

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

Soher E. Aly* and A. S. Nadir**

* Food Toxicology and Contaminants Dept., National Research Center

** Food Technology, Dep., National Research Center, Dokki, Cairo-Egypt

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 (FB₁) 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 B₁ (afl B₁) was detected in 22% of corn samples at a mean level of 30 µg /Kg. Fifteen samples (30%) were contaminated with Fumonisin B₁ (FB₁) at a mean level of 180 µg /Kg. Both afl B₁ and FB₁ 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 FB₁ 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 °C) were decreased compared with the control (73.5 °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

Corresponding to Dr. Soher E. Aly Food Toxicology and Contaminants Dep., National Research Center, Dokki, Cairo, Egypt Fax: 202-337-0931. Emailaddress: Soheribrahim@hotmail.com

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). Fumonisin 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 moniliforme*) , the occurrence of molds on corn and investigate the ability of isolated fungi to produce aflatoxin B₁ and fumonisin B₁. 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 B₁ and fumonisin B₁ 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 B₁ and FB₁ (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 X 10⁵ 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°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, redissolved in 2ml of methanol and re-evaporated to dryness under a nitrogen stream

Derivatization and HPLC analysis

The purified samples was dissolved in 200 ml methanol, 50µl of sample solution derivatized 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 derivatized solution was injected into the HPLC system. The reversed phase column was a spherisorb ODS-2 10µm (25x 0.4cm) 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 fluorescence 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 B₁ 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, 4µ), Mobile phase A: Acetonitrile: H₂O, 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-ratio 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) followed 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*, *Penicillium 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. proliferatum* (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 common 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 fumonisin 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 AfB ₁ +FB ₁ | 6 | 12 | 45 + 160 | 10-80/ 50-280 |
| Uncontaminated | 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 B₁ 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 B₁ at a mean level of 180µg /Kg (maximum 310µg/Kg). Table (1) also showed that both Afl B₁ and FB₁ were Co-occurrence in 6 (12%) samples. These levels were lower than those reported by Ali *et. al.*, (1998), they detected Afl B₁ in 69% of samples and FB₁ in all samples at mean level 895µg /Kg .Also Castell *et. al.*, (1999) were detected FB₁ in 79% 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 free 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 determine the ability of isolates for producing AflB₁ or FB₁, respectively. Data in Table (2) showed that 25% and 20% of the isolates have the ability of producing AflB₁ in amount ranged between 10 to 100 µg/kg and FB₁ level 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 B₁ 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 especially 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 FB₁ production

| Species | No. of isolates | No. of tested isolates | No. of positive isolates | Range [µg/kg] |
|-----------------------|-----------------|------------------------|--------------------------|-------------------------|
| <i>A. flavus</i> | 54 | 20 | 5 (25%) | 10-100 (mean =(55) |
| <i>F. moniliforme</i> | 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 B₁. 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 \pm SE)

| Treatments | Total Sugar | Reducing Sugar | Non R. sugar | Starch | Protein | Lipids | Fiber | Ash |
|--------------------------------------|--|--|--|--|--|---|--|---|
| | g/100 (%) | g/100 (%) | g/100 (%) | g/100 (%) | g/100 (%) | g/100 (%) | g/100 (%) | g/100 (%) |
| Control | 3.71 \pm 0.02 ^a (0.0) | 1.88 \pm 0.08 ^a (0.0) | 1.83 \pm 0.13 ^a (0.0) | 72.72 \pm 0.01 ^a (0.0) | 6.68 \pm 0.09 ^a (0.0) | 6.29 \pm 0.13 ^a (0.0) | 3.3 \pm 0.01 ^a (0.0) | 1.08 \pm 0.06 ^a (0.0) |
| Contaminated with <i>A. flavus</i> | 4.86 \pm 0.12 ^b (+25.60) | 2.28 \pm 0.10 ^b (+21.30) | 2.38 \pm 0.07 ^b (+30.10) | 72.16 \pm 0.03 ^a (-0.77) | 7.17 \pm 0.01 ^b (+7.34) | 6.09 \pm 0.00 ^b (-3.18) | 3.62 \pm 0.06 ^b (+9.70) | 0.88 \pm 0.04 ^b (-17.6) |
| Contamin. With <i>F. moniliforme</i> | 4.09 \pm 0.09 ^c (+10.24) | 2.04 \pm 0.12 ^c (+8.51) | 2.05 \pm 0.13 ^c (+12.80) | 71.60 \pm 0.04 ^a (-1.54) | 7.83 \pm 0.06 ^c (+17.22) | 6.14 \pm 0.09 ^c (-2.38) | 3.73 \pm 0.12 ^b (+13.03) | 1.04 \pm 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 °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 rheological 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 <i>A. flavus</i> | 70.5 | 1620 | 91.5 | 1720 |
| Contaminated with <i>F. moniliforme</i> | 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.

