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Assessment of mycoflora and aflatoxin contamination of stored wheat grains

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Abstract Pakistan exports wheat to many countries. Therefore, post-harvest activities for analyzing good quality of wheat in a scientific manner duly approved by ISO are prerequisite. To meet the challenges of competition in the foreign market, particularly from the long established export giants like USA, Canada, Australia and France this has become essential for all the wheat exporters to establish quality control system. In present study stored wheat grains were randomly sampled. Each sample was divided in to three portions for their complete survey of moisture status, fungal flora and aflatoxin contamination. The moisture content of all samples was found less than 12%. All these samples were found positive for fungal contamination when analyzed by plating under unsterilized and sterilized (with 1 % chlorox) conditions on moistened filter paper, Czepaks agar and *Aspergillus flavus* and *A. parasiticus* Agar medium (AFPA). A total number of 30 species of fungi viz., *A. candidus*, *A. flavus*, *A. fumigatus*, *A. parasiticus*, *A. niger, A. restrictus*, *A. sulphurus*, *A. sydowi*, *Alternaria alternata*, *A. brassicae*, *A. humicola*, *A. solani*, *Rhizopus oryzae*, *R*. spp*., R. stolonifer*, *Acremonium* spp., *Geotrichum candidum*, *Mucor heimalis*, *M. spp.*, *Cochliobolus lunatus*, *Fusarium* spp., *F. culmorum*, *Rhizoctonia*, *Curvularia lunata*, *Cladosporium herbarum*, *Penicillium frequentus*, *Botrytus* spp., *Nigrospora* spp., *Humicola*, *Helminthosporium* spp. were isolated. *A. flavus and A parasiticus* population ranged from 0 – 5.4 CFU/10 seeds and 0 – 5.0 CFU/ 10 seeds respectively. AFPA proved excellent in the isolation of *A. flavus* and *A. pararitticus* strains. None of the samples was found aflatoxin contaminated when analyzed by ELIZA technique. The efficiency of ELIZA confirmed by the percent recovery of aflatoxin from positive and spiked controls and samples was found hundred percent.

Keywords: mycotoxin, ELIZA, spiked control, percent recovery method, *Aspergillus flavus*, *A. parasiticus*, AFPA

Introduction

Pakistan economy is mainly agrarian and earns about 35-40% of the national income. Wheat is the first major cereal crop of Pakistan and is the main staple diet of the people of Pakistan. Pakistan has been exporting wheat in past few years (GOP 2006). Main wheat exporters in the international market are developed countries having developed techniques for wheat production and its quality maintenance according to the FAO and WTO standards. Mycotoxins are secondary metabolites produced by fungi of various generations when they grow on agricultural products before or after harvest or during transportation or storage. Aflatoxins are one of the most potent mycotoxin that occurs naturally. Several surveys had been carried out in world to generate the basic information about the mycoflora and mycotoxin contamination of cereals (Halt 1994; Vrabcheva *et al.,* 1996; Carlos *et al.,* 2000). To compete in the export and the national and regional market, Pakistan has to establish quality control system of foods and feeds. First step to achieve this is to carry out surveys for the estimation of current status of commodities in detail under natural conditions. No such thorough data base is available; surveys carried out in previous century are out dated due to changed environmental conditions and in case of stored commodities, new storage facilities. Present study was conducted to generate data relating to the level of fungal flora population and aflatoxin contamination of stored wheat grains sampled from three provinces of Pakistan. This data will help to set local standards and legislative measures for admissible aflatoxin levels in Pakistani wheat grains.

Material and Methods

Survey and sample collection from wheat storage houses

Survey of Pakistan Administrative Services of Storage Corporation (PASSCO), different food department stores and local farmer storages of wheat grains from following locations was carried out during 2002 to 2005. From upper Punjab districts (Rawalpindi, Gujrat, Gujranwala, Lahore), Central Punjab districts (Muzzafargarh, Sahiwal, Pakpattan, Vehari), Lower Punjab districts (Khanewal, Multan, Lodhran, Bahawalpur), Upper Sindh districts (Karachi, Hyderabad, Badin, Thatta), Lower Sindh districts (Jacobabad, Nawabshah, Naushehro Feroz, Mirpur Khas) and NWFP (Mardan, Haripur,

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Nowshehra, Mansehra and Peshawar). Standard sampling procedure for bulk storage grain (Boxwall, 1986) was followed.

