفطر الأسبرجلس فلافس ، ١٠,٢٤ % للعينات الملوثه بفطرفيوزاريوم مونيليفورم.كذلك حدث زيادة فى السكريات المختزله و الغير مختزله. وصلت الزيادة للبروتين الى ٧،٣٤ % ، ١٧،١٢ % والألياف زادت فى حدود ٩،٧ % و ١٣،٠٣ % فى العينات الملوثه بالأسبرجلس فلافس وفيوزاريوم مونيليفورم على التوالى وعلى العكس من ذلك لوحظ انخفاض فى نسبة النشا فى حدود ١،٥٤ % . تم قياس التغير فى بعض الصفات الريولوجيه للعجين المصنع من الدقيق الملوث صناعيا السابق تجهيزه بالفطر. وقد لوحظ ارتفاع اللزوجه وكذلك انخفاض الحرارة عند اقصى لزوجه وأقل لزوجه بالمقارنة بعينات الكنترول (المصنعة من الدقيق الغير ملوث) .نستدل من هذه الدراسه على اختلاف التأثير الحيوي لكلا الفطرين ا ثناء النمو على الذرة -كذلك أوضح أهمية تخزين الذرة فيبيئه تمنع نمو الفطريات اذ ان الذره وان كان فى بعض الأحيان خاليا من السموم الفطرية ولكنه فى اغلب الأحيان يكون ملوثا بواحد أو أكثر من الفطريات المفرزه للسموم الفطرية والتى تنشط سريعا عند توافر الظروف المناسبة من حرارة ورطوبة.

وبالتالي يمكن القول إن اخطر مرحلة يمكن ان يصاب بها الذرة او تلوث هي مرحلة التخزين و ما يتعلق بظروف من حرارة و رطوبة وتهوية كما دلت الدراسة الأبحاث السابقة وانة مكمن الخطورة فى الذرة المخزنة هى تواجد السموم الفطرية وليس التغير فى التركيب الكيماوي وانما هو فى الدرجة التالية فى الاهتمام حيث يغير من صفات العجين اثناء التصنيع كما انه قد يتغير تلك الصفات من فطريات غير مفرزه لسموم كذلك فان تغير التركيب الكيماوىلا يحدد نوع التو كسين وتركيزة .