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DETECTION OF MYCOTOXINS IN MAIZE SEED SAMPLES

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Abstract

Present results describe the detection of mycotoxins in samples of maize seed collected from different localities of Pakistan viz., Karachi, Hyderabad, NawabShah, Sukhur, Lahore, Quetta, Peshawar and Islamabad. Seed samples were analyzed quantitavely by competitive Direct Enzyme Linked Immunosorbent Assay technique (CD-ELISA). Out of fifty nine samples tested, 50 samples were found to be contaminated with aflatoxin whereas 43 samples contained zearalenone and ochratoxin was detected from 4 seed samples. The amount of aflatoxin was detected in high quantity from five samples, while zearalenone was also detected in highest quantity from six samples and ochratoxin detected only in four samples in low quantity.

Introduction

Maize (*Zea mays* L.) is the important cereal crop after wheat and rice. It is grown on 0.9355 metric hec annually with an average yield of 1857 kg/hec and production of 1.7371 metric tones (Anon., 2005). Maize grain is important for the production of oil, starch and glucose (Krishnamurthi, 1969). Niaz & Dawar (2009) reported that about 70 % samples of maize seed were found to be infested with *Aspergillus* and *Penicillium* spp. Mycotoxins are naturally occurring secondary metabolites produced by several fungi on variety of substrates. More than 300 fungal species are known to produce mycotoxins with variable toxic affects (Anon., 1991). The toxins can be produced in the growing crop and during storage. Animals may die or suffer from growth and reproductive problems following ingestion of mycotoxins. Mycotoxin has also been implicated in non-specific illnesses in the farm animals. Considerable economic losses are attributed to reduced crop yield and grain quality following fungal contamination. Most of the fungi like *Aspergillus, Penicillium, Fusarium, Alternaria* and *Claviceps* produced secondary metabolites on a variety of substrates (Adebajo & Diyaolu, 2003). It may also affect food products used for human consumption. Improper control of moisture content and temperature are the main factors responsible for mycotoxin production (Styriak *et al*., 1998).

Many of the food stuffs spoiled as a result of bad harvesting practices and storage conditions, most of them are due to the growth of toxin producing fungi (Ramesh & Siruguri, 2003). Many crops including peanuts, corn, coffee beans, spices, herbs, barley, wheat, raw or processed fruits and vegetables are highly susceptible for the mycotoxins contamination (Candlish *et al.,* 2000*;* Ramakrishana *et al.,* 1990; Gilbert & Smiley 1995; Desjardins *et al.,* 2000; Milanez *et al.,* 1995; Ramesh & Siruguri, 2003; Fezekas *et al.,* 2005; Kabelitz & Siever, 2004; Giryn & Szteke, 1995). Forgacs (1972) observed that *Fusarium* species caused abnormalities after World War II. Worldwide scientific recognition of mycotoxins problem was, however, only in 1960 when it was discovered that the aflatoxins were responsible for the death of about 100,000 turkey poultry (Turkey X disease) in England (Blount, 1961). Due to the presence of different mycotoxins in human edible crops, different adverse effect was observed in human health. Metabolically active aflatoxins if present in the diet can cause DNA modifications which ultimately causes cell deregulation due to which cell death/transformation occur because it disrupt the synthesis of macromolecules (Eaton & Gallagher, 1994). If diet is contaminated with deoxynivalenol (DON), protein synthesis is inhibited due to which disruption of cytokinin regulation occurs and causes alteration of cell proliferation and cell death (Rotter *et al*., 1996). Amino acid and phenylalanine metabolism are also interrupted by ochratoxin due to reduction of phosphoenol pyruvate carboxykinase enzyme activity causing reduction in gluconeogensis, inhibits protein/DNA synthesis, alters membrane permeability which disrupts Ca++ homeostasis and cell regulation, ultimately causing cell death (Creppy, 1995). Studies were therefore carried out on detection of mycotoxins in maize seed samples collected from different areas of Pakistan.