Determination of moisture content of wheat samples

The moisture content of the wheat grains was calculated as percentage moisture of the stored wheat grains (AOAC, Method No. 925.10 (2000) with slight modification of AOAC, Method No. 22.008 (1965).

Isolation and quantification of fungal flora

Samples were tested for mycoflora status by plating sterilized and unsterilzed wheat seeds on three media. For grains sterilization, in one plate 1% cholorox and in other three plates sterile water was poured. Grains were sterilized in chlorox for 4-5 min and then with the help of sterile forceps seed were washed in three changes of sterile water. These seeds were dried on sterilized filter paper and plated on the three media in triplicate on sterilized moistened filter paper by filter paper blotter method (ISTA, 1985). Samples were also tested on synthetic Czepek medium (SIGMA) and in lab made AFPA medium (Peptone, 10 g; Yeast extracts, 20 g; Ammonium iron citrate, 0.5g; Chloremphenicol, 0.1 g; Dicloran, 2 mg; Agar, 15 g; Distilled water, 1000 ml; pH, 6.2). Plates were incubated at 29°C for 6-10 days depending upon the fungal growth with in the samples. Pure cultures of colonies have been obtained by transferring fungal colonies individually on fresh media plates of synthetic Potato Dextrose Agar. A stereomicroscopic and microscopic study of fungi was carried out and the slides have been continuously observed under various powers of microscope i. e., 10X, 40X and 100X. The sizes of fugal spores, hyphae, heads, phialides, clistothecia, sclerotia, conidia, conidiophore, metulae etc was taken out after the calibration of microscope with ocular and stage micrometer ad were compared with manuals and compedium (Raper and Fennell 1965; Domsch *et al.,* 1980).

Mycotoxin quantification by ELIZA technique

For the quantification of aflatoxin in these samples toxins was extracted and tested using Neogen ELISA kits (Veratox for Aflatoxin HS Product No. 8031) as described by the manufacturer. Positive and negative standards were maintained for making a comparison between the standards and the samples results. A positive control was used as a reference material to make a comparison between the measured value of positive control and the measured sample value and a negative control called the method blank contained no sample material maintained in every analytical batch to monitor the presence of any contamination to which the analytical batch might be able to be exposed during analysis. Spiked controls were also maintained in each trial for the assurance of accuracy of results. The results were also represented by the percent recovery method by the formula.

$$\% \text{ Recovery of Spiked toxin} = \frac{(Xs - X_u)}{K} \times 100$$

$\mathbf{X_s}$ = Measured value in the spiked sample

$\mathbf{X_u}$ = Measured value in the unspiked sample

K = Expected value

Results

The moisture content of all the present study analyzed samples was less than 12%. Experiment conducted to analyze fungal status of all collected samples revealed that wheat grains of upper Punjab districts were infected with eight fungal species belonging to six different genera (Table 1). *A. flavus* was isolated from samples of three (Gujtrat, Gujranwala and Lahore) out of four districts of upper Punjab while *A. parasiticus* was noted only in the samples collected from Lahore district. Overall from all the samples fungal counts were higher on unsterilized seeds than the sterilized ones when samples were plated on three media used in this present study. AFPA a specific medium for the growth of *Aspergillus* and *Penicillium* proved excellent tool for the collection of *A. flavus* and *A. parasiticus* isolates because these species were isolated from Gujranwala and Lahore district samples only when grains were plated on this medium. In the central Punjab district samples *A. flavus* was noted in all of the collected samples and *A. parasiticus* was noted only in the samples of Muzzafargarh and Pakpattan. Moreover, *Alternaria alternata* was found as a dominant fungal contaminant of central Punjab stored wheat grains (Table 2).

As shown in Table 3 in the lower Punjab districts wheat grains twelve fungal species were found in different counts and occurrences. *A. parasiticus* was totally absent from the samples of lower Punjab districts. *A. flavus* was found in the samples collected from Multan and Bahawalpur districts. *Rhizopus oryzae* was found in almost all of the samples of these four districts and ranged from 0.051 to 3.991 CFU/ 10 seeds. From Sindh province samples thirteen fungal species were collected from Karachi, Hyderabad, Badin and Thatta districts (Table 4). Out of these four districts *A. flavus* was noted only in the samples of Karachi district. In the wheat grains of Jacobabad, Nawabshah, Naushehro Feroz and Mirpur khas a large