REFERENCES

- A.A.C.C. (1983). American Association of cereal chemists, cereal laboratory methods, 8th Ed., St. Poul, Minnesota.
- Ali, N. Sardjono ; A. Yamashita and T. Yoshizawa (1998). Natural co-occurrence Aflatoxin B1 and Fusarium mycotoxins (Fumonisin, Deoxynivalenol , nivalenol and Zearalenone) in corn from Indonesia . *Food Additives and Contaminants* ,13: 5337-5338.
- A.O.A.C. (1995). Official Methods of Analysis, 14th Ed., Association of Official Analytical Chemists, Washington, DC.
- Aired, C.E. and O.F. Esuruoso (1987). deterioration of shelled oil palm kernel caused by seed born fungi. *J sci. Food Agric.*, 40: 293-304.
- Atalla, M.M., N.M. Hassanein; A.A. El-Beih and Y.A. Youssef (2003). Mycotoxin production in wheat grains by different *Aspergilli* in relation to different relative humidities and storage periods. *Nahrung.Food.*, 47 : 6-10
- Aziz, N.H.; S.A. El-Zeany and L.A. Mousa (2002). Influence of gamma-irradiation and maize lipids on the production of aflatoxin B1 by *Aspergillus flavus*. *Nahrung*, 46:3277-331
- Bacon ,C.W. and P.E. Nelson (1994). Fumonisin production in corn by toxigenic strains of *F.moniliforme* and *F. proliferatum*. *J Food Prot.* 57,514-521.
- Bekheit, M.A. (1993) . Utilazation of Rice germ flour in biscuit manufacture. *Egypt J. Appl. Sci.* 8, 382-355.
- Bhattacharya, K, and S. Raha (2001). Deteriorative changes of maize ,groundnut and soybean seeds by fungi in storage. *Mycopathologia*.155 :135-141.
- Boyacioglu, D. and N.S. Hettiarachchy (1995). Changes in biochemical components of wheat grain that was infected with *Fusarium graminearum*. *Journal of cereal science* 21, 57-62.
- Brown, R.L.; Z. Y. Chen; T.E. Cleveland and J. S. Russin (1995) .Advances in the development of host resistance in corn to aflatoxin contaminated . *Phytopathology* 89, 113-117.
- Castella, G.; M.R. Bragulat and F.J. Cabanes (1999) Surveillance of Fumonisin in maize-based feeds and cereals from Spain. *J. Agric . Food Chem.*, 47:4707-4710.

