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# SCREENING OF MYCOTOXINS IN WHEAT, FRUITS AND VEGETABLES GROWN IN SINDH, PAKISTAN

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**Abstract**

The main objective of the study was to determine the presence of different types of mycotoxins in different varieties of wheat, fruits and vegetables. The toxins included aflatoxin (total), deoxynivalenol (DON), fumonisin and ochratoxin (total). Sixty grain samples of wheat were randomly collected from different farmers' fields, godowns and flour mills of Sindh, and 20 vegetables and fruits samples from open market and exporters. Wheat samples were analyzed quantitatively by Competitive Direct Enzyme Linked Immunoscorbent Assay (CD-ELISA) and vegetables and fruits were analyzed qualitatively by thin layer chromatography (TLC). The quantity of mycotoxins in most of the wheat, fruits and vegetables were not detected within the detectable limits, and a few were found contaminated with the deoxynivalenol (DON) as in wheat, and aflatoxin in fruits and vegetables. However the values were below the permissible safe limits for human consumption and health.

## Introduction

Mycotoxins are chemically diversified low molecular weight compounds produced as secondary metabolites of fungal genera such as *Aspergillus*, *Penicillum*, *Fusarium*, *Altenaria* and *Claviceps* over a variety of food stuff having deleterious biological effect in animal and human (Adebajo & Diyaolu, 2003). Mycotoxin contamination occurs not only in crops at the field level and during storage; it may also affect food products destined for human consumption. The main factors responsible for the mycotoxins production are improper control of moisture content and temperature (Styriak *et al.*, 1998). Due to bad harvesting practices and storage condition many of the food stuff become spoiled due to the proliferation of toxin producing fungi (Ramesh & Siruguri, 2003). The food stuffs which are highly susceptible for the mycotoxin contamination are corn (Candlish *et al*., 2000), barley (Ramakrishna *et al*., 1990), wheat (Gilbert *et al*., 1995., Desjardins *et al*., 2000), coffee beans (Milanez *et al*., 1995), peanuts (Ramesh & Siruguri, 2003), spices (Fezekas *et al*., 2005), herbs (Kabelitz & Siever, 2004) and raw or processed fruits and vegetables (Giryn & Szkete, 1995). Biological detoxification methods such as fermentation can eliminate some classes of mycotoxins. Chemical detoxification is also done to minimize the toxic effect. Dry milling seems to concentrate fumonisin in the germ and bran fractions whereas wet milling tends to concentrate the fumonisin into gluten, germ and fiber fractions. Milling substantially reduces the level of ochratoxin A in white flour, but has little effect on levels in whole meal flour. The severity of mycotoxin problem was realized during World War II when Russians were eating moldy winter grains, they suffered with severe dermal necrosis, leucopenia, hemorrhages and destruction of bone marrow, *Fusarium* was found to be the causal organism of these abnormalities emerged after World War II (Forgacs, 1972). World–wide scientific recognition of mycotoxin problem was, however, only in 1960 when it was discovered that the aflatoxins were responsible for the death of about 100,000 turkey

poults (Turkey X disease) in England (Blount, 1961). Due to the presence of different mycotoxins in human edible crops, different adverse effects were observed in human health. Metabolically active aflatoxin if present in the diet can cause DNA modification which ultimately cause cell deregulation due to which cell death / transformation occur because it disrupt the synthesis of macromoleculer (Eaton & Gallagher, 1994). If diet is contaminated with deoxynivalenol (DON), protein synthesis is inhibited due to which disruption of cytokinin regulation occurs, which cause alteration of cell proliferation and cell death (Rotter *et al*., 1996). Fumonisin is another mycotoxin, if present in high concentration, then alteration of sphingoamine –N-acyltransferase occur which disrupt lipid metabolism and arachidonic acid metabolism, resulting in cell apoptosis (Merrill *et al*., 1996). Amino acid metabolism is also interrupted by ochratoxin and phenylalanine metabolism was disrupted by this due to reduction of phosphoenol pyruvate carboxykinase enzyme (PEPCK) activity causing reduce gluconeogensis, inhibits protein/DNA synthesis, alters membrane permeability which disrupts $Ca^{++}$ homeostasis & cell regulation, ultimately causing cell death (Creppy, 1995). Studies were conducted to define the formation of mycotoxins in wheat, fruits and vegetables grown in Nawabshah and Sukkur regions of Pakistan.