Table 1. Fungal flora (CFU/10 seeds) isolated from stored wheat grains of upper Punjab

Districts	Media		Total fungal flora	*Aspergillus flavus*	*Aspergillus parasiticus*	*Aspergillus niger*	*Rhizopus oryzae*	*Mucor spp.*	*Alternaria alternata*	*Fusarium spp.*	*Penicillium frequentus*	*Aspergillus restrictus*
Rawalpindi	FP	Un	9.333			2.530	1.333	1.133	6.166	0.533	0.800	0.000
		Ster	8.166			3.333	1.500	0.664	3.333	0.600	0.800	0.000
	Czepaks	Un	9.000			1.333	0.500	1.066	2.666	0.400	0.533	0.000
		Ster	8.500			2.833	2.666	0.066	3.500	0.200	0.533	0.000
	AFPA	Un	7.500			2.500	1.333	1.133	4.166	0.533	0.800	0.000
		Ster	6.667			2.666	1.333	0.664	2.833	0.600	0.800	0.000
Gujrat	FP	Un	9.933	0.400	0.000			1.133	7.866	0.533	0.800	0.000
		Ster	8.600	0.333	0.000			0.664	7.066	0.600	0.800	0.000
	Czepaks	Un	9.533	1.266	0.000			1.066	6.799	0.400	0.533	0.000
		Ster	9.733	0.066	0.000			1.066	7.399	0.200	0.533	0.000
	AFPA	Un	10.00	0.399	0.000			0.800	8.333	0.466	0.800	0.000
		Ster	9.000	0.200	0.000			0.799	6.800	0.600	0.800	0.000
Gujranwala	FP	Un	8.600	0.000	0.000	0.000			8.600			0.000
		Ster	9.200	0.000	0.000	0.000			9.200			0.000
	Czepaks	Un	9.933	0.000	0.000	0.000			9.066			0.866
		Ster	9.733	0.000	0.000	0.000			8.533			1.200
	AFPA	Un	8.866	2.133	0.000	2.266	1.333		3.466			0.866
		Ster	7.800	2.266	0.000	2.133	0.000		3.066			0.333
Lahore	FP	Un	10.00	2.133	0.000				8.733			
		Ster	9.733	1.160	0.000				8.066			
	Czepaks	Un	9.733	1.199	0.000				8.266			
		Ster	9.600	1.066	0.000				7.200			
	AFPA	Un	9.866	1.266	0.000				8.733			
		Ster	9.000	1.200	0.000				7.466			

Each value is the mean of three replicates X all samples collected from different centers of each district. The value of Standard Error all the values is less than ±2.3026. Where Un=unsterilized, Ster= sterilized, FP= Filter Paper, AFPA= *Aspergillus flavus* and *A. parasiticus* Agar

Table 2. Fungal flora (CFU/10 seeds) isolated from stored wheat grains of central Punjab

District												
	Media		Vehari			Pakpattan			Sahiwal			Muzaffargarh		
			AFPA	Czepaks	FP	AFPA	Czepaks	FP	AFPA	Czepaks	FP	AFPA	Czepaks	FP
Total fungal flora		**Un**	8.142	7.952	8.085	8.686	8.700	8.726	8.801	9.368	9.228	9.466	9.866	10.00
		Ster	7.538	7.738	8.142	8.673	8.666	8.613	9.350	9.339	9.127	9.600	10.00	9.866
Aspergillus flavus		**Un**	0.805	0.890	0.766	1.193	1.353	1.293	1.739	2.164	1.904	2.533	0.000	0.000
		Ster	0.971	0.785	0.799	1.180	1.253	1.326	1.706	1.416	1.683	3.333	0.000	0.000
Aspergillus parasiticus		**Un**				0.460	0.466	0.493				2.266	0.000	0.000
		Ster				0.420	0.486	0.480				1.866	0.000	0.000
Alternaria alternata		**Un**	5.533	5.742	5.962	6.153	6.160	6.120	4.956	5.460	5.430	2.066	6.400	6.666
		Ster	5.590	5.562	5.861	6.153	6.233	6.133	5.552	5.381	5.760	2.066	6.600	6.466
Aspergillus niger		**Un**	1.023	0.981	1.000				0.731	0.666	0.747			
		Ster	1.000	1.004	0.999				0.652	0.635	0.643			
Rhizopus stolonifer		**Un**	0.223	0.288	0.228				0.652	0.579	0.662			
		Ster	0.214	0.288	0.214				0.702	0.649	0.812			
Rhiozpus oryzae		**Un**				0.933	0.720	0.746				2.600	0.000	0.000
		Ster				0.506	0.786	0.693				2.333	0.000	0.000
Geotrichum candidum		**Un**										0.000	2.066	2.000
		Ster										0.000	2.400	2.266
Fusarium culmorum		**Un**										0.000	1.266	1.333
		Ster										0.000	1.000	1.133