- Castella, G.; M.R. Bragulat and F.J. Cabanes (1999) Fumonisin production by *Fusarium* species isolated from cereals and feeds in Spain. *J Food protection*, 62:811-813.
- Castelo, M.M.; S.S. Sumner and L.B. Bullerman (1998). Occurrence of fumonisin in corn and corn based food products. *J food prot.*, 61 :704-707
- Clavero, R.S., Y.C. Hung; L.R. Beuchat and T. Nakayama (1992.) Separation of aflatoxin contaminated kernels from sound kernel by hydrogen peroxide treatment. *J Food prot.*, 56:130-133
- Farag, R.S.; Mohsen, S.M.; F.A. Khalil and A. E. Basyony (1985). Effect of fungi infection on the chemical composition of wheat soybean and sesam seeds. *Aceites*, 36: 357-361.
- Girolamo, A.; M. Solfrizzo and A. Visconti (2001). Effect of processing fumonisin concentration in corn flakes. *J. Food prot.*, 64: 701-705.
- Hamilton, R.M.G. and H. L. Trenholm (1984). The chemical and nutritive content of wheat winter and spring wheat contaminated with deoxynivalenol (Vomitoxin). *Animal Feed Science. Technology*, 11: 293-300.
- Hirooka, E.Y.; M.M. Yamaguchi; S. Aoyama; Y. Sugiura and Y. Ueno (1996). The natural occurrence of fumonisins in Brazilian Corn kernels. *Food Additives and Contaminants* 13,173-183.
- Ichinoe, M.; H. Kurata; Y. Sugiura and Y. Ueno (1983). Chemotaxonomy of *Gibberella Zeae* with special reference to production of Trichothecenes and Zearalenone. *Applied and Environmental Microbiology*, 46: 1364-1369.
- Jintian, F.; S.R. Poul and E.J. Pinthus (1997). Physicochemical changes in starch during deep-fat frying of a molded corn starch patty. *J. food processing and preservation*, 21: 443-460.
- Katta, S.K. A.E. Cagampang; L.S. Jackson and L.B. Bullerman (1997). Distribution of *Fusarium* molds and Fumonisin in dry milled corn fractions. *Cereal Chemistry*, 74, 858-863.
- Kpodo, K.; A.K. Sorensen and M. Jakson (1995). The occurrence of mycotoxins in fermented maize products. *Food Chemistry*, 56: 147-153.
- Lacey, J.; Ramakrishna, N.; Hamer, A.; Magan N. and Marfleet, I.C. (1991). Grain fungi. In *Handbook of Applied Microbiology Vol. II, Food and Feed*, (D.K. Arora, K.G. Mukerji and E.H. Marth, eds.) pp. 121-127, Marcel Dekker, New York.
- Madhyastha, S.; R.r. Marquardt and A. David (1993). Effect of ochratoxin producing fungi on the chemical composition of wheat and barley. *Journal of Food quality*, 16: 87-299.
- Medina, M.M. S. and A. J. Martinez (2000). Mold occurrence and Aflatoxin B1 and Fumonisin B1 determination in corn samples in Venezuela. *J.Agric Food Chem.*, 47: 2833-2836.
- Mislivec, P.B. (1977). Direct plate versus, dilution plating in qualitatively determining the mold flora of dried beans and soy beans. *J.A.O.A.C.*, 60 : 741 - 743.

- Nelson, P. E.; T.A. Toussoun and W. F. O Marasas (1983). *Fusarium* species. An Illustrated manual for identification. Published by The Pennsylvania State University press, University park and London., P. 193.
- Ono, E.Y.; E.Y. Sasaki; E.H. Hashimoto; L. N. Hara; B. Correa; E. N. Itano; T. Sugiura; Y. Ueno and E.Y. Hirooka (2002). Post-harvest storage of corn : effect of beginning moisture content on mycoflora and fumonisin contamination. *Food Additive and Contaminant*, 19 :1081-1090
- Raper, K.B. and D.I. Fennel (1965). " *The Genus Aspergillus* " Williams and Wilkins, Co., Baltimore
- Riley, R. T.; E. Wang; J. J. Schroeder; E. R. Smith; R. D. Plattner; H. Abbas; S. Yoo H. and A. H. Jr. Merrill (1996). Evidence for sphingolipid metabolism as a contributing factor in the toxicity and carcinogenicity of fumonisins. *Nat. Toxins*, 4:3-15.
- Rottinghaus, G. E.; Coatney, C. E. and Minor, H.C. (1992). A rapid, sensitive thin layer chromatography procedure for detection of Fumonisin B₁ and B₂. *J. Vet. Invest* 4, 326-329.
- Sahab, A. F.; E. S. Aly; H. A. Amraand; M. I. Mohamed (1999). Factors influencing activity of *Fusarium moniliforme* growth and Fumonisin production on some corn cultivars during storage. *J. Agric. Mansoura Unive*, 5: 2619-2628.
- SAS Institute Inc. (1982) S A S User s guide; tatistics. nstitute, Cary, N.C.
- Shephard, G.S.; E.W. Sydenham; P.G. Thiel; and W.C.A. Gelderblom (1990). Quantitative determination of Fumonisin B₁ and B₂ by High Performance Liquid Chromatography with fluorescence detection . *Liquid Chromatography*, 13: 2077-2087.
- Sydenham, E.W.; Thiel, P.G.; Marasas ,W.F.O.; Shephard, G.S.; Van Schalkwyk, D.J. and Koch, K. R. (1990). Natural occurrence of some *Fusarium* mycotoxins in corn from low and high esophageal cancer prevalence areas of the Traskei. *J. Agric. and Food Chem.* 38, 1900-1903.
- Waller, R. A. and Duncan, D. B. (1969). A Bayes rule for the symmetric multiple comparison problems. *J. Am. Stat. Assoc.* 64, 1484-1503.
- Yoshizawa, T.; Yamashita, A, and Chokethaworn ,N. (1996). Occurrence of Fumonisins and Aflatoxin in corn from Thailand. *Food Additives and Contaminants* 13, 163-168
- Ueno, Y., (2000). Risk of Multi-exposure to natural toxins. *Mycotoxins* 50, 13-22