## Materials and Methods

Fruits and vegetables samples were collected from open market and from exporters during moist hot season and wheat varieties samples were collected from two districts of Sindh (Nawabshah and Sukkur). These samples were well mixed, homogenized and placed in glass bottle and stored at 2–8°C until further analysis. For the qualitative analysis of aflatoxin, TLC (thin layer chromatography) technique was used. 50g of each of fruit and vegetable samples were ground, mixed with 50ml distilled water and 200ml acetone which was then blended and filtered. Filtrate (150ml) was taken as a representative sample for analysis. The extract was then spotted (two spots of 1 ul and 5 ul of each dilution) on TLC plate. The lower 2 cm part of silica plate was scratch and developed plate in $CH_3Cl$ - acetone (85:15) solution. After development, TLC plate was examined under long wave UV light (150-350 nm) and compared the colour of spots with standards (Stack *et al*., 1975). For the quantitative analysis of mycotoxins (aflatoxin, fumonisin, ochratoxin and deoxynivalenol), Enzyme Linked Immunoscorbent Assay technique (ELISA) was used. Ground wheat samples of 25g were taken in 50ml of 70% methanol for aflatoxins and fumonisin analyses, while 40ml of 50% methanol for ochratoxin analysis, 10g of ground wheat sample was taken in 50ml of deionized water for deoxynivalenol (DON) analysis. Wheat samples were blended individually with high speed blender for three minutes. After blending the material was filtered with Whatman filter paper number 1, filtrate was used for further analysis. Commercially available immunoassay kit Veratox for quantitative analysis of aflatoxin, ochratoxin, fumonisin and deoxynivalenol (DON) test – NEOGEN Crop., Lansing, MI. were used to estimate these above mentioned mycotoxins. The assay kit was based on Competitive Direct Enzyme Linked Immunoscorbent Assay (CD-ELISA) format. The antibodies captured the analyte and conjugated to the enzyme i.e., 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 mycotoxin was calculated by Log/logit Software (Awareness Technology Inc).

**Table 1. Qualitative analysis of Aflatoxin in fruits and vegetables samples by TLC.**

| S. No. | Name of sample | No. of samples analyzed | Observation | Type of aflatoxins |
|---|---|---|---|---|
| 1. | Tomato | 3 | Aflatoxin | $B_1$ |
| 2. | Potato | 2 | *ND | ND |
| 3. | Peas | 4 | ND | ND |
| 4. | Beet Root | 3 | ND | ND |
| 5. | Pumpkin | 4 | Aflatoxin | $G_1$ |
| 6. | Garlic | 3 | ND | ND |
| 7. | Ginger | 3 | ND | ND |
| 8. | Onion | 3 | ND | ND |
| 9. | Chillies (powder) | 4 | Aflatoxin | $B_1$ |
| 10. | Carrot | 2 | ND | ND |
| 11. | Coriander(dry) | 3 | Aflatoxin | $B_1$ |
| 12. | Cucumber | 3 | Aflatoxin | $B_1$ |
| 13. | Grapes | 3 | ND | ND |
| 14. | Pomegranate | 3 | ND | ND |
| 15. | Persimmon | 3 | Aflatoxin | $G_1$ |
| 16. | Peanuts(dry) | 4 | Aflatoxin | $B_1$ |
| 17. | Dates | 3 | ND | ND |
| 18. | Peach(dry) | 3 | Aflatoxin | $B_1$ |

*ND (Not Detected)