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Table 3. Fungal flora (CFU/10 seeds) isolated from stored wheat grains of lower Punjab

District		Khanewal			Multan			Lodhran			Bahawalpur		
Media		FP	Czepaks	AFPA	FP	Czepaks	AFPA	FP	Czepaks	AFPA	FP	Czepaks	AFPA
Total fungal flora	Un	8.240	7.474	7.870	9.255	9.170	8.506	9.250	9.266	9.167	10.00	9.133	9.800
	Ster	7.755	7.737	7.885	9.144	9.229	9.548	9.300	9.216	9.200	9.000	8.733	9.600
Aspergillus flavus	Un				3.000	2.085	2.651				0.200	0.066	2.400
	Ster				2.088	2.155	2.726				0.533	0.066	3.000
Alternaria alternata	Un	0.137	0.177	0.270							5.600	5.466	1.66
	Ster	0.159	0.214	0.318							5.266	4.666	1.933
Aspergillus niger	Un	2.060	2.150	2.170							0.933	0.933	1.800
	Ster	2.150	1.981	1.814							0.666	0.866	2.733
Mucor heimalis	Un	2.051	1.759	1.974									
	Ster	2.011	1.781	1.925									
Rhizopus oryzae	Un	3.081	2.392	2.318	1.451	1.459	1.259	2.085	1.933	1.883	2.466	2.133	3.199
	Ster	2.344	2.544	2.711	1.455	1.381	1.303	2.067	1.800	2.050	2.000	2.000	2.133
Rhizoctonia	Un	0.611	0.466	0.529									
	Ster	0.485	0.618	0.518									
Aremonium spp.	Un	0.000	0.104	0.765	2.922	2.840	3.000						
	Ster	0.317	0.394	0.588	2.777	2.770	3.050						
Mucor spp.	Un				0.114	0.277	0.207	2.310	1.600	1.650			
	Ster				0.270	0.281	0.170	1.566	1.633	1.750			
Cochliobolus lunatus	Un				2.659	2.670	2.459						
	Ster				2.313	2.459	2.322						
Helminthosporium spp.	Un							4.366	4.500	4.533			
	Ster							4.433	4.550	4.730			
Alternaria solani	Un							1.350	1.283	1.050	5.600	5.466	1.666
	Ster							1.300	1.233	0.683	5.266	5.666	1.933
Penicillium frequentus	Un										0.800	0.600	0.000
	Ster										0.466	0.533	0.000

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Table 4. Fungal flora (CFU/10 seeds) isolated from stored wheat grains of upper Sindh

Thatta			Badin			Hyderabad			Karachi			District	Total fungal flora
AFPA	Czepaks	FP	AFPA	Czepaks	FP	AFPA	Czepaks	FP	AFPA	Czepaks	FP	Media	
6.906	8.968	8.985	9.333	9.222	8.221	9.888	9.999	8.554	7.781	8.472	7.892	Un	
9.040	8.979	8.997	10.11	8.666	8.887	9.888	9.776	8.221	8.260	8.029	8.157	Ster	
			3.000	3.222	2.889	2.333	1.555	2.777	2.558	3.771	2.635	Un	*Aspergillus niger*
			3.444	3.222	2.777	1.666	0.777	2.666	2.964	2.558	3.206	Ster	
									0.564	0.552	0.562	Un	*Alternaria brassicae*
									0.587	1.118	0.558	Ster	
									0.539	0.565	0.553	Un	*Rhizopus spp,*
									0.925	0.453	0.565	Ster	
									0.396	0.397	0.403	Un	*Acremonium Spp.*
									0.378	0.397	0.400	Ster	
0.430	0.430	0.400	6.333	6.000	0.000				3.107	2.484	2.958	Un	*Rhizopus oryzae*
0.430	0.430	0.411	6.666	5.444	0.000				2.793	2.859	2.679	Ster	
									3.107	2.484	2.958	Un	*Aspergillus flavus*
									2.793	2.859	2.769	Ster	
			0.000	0.000	2.444	7.555	8.000	2.555				Un	*Alternaria alternata*
			0.000	0.000	2.777	8.222	8.444	2.555				Ster	
			0.000	0.000	2.888							Un	*Aspergillus candidus*
			0.000	0.000	3.333							Ster	
						0.000	0.444	0.000				Un	*Rhizopus stolonifer*
						0.000	0.555	0.000				Ster	
						0.000	0.000	3.222				Un	*Aspergillus fumigatus*
						0.000	0.000	3.000				Ster	
4.210	6.350	6.430										Un	*Alternaria solani*
6.266	6.340	6.420										Ster	
1.233	1.155	1.089										Un	*Nigrospora Spp.*
1.289	1.176	1.100										Ster	
1.033	1.033	1.066										Un	*Humicola*
1.055	1.033	1.066										Ster	
1.033	1.033	1.066							0.617	0.703	0.781	Un	*Geotrichu, candidum*
1.055	1.033	1.066							0.613	0.644	0.749	Ster	