Materials and Methods

Fifty nine maize seed samples were collected from different localities of Pakistan including Karachi (8), Hyderabad (11), Nawabshah (8), Sukkur (2), Lahore (8), Quetta (1), Peshawar (2) and Islamabad (19). Samples were homogenized and kept in glass bottle and stored at 2-8^{o}C until further analysis. For the quantitative analysis of mycotoxins (aflatoxin ochratoxin and Zearalenone). Enzyme Linked Immounosorbent Assay technique (ELISA) was used. Maize seeds (10 g) were taken in 50ml of 70% methanol for aflatoxins, zearalenone and ochratoxin analysis. Maize seed powder was blended individually with high speed blender for three minutes. After blending the material was filtered with Whatman filter paper number 1, the filtrate was used for further analysis. Commercially available immunoassay kit Veratox for quantitative analysis of aflatoxin, zearalenone and ochratoxin test-NEOGEN Crop, Lansing, MI was used. The assay kit was based on Competitive Direct Enzyme Linked Immunosorbent Assay (CD-ELISA) format (Stoloff *et al.,* 1991). The antibodies captured the analyte and conjugated to the enzyme (horse reddish peroxidase). Tetra methylbenzidine/ hydrogen peroxide was used as a substrate for colour development. Finally stopping solution was added to stop the reaction. The colour intensity was inversely proportional to the mycotoxin concentration and measured with the ELISA reader. All necessary reagents were present in the kit. Concentration of mycotoxins was calculated by Log/logit Software Awareness Technology Inc (Anon., 2000; Stoloff *et al.,* 1991).

Table 1. Quantitative analysis of mycotoxin in maize seed samples collected from different localities of Pakistan by using ELISA technique.

District	No. of varieties / sample	Mycotoxin (ug/kg)					
		Aflatoxin PL 0.9999		Zearalenone PL. 0.9992		Ochratoxin P.L : 25	
Sahiwal	Golden	OD	Results	OD	Results	OD	Results
	Sahiwal-2002	2.032	0.0	1.641	0.0	0.0	0.0
	EV-5098	1.485	5.0	1.061	25.5	0.0	0.0
	Agaiti-85	0.917	15.3	0.783	70.2	3.275	1.3
	Agaiti-2002	0.389	49.5	0.563	158.0	0.0	0.0
	EV-1098	1.889	1.1	0.315	496.7	0.0	0.0
	Sadaf	0.900	15.8	1.465	2.8	0.0	0.0
	EV-6089	1.799	1.8	1.189	15.1	0.0	0.0
	EV-6098	1.530	4.4	1.439	3.5	0.0	0.0
	Pak Afgoyee	1.079	11.3	0.705	92.8	0.0	0.0
Islamabad	Islamabad white	1.785	2.0	0.948	39.0	0.0	0.0
	Islamabad gold	1.082	11.2	1.128	19.5	0.0	0.0
	Margala	1.841	17.7	1.098	22.1	3.352	1.0
	Soan-3	0.711	22.8	0.957	37.7	0.0	0.0
	EV-1097	1.812	1.7	0.708	91.8	0.0	0.0
	EV-7004	1.206	8.9	1.165	16.7	0.0	0.0
	Pakaposhi	1.307	7.3	1.049	26.7	0.0	0.0
	BS-2	3.597	0.0	0.953	38.3	0.0	0.0
	BS-1	2.518	5.1	1.684	0.0	0.0	0.0
	P0P-2004	1.563	14.2	1.265	25.5	0.0	0.0
	POP-2006	0.574	51.3	0.931	74.3	0.0	0.0
	SWAN-3	3.352	1.0	0.698	144.8	0.0	0.0
	Manschra BSO-1	4.170	0.0	0.333	512.3	0.0	0.0
	EV-3001-NARC	2.832	3.4	1.578	3.8	0.0	0.0
	POP-2004 CCRI	3.779	0.0	1.635	1.4	0.0	0.0
	Agati-2002	3.190	1.7	1.565	4.4	0.0	0.0
	EV1097Islamabad Gold	2.669	4.2	1.510	7.2	1.671	0.6
	Agaiti-85	3.348	1.0	1.671	0.3	0.0	0.0
	Margalla NARC	2.114	8.1	1.515	6.9	0.0	0.0
Peshawar	Azam	3.275	1.3	1.793	0.0	1.635	1.4
	Sarhad white	3.122	2.0	1.564	4.4	0.0	0.0
	Kisan(New version)	0.089	324.6	1.605	2.6	0.0	0.0
Karachi	Unknown varieties	2.968	2.7	1.327	19.9	0.0	0.0
		1.896	10.1	1.507	7.7	0.0	0.0
		1.278	19.3	1.591	3.2	0.0	0.0
		2.957	2.7	1.454	10.5	0.0	0.0
		0.240	125.5	1.484	8.7	0.0	0.0
		2.052	8.6	0.942	72.0	0.0	0.0
		2.719	4.0	1.193	33.1	0.0	0.0
		2.667	4.3	1.305	21.8	0.0	0.0
Larkana	Unknown varieties	2.424	0.0	1.484	8.7	0.0	0.0
		1.974	5.9	1.833	0.0	0.0	0.0
		1.729	11.0	1.484	24.1	0.0	0.0
		0.868	57.9	1.073	80.0	0.0	0.0
		2.584	0.0	0.819	147.7	0.0	0.0
		2.806	0.0	0.389	492.8	0.0	0.0
		2.611	0.0	1.593	14.6	0.0	0.0
		2.52	0.0	1.827	0.2	0.0	0.0
		1.278	17.2	1.793	ND	0.0	0.0
		1.327	18.1	1.833	ND	0.0	0.0
		1.305	21.9	1.827	ND	0.0	0.0
Nawabshah	Unknown varieties	1.165	17.1	1.793	ND	0.0	0.0
		28.053	0.1	1.973	ND	0.0	0.0
		28.0530	13.5	1.833	ND	0.0	0.0
		28.0501	21.4	1.671	ND	0.0	0.0
		28.0537	30.8	1.641	ND	0.0	0.0
		1.484	8.6	1.684	ND	0.0	0.0
		1.454	9.0	1.635	ND	0.0	0.0
		1.974	5.7	1.465	ND	0.0	0.0

