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Identification of Fungal Genus and Detection of Aflatoxin Level in Second Crop Corn Grain

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Abstract: This study was carried out to determine post harvest mycoflora and establish the presence of aflatoxin in second crop corn after harvest in Kahramanmaras, Turkey. During the study, total of thirty corn samples were collected from Turkoglu, Pazarcik, Narli countries in 2005-2006 growing season. Most of the samples tested in this study came from neighboring villages to Kahramanmaras and from Turkoglu county. Mold counts per sample were ranged from 1×10^5-1×10^7. Significantly higher number of mold counts (1×10^7) were obtained in a sample came from Turkoglu. Through, this study, incidence of *Penicillum* sp. was significantly higher than *Fusarium* and *Aspergillus* sp. total of 28 corn samples were tested for the presence of aflatoxin B1. Of the samples, 12 were >5 ppb. Aflatoxin levels were ranged from 7.70-108.86 ppb. Of total 28 samples, 9 were >9 ppb for total aflatoxin although, total aflatoxin levels were ranged from 14.03-116.72. In summary, 43% of samples were contaminated with aflatoxin B1 although, 32% of samples were contaminated with total aflatoxin.

Key words: Fusarium, penicillium, aspergillus, aflatoxin, corn, HPLC

INTRODUCTION

Corn was introduced to Europe after discovered by Columbus's men on March 6, 1492 on the island of Cuba (Jeffers, 2004). It is commonly grown in southern part of Kahramanmaras region which is located on eastern Mediterranean. Corn diseases cause a reduction in quantity and quality of grain harvested. Losses due to diseases vary between 2 and 20% a year. Fungi cause numerous corn diseases and post harvest spoilage, which results in production of toxic compounds called mycotoxin (Seo and Yu, 2005). Mycotoxins are commonly produced by fungi belong to the genus *Aspergillus*, *Fusarium* and *Penicillium*. The most commonly found mycotoxins in corn are aflatoxin, fumonosin, Deoxynivalenol (DON). Aflatoxin, the most known mycotoxin, is produced by the fungus *Aspergillus flavus*.

Aspergillus flavus is a saprophytic fungus and over winters in crop residues such as corn stalks and cobs (Jaime-Garcia and Cotty, 2004). Fungus produces microscopic spores on corn residue and in survival bodies at soil surface. Spores blown on the silk germinate and colonize the silk during hot and humid conditions. Fungal hyphae can grow down to the silk channel. Pollinated, yellow-brown silks are more susceptible to fungal colonization and invasion than fresh unpolinated silks. Once, fungus establishes under husk, it may infect injured kernels when the plant is stressed by drought during dough stage.

Aflatoxin production by the fungus is triggered by drought and high temperature during grain fill. Nitrogen deficiency, excessive plant population, poor root development and insect damage of kernels may also induce aflatoxin production in the field. When, the weather conditions are favorable to the fungus, the fungus may produce aflatoxins at any stages of production and transformations.

Corn grain as energy source is widely used in both human and animal nutrition such as cows, sheep, goat, poultry and fish etc. The nutritive value of corn grain depends on the nutrient contents and digestibility. However, the anti-nutritive factors in corn grain such as aflatoxin content due to improper storage condition or climatic conditions at harvest limits their utilization in human and animal diets. The low level of aflatoxin may affect the animal performance. Therefore, it is very important to determine the alflatoxin level of corn grain before inclusion into diets of human and animal diets to prevent the deleterious effect on the health of human and livestock.

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The aim of the current study was to determine the fungal genus and aflatoxin contents of corn kernels and relationship among the relative humidity, mold count and aflatoxin levels of second crop corn kernels.

MATERIALS AND METHODS

Sampling: Corn samples were obtained by a probe (170×2.5 cm) from loaded trucks came to local bins. The samples were placed into a paper bag and labeled. Relative humidity (%) for each sample was recorded. Samples were brought to the laboratory and stored. Each sample was milled to pass through a no. 20 sieve. A 50 g of sub sample was taken for analysis.