the mean of three replicates X all samples collected from different centers of each district. The value of Standard Error all the values is less than ±2.3026. Where Un=unsterilized, Ster= sterilized, FP= Filter Paper, AFPA= *Aspergillus flavus* and *A. parasiticus* Agar

Table 5. Fungal flora (CFU/10 seeds) isolated from stored wheat grains of lower Sindh

District	Media	Total fungal flora		*Aspergillus niger*		*Rhizopus oryzae*		*Alternaria alternata*		*Aspergillus flavus*		*Aspergillus parasiticus*		*Alternaria humicola*		*Botrytus*	
		Un	Ster	Un	Ster	Un	Ster	Un	Ster	Un	Ster	Un	Ster	Un	Ster	Un	Ster
Jacobabad	FP	8.216	7.733	0.750	0.750	0.200	0.200	5.700	5.783							1.566	1.000
	Czepaks	7.776	7.799	0.716	0.750	0.150	0.150	5.866	5.866							1.044	1.033
	AFPA	7.766	7.763	0.750	0.780	0.150	0.150	5.833	5.800							1.033	1.033
Nawabshah	FP	10.432	9.765			4.733	4.366	4.199	4.233	0.000	0.000	1.500	1.166				
	Czepaks	9.732	10.065			4.866	4.833	3.600	3.833	0.000	0.000	1.266	1.399				
	AFPA	9.524	9.698			1.499	1.066	3.466	4.133	1.899	1.899	2.660	2.600				
Naushehro Feroz	FP	9.732	9.932	0.000	0.000	3.466	3.866	4.266	4.133			2.000	1.933				
	Czepaks	9.932	9.866	0.000	0.000	3.800	4.133	4.266	3.933			1.866	1.800				
	AFPA	9.931	9.132	2.333	2.200	3.533	1.066	2.666	3.466			1.399	2.400				
Mirpur Khas	FP	9.905	9.187	2.000	1.238	3.905	3.809			0.000	0.000			4.000	4.140		
	Czepaks	9.999	10.713	1.380	2.000	4.524	5.428			0.000	0.000			4.095	3.285		
	AFPA	9.474	9.047	2.523	2.809	3.142	1.000			1.476	2.524			2.333	2.714		

Each value is the mean of three replicates X all samples collected from different centers of each district. The value of Standard Error all the values is less than ±2.3026. Where Un=unsterilized, Ster= sterilized, FP= Filter Paper, AFPA= *Aspergillus flavus* and *A. parasiticus* Agar

Table 6. Fungal flora (CFU/10 seeds) isolated from stored wheat grains of NWFP province of Pakistan