## Results

Qualitative analysis of mycotoxins presented in Table 1 shows that some fruits and vegetables had two types of aflatoxin. Aflatoxin $B_1$ was detected in tomato, chilli powder, coriander (dry), cucumber, peanuts (dry) and peach (dry) samples whereas aflatoxin $G_1$ was found in pumpkin and persimmon samples. Aflatoxin in other fruits and vegetables were not detectable (within the detection limit). Quantitative analysis of mycotoxins in some wheat varieties collected from Nawabshah, are presented in Table 2. Results showed that Bhittai, TJ-83 and Imdaad respectively contained 1.3 ug/kg, 1.0 ug/kg and 1.4 ug/kg ochratoxin whereas deoxynivalenol (DON) was found at 100 ug/kg in Rabel, SKD-1, Imdaad and Moomal 2006. Aflatoxin, fumonisin, ochratoxin and deoxynivalenol (DON) were not detected in all other varieties. Wheat samples collected from Sukkur district revealed that ochratoxin 0.6 ug/kg and deoxynivalenol (DON) 100 ug/kg were found in Yakria and Bakkar respectively while aflatoxin, fumonisin, ochratoxin and deoxynivalenol (DON) were not detected in all the other varieties.

## Discussion

Most of the commodities under test were, free from mycotoxin contamination, some had a trace amount of mycotoxins which however were below permissible limit for human consumption and health. The study suggests that the mycotoxin contamination and fungal proliferation need to be controlled in field and storage before consumption to cease the deleterious effect on health. Mycotoxin contamination in different food product occur due to there high moisture content and improper storage temperature. Therefore all the edible commodities should be maintained at their specific moisture content and storage temperature to prevent the toxin proliferation. Post harvest measures like drying; cleaning, decontamination, detoxification and washing may also reduce contamination risks and enhance the food purity.

**Table 2. Quantitative analysis of Mycotoxin in wheat samples collected from Nawabshah and Sukkur District via ELISA.**

| District | Varieties | No. of samples | Mycotoxin (ug/kg) | | | |
|---|---|---|---|---|---|---|
| | | | Aflatoxin | Fumonisin | Ochratoxin | Deoxynivalenol (DON) |
| | | | *P.L: 20 | *P.L: 2000 | *P.L: 25 | *P.L: 1000 |
| Nawabshah | Bhittai | 2 | **ND | ND | 1.3 | ND |
| | TJ-83 | 2 | ND | ND | 1.0 | ND |
| | I-711 | 3 | ND | ND | ND | ND |
| | Inqlab-91 | 3 | ND | ND | ND | ND |
| | TD-1 | 1 | ND | ND | ND | ND |
| | V-7001 | 1 | ND | ND | ND | ND |
| | Anmol-91 | 3 | ND | ND | ND | ND |
| | Rabel | 2 | ND | ND | ND | 100 |
| | SKD-1 | 3 | ND | ND | ND | 100 |
| | Mehran-89 | 4 | ND | ND | ND | ND |
| | Abadgar-93 | 1 | ND | ND | ND | 100 |
| | Hamal Faqeer | 3 | ND | ND | ND | ND |
| | Imdaad | 2 | ND | ND | 1.4 | 100 |
| | Moomal-2002 | 3 | ND | ND | ND | 100 |
| | Sarsabz | 3 | ND | ND | ND | ND |
| | Wattan | 2 | ND | ND | ND | ND |
| Sukkur | Bakkar | 1 | ND | ND | ND | 100 |
| | Bhattai | 4 | ND | ND | ND | ND |
| | Marri | 3 | ND | ND | ND | ND |
| | Sona Leeka | 2 | ND | ND | ND | ND |
| | Inquilab-91 | 4 | ND | ND | ND | ND |
| | Yakria | 4 | ND | ND | 0.6 | ND |

*P.L= Permissible limit (Stoloff *et al*., 1991)
**ND = Not Detected.

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