Isolation of mycoflora and evaluation: Ten grams of sub sample from each sample was taken and placed into 250 mL flask that contains 90 mL sterile physiological salt solutions (1 g casein + 8.5 g NaCl and 1 L ddH_2O) to obtain a 10^{-1} dilution. Flasks were shaked for 1 min and 1 mL suspension was removed with a pipette and poured into the tubes with 9 mL sterile physiological salt solutions. From this initial suspension, serial dilutions were prepared (10^{-2}-10^{-6}). One milliliter of suspension from the last dilution (10^{-6}) was pipetted and added onto pre-poured PDA in 9 cm Petri dish. The inoculums were spreaded onto PDA medium via a glass spreader. The inoculated dishes were incubated at 25°C. The typical colonies of fungi developed on the surface of medium was counted and recorded. Fungal colonies were allowed to form spores and colors. Fungal genus was identified according to Samson and Hoekstra (1988). Colony growth was monitored in Petri dishes and total fungal colonies recorded at the end of 7 days.

Extraction and clean-up of corn samples: Fifty grams from each finely milled corn samples were homogenized in a blender with 100 mL methanol: water (80:20) and 5 g NaCl for 1 min. The mixture was filtered through a Whatman No 4 filter paper. A 10 mL aliquot was pipetted in a 100 mL beaker and volume was brought to 50 mL with distilled water. The filtrate was passed through a micro fiber paper. Ten mL of this filtrate was applied to an immunoaffinity column (Aflates P® Vicam). After sample passage was completed, the column was washed twice with 10 mL distilled water. Then, 1 mL methanol was added to the column to elute aflatoxins bound to monoclonal antibodies and extracted aflatoxins was collected in a 25 mL sintilation vials.

Analysis of AFB1, AFB2, AFG1 and AFG2 by HPLC: Determination of AFB1, AFB2, AFG1 and AFG2 levels in standards and the derivatized samples were carried out by HPLC equiped with a Dionex P680 HPLC Pump, Dionex ASI-100 Automated Sample Injector, Dionex RF 2000 Fluorescence Detector (FLD), Dionex Thermostatted Column Compartment TCC-100, HPLC colomn (C18 -250 mm-5 μm- 4.6 mm) and a Coputer with a software (Packard Bell, Chromeleon). The mobile phase consists of deionized water:acetonitrile: methanol (55:20:30, v/v/v). The flow rate was 1 mL min^{-1} and the injection volume was 100 μL.

Statistical analysis: A simple correlation analysis was used to establish the relationship between relative humidity, mold count, B1 and total aflatoxins contents.

RESULTS AND DISCUSSION

The relative humidity, mould counts and aflatoxin contents of corn grain from different sites in Kahramanmaras, Turkey was given in Table 1.

Total of 30 corn samples came from different counties in Kahramanmaras region were tested for fungal flora and aflatoxin levels. During this study, most of the samples were obtained from villages in Kahramanmaras and Turkoglu county. Colony forming units (cfu g^{-1}) per sample was changed from 5×10^{5}-5.2×10^{7}. Incidence of *Penicillium* sp. was significantly higher than Fusarium and Aspergillus.

Aflatoxin B1 was detected in 21 out of 30 samples tested. Thus, 72.4% of samples were aflatoxin B1 positive. Aflatoxin B1 concentration was ranged from 0.63-108.86 ppb. Of aflatoxin B1 positive samples, twelve were >5 ppb. For total aflatoxin (B1 + B2 + G1 + G2), 64.29% was positive. Total aflatoxin level varied between 0.62 and 116.72 μg Kg^{-1}. Of 30 samples, however, 9 samples were >10 ppb.

Of the samples, 43% was contaminated with aflatoxin B1 above the limit (>5 ppb) although 32% had total aflatoxins >10 ppb.

Mycotoxins are secondary methabolic compounds produced by fungi such as *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* and *Claviceps* some of which are very common on corn. Mycotoxin production by fungi is induced by insect hazards, relative humidity and high temperature. Aflatoxin level was varied between 0 and 53 ppb in second crop corn in Osmaniye, 100 km away from our sampling locations. Although, 30 of 37 samples were positive with aflatoxin, only 11 samples had aflatoxin level above the limit (10 ppb) (Tatli and Ozdemir, 2005). The result obtained in the current study was consistent with finding of Tatli and Ozdemir (2005). In the current study, of 30 samples, only 9 had >10 ppb total aflatoxin.