District	Media	Total fungal flora		*Aspergillus restricts*		*Alternaria alternata*		*Curvularia lunata*		*Aspergillus flavus*		*Aspergillus sulphurus*		*Cladosporium herbarum*		*Aspergillus niger*		*Geotrichum candidum*		*Aspergillus sydowii*		*Penicillium frequentus*		*Acremonium spp.*	
		Un	Ster	Un	Ster	Un	Ster	Un	Ster	Un	Ster	Un	Ster	Un	Ster	Un	Ster	Un	Ster	Un	Ster	Un	Ster	Un	Ster
Mardan	FP	10.00	9.260	8.800	7.730	1.200	1.530																		
	Czepaks	9.730	9.730	8.000	8.200	1.730	1.530																		
	AFPA	9.990	9.450	8.460	8.190	1.530	1.260																		
Haripur	FP	10.00	9.999			8.800	7.933	1.200	2.066																
	Czepaks	9.926	9.132			7.866	7.266	2.060	1.866																
	AFPA	10.000	10.000			10.000	10.000	0.000	0.000																
Nowshehra	FP	9.998	8.532	5.533	4.733	3.199	3.066			1.266	0.733														
	Czepaks	9.992	9.733	5.460	5.800	3.799	3.800			0.733	0.133														
	AFPA	9.998	9.305	4.533	5.133	4.199	3.399			1.266	0.773														
Mansehra	FP	7.854	7.395									2.403	2.431	1.795	1.286	1.231	1.208	2.425	2.470						
	Czepaks	7.190	8.503									2.248	2.063	1.247	1.055	1.220	2.730	2.475	2.655						
	AFPA	7.211	6.952									2.090	2.145	0.971	0.961	1.255	1.291	2.895	2.555						
Peshawar	FP	7.095	7.125			0.706	0.442	0.816	0.872							1.011	1.000			1.400	1.422	3.089	2.966	0.779	0.865
	Czepaks	8.428	5.958			0.881	0.111	0.934	0.223							1.022	1.033			1.422	1.466	2.878	2.733	2.172	0.503
	AFPA	7.010	5.826			0.365	0.431	0.899	0.527							1.033	1.011			1.466	1.477	2.633	2.566	0.979	0.245

Each value is the mean of three replicates X all samples collected from different centers of each district. The value of Standard Error all the values is less than ±2.3026. Where Un=unsterilized, Ster= sterilized, FP= Filter Paper, AFPA= *Aspergillus flavus* and *A. parasiticus* Agar