تقييم مخاطر تواجد بعض الفطريات المفرزة للسموم على الذرة الملقحة : مع التمثيل بالتغير في التركيب الكيماوي والصفات الريولوجية

سهير السيد على* - عبد العزيز ندير**
* قسم سموم وملوثات الغذاء، المركز القومي للبحوث
** قسم تكنولوجيا الأغذية، المركز القومي للبحوث

يحتل الذرة أهمية كبيرة نظرا لتحويله في العديد من الصناعات الغذائية كذلك لاستعماله كغذاء للحيوان - تعتبر الفطريات من أهم الملوثات التي تصيب الذرة أثناء الزراعة والحصاد والتخزين وخاصة الفطريات المفرزة للسموم الفطرية وتسبب التلوث فقد في المحصول وانخفاض في الجودة هدفنا بدراسة تحديد دور كل من الفطرين الأكثر انتشارا في ذهور الذرة المصاب أثناء التخزين وبصفه خاصة الفطريات المفرزة للسموم الفطرية.

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 كمدرت قدرة العزلات من جنس اسبرجلس فلافس و الفيوزاريوم مونيليفورم على إفرار الأفلاتوكسين والفيومونزين.

دللت النتائج على أن نسبة الاصابه بالفطريات ٧٤% و بتعريف الفطريات لوحظ تواجد اسبرجلس فلافس بنسبة ٤٦% يليها اسبرجلس نيجر و الفيوزاريوم مونيليفورم وقد تراوحت نسبة تواجدهما ٢٠% كذلك تم عزل اسبرجلس سيدري بنسبه مخفضه.

درست مدى قدرة العزلات على إفرار السموم وقد وجد أن ٢٥% من عزلات الأسبرجلس فلافس و ٢٠% من فيوزاريوم مونيليفورم كان لها قدره على إفرار السموم الفطرية . كانت التركيزات المفترزه من الأفلاتوكسين ١٠ - ١٠٠ ميكروجرام /كيلوجرام و من الفيومونزين ٨.٦ - ٢٨٠ ميكروجرام /كجم . دللت النتائج ان نسبة ٢٢% من العينات كانت ملوثة بالأفلاتوكسين 1 بتتراوح التركيزات بين ١٠٠ ، ١٠٠ ميكروجرام /كجم الأفلاتوكسين . بينما ٣٠% من العينات كانت ملوثة بالفيومونزين وصل اعلى تركيز إلى ٢١٠ ميكروجرام /كجم وكانت نسبة العينات التي تواجد فيها الفيومونزين والأفلاتوكسين معا ١٢% داخل حدود التركيزات السابقه . وان ٤٨% من العينات كانت خاليه من الأفلاتوكسين و الفيومونزين .

تم حقن الذرة بأحد الفطريات المفرزة للأفلاتوكسين وأحد الفطريات المفرزة للفيومونزين كلا على حده للحصول على ذرة ملوثة بتركيز مرتفع من السموم لأجراء التحليل الكيماوي والاستدلال على التغيرات في التركيب الكيماوي للذرة الناتجه عن نمو الفطريات في الذرة.

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

وبالتالي يمكن القول إن اخطر مرحلة يمكن ان يصاب بها الذرة أو تلوث هي مرحلة التخزين و ما يتعلق بظروف من حرارة و رطوبة وتهوية كما دللت الدراسة الأبحاث السابقة وانه ممكن الخطورة في الذرة المخزنة هي تواجد السموم الفطرية وليس التغير في التركيب الكيماوي وانما هو في الدرجة التالية في الاهتمام حيث ينير من صفات العجين أثناء التصنيع كما انه قد يتغير تلك الصفات من فطريات غير مفترزه لسموم كذلك فان تغير التركيب الكيماوي لا يحدد نوع التوكسين وتركيزه .