Table 1: The relative humidity, mould counts and aflatoxin contents of corn grain from different sites in Kahramanmaras, Turkey

Site 1	Site 2	RH	MC	B1	B1+B2 + G1+G2
Turkoglu	Karacasu	27	5×10^6	2.16	2.16
Turkoglu	Karacasu	25	2×10^6	12.68	19.95
K.Maras	Serefoglu	22	4×10^6	<0.2	<0.5
K.Maras	Serefoglu	21	1×10^6	22.09	24.46
K.Maras	Serefoglu	22	1×10^6	0.31	<0.5
Turkoglu	Merkez	21	1.5×10^6	<0.2	<0.5
Turkoglu	Merkez	23	1.5×10^6	<0.2	<0.5
Turkoglu	Merkez	22	1.5×10^6	<0.2	<0.5
K.Maras	Abbaslar	27	4.5×10^6	<0.2	<0.5
K.Maras	Abbaslar	27	5×10^6	8.49	8.93
K.Maras	Abbaslar	27	1.7×10^6	15.32	16.20
Turkoglu	Arapli	23	7.5×10^6	0.63	0.63
Turkoglu	Arapli	26	1.3×10^6	3.60	8.17
Turkoglu	Arapli	23	1.5×10^6	<0.2	<0.5
Turkoglu	Yenikoy	20	1.5×10^6	<0.2	<0.5
K.Maras	Fatihler	22	2×10^6	12.30	14.03
K.Maras	Fatihler	27	3.5×10^6	2.05	2.18
K.Maras	Serefoglu	24	2.5×10^6	<0.2	<0.5
K.Maras	Serefoglu	25	2.5×10^6	92.90	100.70
Turkoglu	Arapli	28	5×10^6	0.60	0.62
Turkoglu	Yenikoy	28	1.1×10^6	98.24	112.09
Turkoglu	Cakallicullu	27	9×10^6	7.70	8.20
Turkoglu	Yenikoy	27	3.5×10^6	20.60	46.98
Turkoglu	Arapli	30	7×10^6	3.55	3.73
Turkoglu	Yenikoy	29	3×10^6	29.92	33.97
K.Maras	H.Mustafa	27	1.2×10^6	108.86	116.72
Turkoglu	Arapli	26	1.3×10^6	3.60	8.17
Turkoglu	Yenikoy	28	1×10^6	0.63	0.63
K.Maras	Sivricehoyuk	30	1×10^6	ND	ND
Turkoglu	Merkez	29	5.2×10^6	ND	ND

RH: Relative Humidity (%), MC: Mold Count (cfu g^{-1}), B1: Aflatoxin B1, ND: Not Detected

Table 2: The correlation coefficients (r) of relationship among mold count, relative humidity and aflatoxin contents of second crop corn kernels

Parameters	RH	Mold count	B1
Mold count	0.378^{NS}	-	-
B1	0.258^{NS}	-0.249^{NS}	-
B1+B2+ G1+G2	0.274^{NS}	-0.252^{NS}	0.990 ***

***p<0.001, NS: Non-Significant (p>0.05)

The correlation coefficients (r) of relationship among mold count, relative humidity and aflatoxin contents of second crop corn kernels are given in Table 2.

As can be shown in Table 2 there is no significant (p>0.05) relationship among RH and Mould count, B1 and total aflatoxin however there is significant (p<0.001) correlation between B1 and total aflatoxin contents of corn grains.

Physiological maturity of corn starts, when the grain moisture content reaches about 31-33%. Dry matter does not increase after this stage. Corn should be harvested at moisture contents below 28% because harvesting at higher than this moisture contents may cause significant damage to the grain. In order to obtain high quality corn, harvest moistures must be as low as 20-22%. Invasion of corn by *Aspergillus flavus* is prevented at moisture content below 13%. In the current study, moisture contents of corn samples ranged from 21-30%. Therefore, second crop corn must be dried until its moisture contents drops to 13% in bins before storage. Optimum growth of *Aspergillus flavus* occurs on corn at 18% moisture. Fungus growth elevates respiration that release heat and moisture into the surrounding environment in the grain mass. Increased moisture content and temperature of the surrounding corn result in a hot spot. In order to minimize risk of mycotoxin contamination in corn, early planting, irrigating to minimize drought stress, early harvesting, avoiding kernel damage and drying and storing at moisture content 13% or less are practices that should be followed.

CONCLUSION

The current study clearly showed that there is considerable variation among the growing sites of corn in terms of mold count, relative humidity and aflatoxin contents. The total aflatoxin content of corn grain can be estimated from Aflatoxin B1 due to significantly higher correlation between B1 and total aflatoxin contents.

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