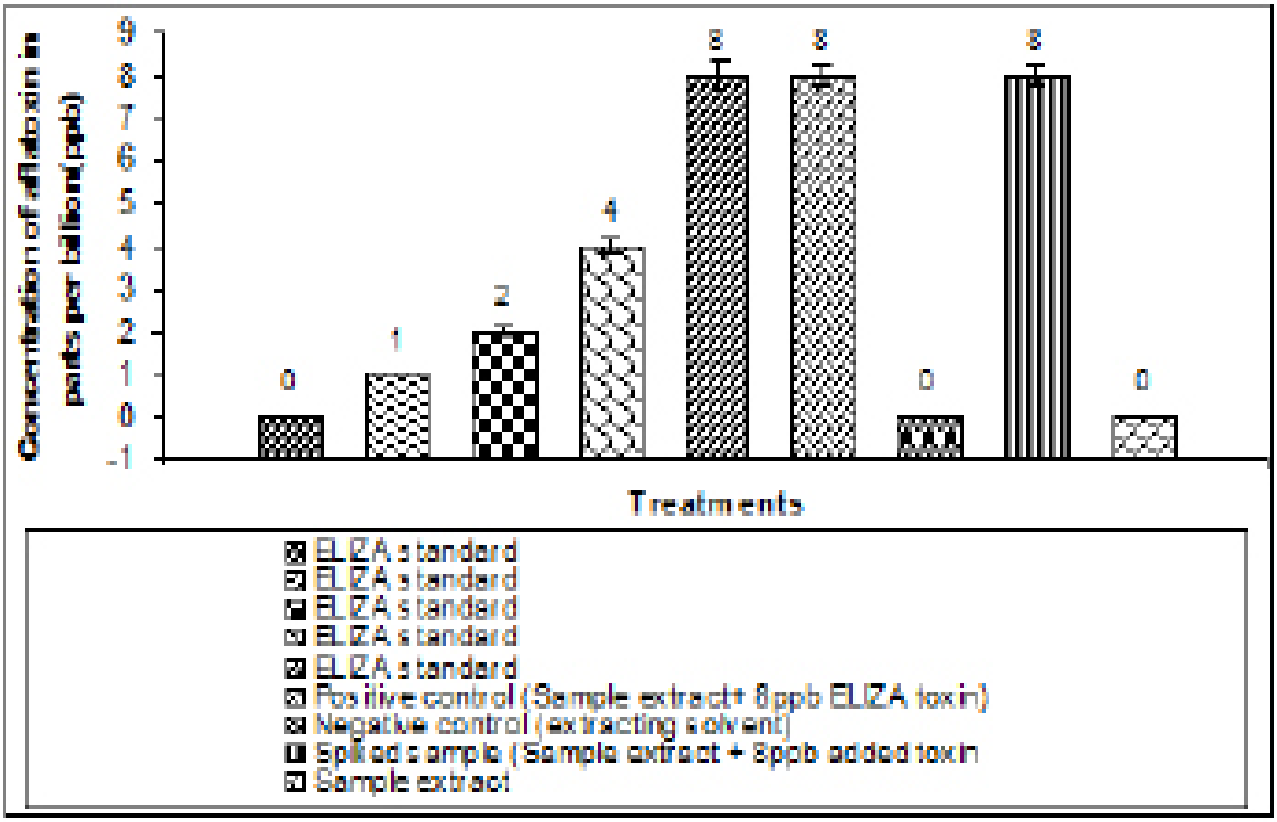

Figure 1. Total Aflatoxin in the stored wheat grains sampled from Punjab, Sindh and NWFP provinces of Pakistan by Enzyme Linked Immuno Assay technique.
Each bar value is the mean of three replicates of each treatment X values recorded for all analyzed batches of collected samples. Error bars are the Representatives of ±SE of each treatment.

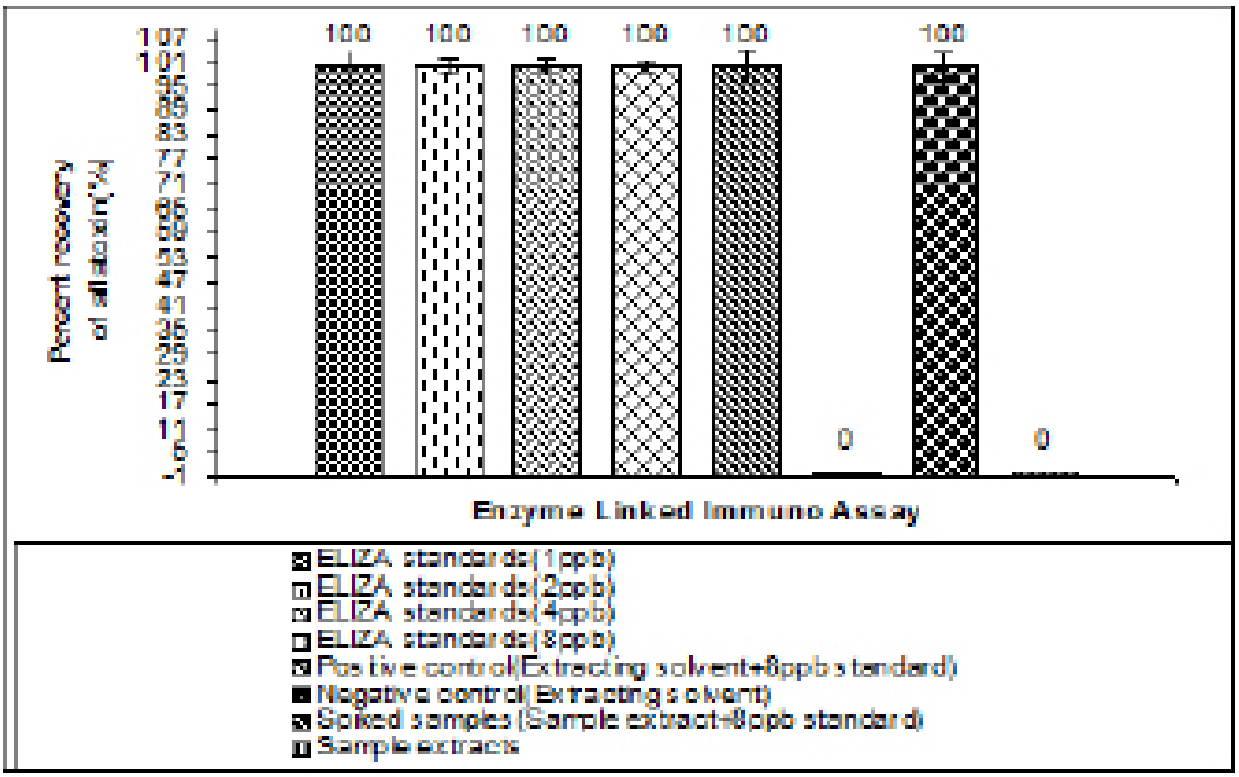

Figure 2. Assessment of Enzyme Linked Immuno Assay technique efficiency by percent recovery method during total Aflatoxin.
Each bar value is the mean of three replicates of each treatment X values recorded for all analyzed batches of collected samples. Error bars are the Representatives of ±SE of each treatment.