P.L = Permissible limit, ND = Not detected

Results and Discussion

For the detection of different types of mycotoxins, 59 maize seed samples were analyzed quantitatively by CD-ELISA technique (Competitive Direct Enzyme Linked Immunosorbent Assay). The quantity of mycotoxins in most of the seed samples were not detected within the detectable limits. However, in few samples, values of mycotoxins were above the permissible safe limits for human consumption and health. Quantitative analysis of mycotoxins in 59 maize seed samples/ varieties collected from different parts of Pakistan. Variety kisan, Mix variety, Pop 2006, Agaiti-2002 and Soan-3 contained highest quantity of aflatoxin, whereas some have very low quantity of aflatoxin. Zearalenone was also detected in highest quantity from six samples. In some sample zearalenone was not detected. Ochratoxin was detected only in four maize seed samples in low quantity. Present results obtained showed that out of 59 maize samples tested for the detection of mycotoxins like aflatoxtin, zearalenone and ochratoxin, 50 samples contained aflatoxin whereas, zearalenone was detected in 43 samples and ochratoxin was detected in 4 samples (Table 1).

Mycotoxins can cause severe damage to liver, kidney and nervous system of man even in low dosages (Rodricks, 1976). *Fusarium* and *Aspergillus* species are common fungal contaminants of maize and also produce mycotoxins (Bakan *et al*., 2002; Verga & Teren, 2005). *Aspergillus flavus* produces aflatoxin B_1, B_2, G_1 G_2 which is carcinogenic and produce liver cancer (Purchase, 1974; Diener & Davis, 1969; Pesta & Bonday 1990). *A. candidus* produce citrinin, harmful to kidney (Domsch *et al*., 1980). *Fusarium solani* cause corneal ulcer while *F. oxysporum* produce Zeralenone α and β causing haemorrhage and necrosis in bone marrow. *F. proliferatum* and *F. verticillioides* cause epidemiologically human esophageal cancer (Desjardins *et al*., 2006). Anne *et al*., (2000), Curtui *et al*., (1998) and Susan *et al*., (2005) isolated several *Fusarium* species from maize seed viz., *Fusarium moniliforme*, *F. graminearum*, *F. proliferatum*, *F. acuminatum*, *F. avenaceum*, *F. chlamydosporum*, *F. equiseti*, *F. oxysporum*, *F. semitectum* and *F. torulosum* which produce mycotoxins viz., Toxins deoxynivalenol (DON), 3-acetyl DON, 15-acetyl DON, Fusarenon X(FX),T-2 Toxin (T2), Diacetoxyscir phenol (DAS), Zearalenon (ZEA), Fumonisin, Aflatoxin B_1, Ochratoxin A (OA) and Citrinum (CT) respectively. Don and acetyle Don were the major mycotoxin in *Fusarium* species. *A.terreus* attacks human skin and nail and is parasitic on human ear. *A. wentii* produce kojic acid causing cardiovascular and brain disorder (Domsch *et al*., 1980).

In the present investigation some samples of seed were found to be contaminated with highest amount of mycotoxins. There is need to control mycotoxin contamination and provide mycotoxin free seeds for human consumption.

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