diversity of fungal species was observed (Table 5) *A. flavus* was isolated from Nawab Shah and Mirpurkhs samples when grains were plated on AFPA medium (Table 5). Wheat grains collected from five districts of NWFP were found infected with eleven different fungal species (Table 6). *A. flavus* was isolated from different centers of Attok district in the range of 0.133-1.266 CFU/10 seeds. All the samples of NWFP were found free for the *A. parasiticus* contamination. *A. restrictus* as a major fungal contaminant was observed only in the Mardan and Attock grains of NWFP province. Peshawar district samples exhibited a large variation in their fungal population without any fungus as a dominant contaminant (Table 6).

Overall from twenty five districts *A. flavus* ranged from zero to 5.4 CFU/10 seeds and was counted in fifteen district samples while *A. parasiticus* (range: 0.0 – 5.0 CFU/10 seeds) was isolated only in grains of five districts. Samples analyzed for their aflatoxin level by ELIZA showed that none of the samples was contaminated as shown in the Figure 1, and the percent recovery of the positive and spiked controls and samples was found 100 % represented in Figure 2. This average percent recovery shows that even a minute toxin in the samples could be detected by the used technique.

Discussion

Moisture level of sampled wheat grains of present work was found less than 12 % and it is well documented that fungi require moisture to cause food spoilage by the production of secondary metabolites within moisture limits of 13-13.2% (George, 1988). Pamela (2001) stated that degree of mould contamination in stored grains and animal feeds is a measure of their quality assurance. Survey of wheat grains for fungal contamination indicated that all samples were positive for fungal infection but their counts were in less number. *A. flavus* isolates in present studies were more in number than the *A. parasiticus* isolates this was also observed by Barros et al. (2005) they isolated more *A. flavus* strains from peanuts 73% than the wheat grains (13%). The morphological and structural sizes of this study isolates were in accordance with the results of Raper and Fennell (1965) and Domsch *et al*. (1980). Moreover in districts of Punjab, Sindh, and NWFP provinces *A. parasiticus* was isolated on AFPA medium. Thus this medium was found suitable for the detections of this fungus. AFPA medium used in the present work was in house made not the synthetic one. Frändberg (2003) also found that home made AFPA was equally efficient as the synthetic one. None of the sample was found aflatoxin contaminated in present study same was observed by Carlos *et al*. (2000) when they analyzed rice for aflatoxin contamination. Halt (1994) found aflatoxin in wheat samples but with the permissible limit i.e. 20 kg/kg (FAO and WTO standards). Escobar and Reguerio (2002) when surveyed a lot of commodities for aflatoxin contamination they found aflatoxin in wheat but less than other analyzed commodities like Sorghum, Peanut and corn. Polley *et al*. (1991) reported that mycotoxin levels in United Kingdom wheat have usually been found low though variable in quantity.

In present study percent frequency of positive and spiked controls and samples were found 100% by ELIZA, which proved very helpful for the analysis of aflatoxin free seed of present study. Similarly Ramón et al (2002) measured average accuracy of the ELIZA technique by a recovery assay in their analyzed samples that contained aflatoxin B_1 and they reported 107% aflatoxin recovery. Younis and Kamal (2003) surveyed peanuts and peanut products for aflatoxin contamination by HPLC and TLC techniques and when for the accuracy assessment of these two methods they spiked these samples with known amount of aflatoxin they found 79% and 95% toxin recovery by TLC and HPLC methods. Thus it has been cleared by present study and the reported ones that ELIZA technique is useful tool to detect the aflatoxin contamination in foods.

The present investigations revealed that these wheat grains stored in different store houses are of good quality. This data could be helpful to set local standards and legislative measures for admissible aflatoxin levels in Pakistani wheat grains. Present results recommends that similar surveys for other commodities in field and stored conditions should be carried out to make a clear picture for quality assurance and also in the development of advanced quality system by advanced techniques in agriculture of Pakistan.

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